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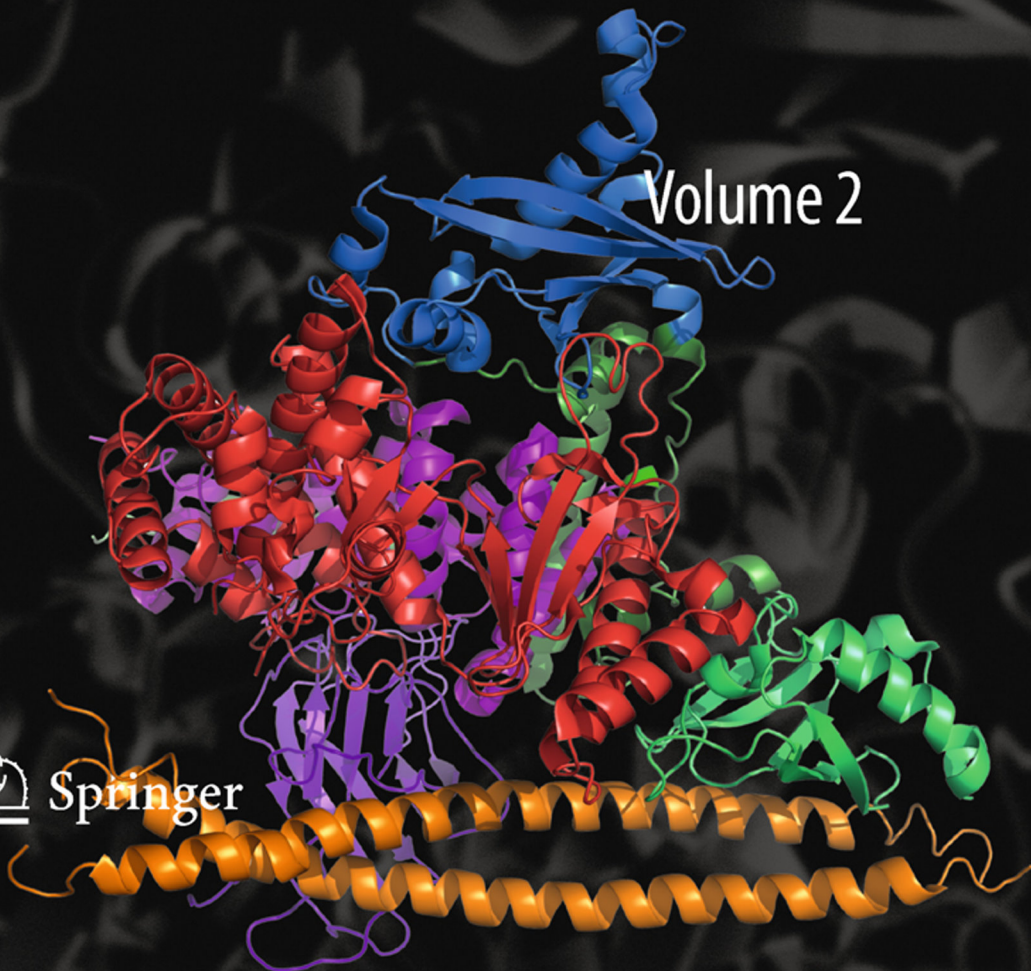
Christian Rommel
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Editors

Phosphoinositide 3-kinase in Health and Disease

Volume 2



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Christian Rommel • Bart Vanhaesebroeck •
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Phosphoinositide 3-kinase in Health and Disease

Volume 2

 Springer

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PI3K: From the Bench to the Clinic and Back

Bart Vanhaesebroeck, Peter K. Vogt, and Christian Rommel

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Abstract From humble beginnings over 25 years ago as a lipid kinase activity associated with certain oncoproteins, PI3K (phosphoinositide 3-kinase) has been catapulted to the forefront of drug development in cancer, immunity and thrombosis, with the first clinical trials of PI3K pathway inhibitors now in progress. Here, we give a brief overview of some key discoveries in the PI3K area and their impact, and include thoughts on the current state of the field, and where it could go from here.

PI3K has become a very intense area of research, with over 2,000 publications on PI3K in PubMed for 2009 alone. The expectations for a therapeutic impact of intervention with PI3K activity are high, and progress in the clinical arena is being monitored by many. However, targeted therapies almost invariably encounter road-blocks, often exposing unresolved questions in the basic understanding of the

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target. PI3K will most likely be no exception. Below, we describe some of these early “surprises” and how these inform and shape basic science investigations.

1 The Discovery of the PI3K Signalling Pathway and Its Potential as a Therapeutic Target

Early work showed that a phosphatidylinositol kinase activity co-purified with various viral oncoproteins expressed in mammalian cells (Macara et al. 1984; Sugimoto et al. 1984) and that cellular transformation mediated by such oncoproteins was to some extent dependent on the association with this lipid kinase activity (Whitman et al. 1985). This oncoprotein-associated lipid kinase could phosphorylate phosphatidylinositol on the 3-OH position of the inositol ring, hereby generating PI3P, a novel type of phosphoinositide (Whitman et al. 1988). This finding was followed by the discovery of PI(3,4,5)P₃ (phosphatidylinositol(3,4,5)trisphosphate; PIP₃) in GPCR-stimulated neutrophils (Traynor-Kaplan et al. 1988, 1989) and upon acute stimulation with tyrosine kinase agonists (Auger et al. 1989; Hawkins et al. 1992; Jackson et al. 1992). It was not known at the time that agonist-stimulated PI3K is a heterodimer made up of a p110 catalytic subunit and a regulatory subunit, namely p85 in the case of class IA PI3Ks and p101 in the case of the class IB p110 γ . Early studies very much focused on a tyrosine-phosphorylated 85 kD protein found in PDGF-stimulated or polyoma middle T-transformed cells which associated with PI3K activity (Courtneidge and Heber 1987; Kaplan et al. 1987). This protein turned out to be the p85 regulatory subunit of PI3K, and its cDNA was cloned by several groups (Escobedo et al. 1991; Otsu et al. 1991; Skolnik et al. 1991). Several teams also purified the PI3K enzyme activity biochemically from various tissues (Carpenter et al. 1990; Fry et al. 1992; Morgan et al. 1990; Shibasaki et al. 1991; Stephens et al. 1994). Protein microsequencing allowed the design of oligonucleotide probes to isolate the first cDNA of a PI3K catalytic subunit, namely p110 α (Hiles et al. 1992). This work revealed that the sequence of p110 was closely homologous to that of the product of *vps34*, a *S. cerevisiae* gene involved in endosomal sorting of proteins towards the vacuole, the yeast equivalent of the mammalian lysosome (Herman and Emr 1990). Follow-up work revealed that *vps34* indeed had PI3K activity, but with a substrate specificity that was different from p110 α , in that it can only phosphorylate PI (phosphatidylinositol) but not PI(4,5)P₂ (phosphatidylinositol(4,5)bisphosphate) (Schu et al. 1993).

A concerted effort of many laboratories, using various techniques, including biochemical purification and degenerate PCR approaches, revealed the existence of multiple PI3K isoforms in mammals (Arcaro et al. 1998; Brown et al. 1997; Chantry et al. 1997; Domin et al. 1997; Hu et al. 1993; Misawa et al. 1998; Ono et al. 1998; Stephens et al. 1997; Stoyanov et al. 1995; Vanhaesebroeck et al. 1997b; Virbasius et al. 1996), but also in *D. melanogaster* (MacDougall et al. 1995), *C. elegans* (Morris et al. 1996), *Dictyostelium* (Zhou et al. 1995) and other

species, even in plants. These findings led to the realisation that PI3Ks are an evolutionarily conserved family of enzymes which on the basis of structural and biochemical characteristics was divided into three classes (Vanhaesebroeck et al. 1997a; Zvelebil et al. 1996). Mammals have eight isoforms of PI3K (class IA: p110 α , p110 β , p110 δ ; class IB: p110 γ ; class II: PI3K-C2 α , PI3K-C2 β , PI3K-C γ , and class III: vps34p). A single representative of each of the three PI3K classes is present in *C. elegans* and *D. melanogaster*. In yeast, only a class III PI3K is found (reviewed in Vanhaesebroeck et al. 2001).

The analysis of PI3K functions in the cell was greatly aided by two small molecule inhibitors, wortmannin and LY294002. Wortmannin was identified as a PI3K inhibitor in 1993 (Arcaro and Wymann 1993; Okada et al. 1994; Powis et al. 1994; Yano et al. 1993), and in 1994, Lilly laboratories published the LY294002 inhibitor (Vlahos et al. 1994). Interestingly, all these papers almost exclusively focused on probing the immunological aspects of PI3K function using these compounds. LY294002 and wortmannin have undoubtedly been instrumental in providing first insights into the cell biology of PI3Ks but may also have generated some false expectations due to lack of specificity (see below).

Concurrent with the isolation of the genes for the different PI3Ks was the realisation that the 3-phosphoinositides could selectively bind to defined target modules in proteins, thereby altering the localisation of such proteins and their conformation and activity. Among numerous protein domains that were defined during this time was the PH (pleckstrin homology) domain, a module that occurs in many proteins (Haslam et al. 1993; Mayer et al. 1993). A major discovery was that some PH domains could bind phosphoinositides (Harlan et al. 1994). The characterisation of other 3-phosphoinositide binding domains soon followed, including the FYVE (Fab 1, YOTB, Vac 1, EEA1) domain (Gaulhier et al. 1998; Mu et al. 1995; Stenmark et al. 1996) and PX (Phox) domain (Cheever et al. 2001; Ellson et al. 2001; Kanai et al. 2001; Song et al. 2001; Xu et al. 2001) which both bind PI3P (phosphatidylinositol 3-phosphate).

One of the proteins that was reported (Haslam et al. 1993; Mayer et al. 1993) to have a PH domain was the Ser/Thr kinase Akt, which is the mammalian cellular homologue of the retroviral transforming gene *v-Akt* (Bellacosa et al. 1991). Akt was also independently cloned as a protein kinase related to PKA and PKC, hence its alternative names PKB (Coffer and Woodgett 1991) and Rac (related to A and C kinases) (Jones et al. 1991). Akt was subsequently confirmed as a PI3K target in cells stimulated with tyrosine kinase agonists, including PDGF and insulin (Burgering and Coffer 1995; Franke et al. 1995), and through its PH domain shown to bind PIP₃ and PI(3,4)P₂ with high specificity and affinity (Andjelkovic et al. 1997; Frech et al. 1997; Stokoe et al. 1997). An intact PH domain in Akt is crucial for its function (Stocker et al. 2002).

The regulation of Akt itself turned out to be rather complex. The PH domain recruits Akt to PIP₃ and PI(3,4)P₂ and the plasma membrane, where it becomes a substrate for the membrane-bound PDK1 kinase, which phosphorylates Akt on Thr308 (Alessi et al. 1997a, b; Stephens et al. 1998; Stokoe et al. 1997). Very early on, it was documented that Akt is also phosphorylated on Ser473 (Alessi et al. 1996),

but it took more than a decade to identify the kinase that performs this phosphorylation. It turned out to be mTOR complexed with the Rictor protein, also referred to as mTORC2 (Sarbasov et al. 2005) (as opposed to mTORC1, the “classical” mTOR in complex with Raptor).

A next step was to identify downstream substrates of Akt protein kinase activity. Akt was found to control other protein kinases either directly, such as GSK β (Cross et al. 1995) or indirectly, such as p70 S6 kinase (Burgering and Coffey 1995). One of the Akt substrates turned out to be the pro-apoptotic protein BAD, which is inhibited in its apoptotic function upon phosphorylation by Akt (Datta et al. 1997; del Peso et al. 1997). Given that wortmannin and LY294002 had previously been shown to be able to induce cell death (Yao and Cooper 1995), these observations suggested the existence of a PI3K-Akt cell survival pathway.

It is often overlooked that studies in *D. melanogaster* and especially in *C. elegans* have been instrumental in delineating the generic layout of the PI3K pathway and key aspects of its biology. For example, studies in *C. elegans* uncovered the link between the insulin-receptor, PI3K and the FOXO transcription factors (Ogg et al. 1997), and between Akt and FOXO (Paradis and Ruvkun 1998). FOXO transcription factors were later shown to be a target for direct phosphorylation by Akt in mammalian cells (Brunet et al. 1999; Kops et al. 1999). Further seminal work in model organisms included the identification of AGE-1 as the *C. elegans* p110 paralog with a key function in the control of lifespan (Morris et al. 1996) and the identification of PI3K in *Drosophila* as an important determinant in the regulation of cell growth and size (Leever et al. 1996).

Work from many groups further uncovered new elements of PI3K signalling, revealing the involvement of other PH domain-containing proteins, including regulators of small GTPases (GEFs and GAPs) (Klarlund et al. 1997; Krugmann et al. 2002; Welch et al. 2002) and various scaffolding and adaptor proteins (such as Gab1, Bam32, DAPP1) (Isakoff et al. 1998). These pathways have received much less attention over the years than Akt, and this may have had the effect of underestimating the importance of Akt-independent biology in PI3K action.

2 PI3K and Human Disease

Although the link between oncoproteins, growth factors and PI3K signalling, including the identification of PI3K as a Ras effector (Rodriguez-Viciana et al. 1994; Sjolander et al. 1991) and the demonstration that PI3K could act as a retroviral oncogene (Chang et al. 1997), provided some circumstantial evidence for a role of PI3K in cancer, genetic evidence from human cancer emerged only relatively late. An important breakthrough was the identification of the PTEN tumour suppressor as a PIP₃-phosphatase (Maehama and Dixon 1998). The frequently occurring inactivation of PTEN in cancer leads to constitutive activation of the PI3K pathway. It was not until 2004, however, that cancer-specific activating mutations were reported in *PIK3CA*, which encodes the p110 α isoform of PI3K

(Campbell et al. 2004; Samuels et al. 2004). Surprisingly, no mutations in non-p110 α isoforms have been detected thus far (Parsons et al. 2008; Samuels et al. 2004; TGCA 2008; Thomas et al. 2007; Wood et al. 2007). Mutations in the regulatory subunit, p85 α , encoded by *PIK3RI*, have been also discovered, although they occur at low frequency (Jaiswal et al. 2009; Philp et al. 2001; TGCA 2008). Interestingly, these mutations can also activate p110 β and p110 δ , possibly providing a broader activation of the class IA PI3K pathway than *PIK3CA* mutations (Jaiswal et al. 2009). The sheer number of mutations directed to PI3K signalling in *PTEN*, *PIK3CA*, *PIK3RI* and several upstream receptor tyrosine kinases makes this pathway one of the most deregulated and druggable biochemical activities in human cancer.

Since the mid-1990s, evidence for non-redundant functions of the class IAPI3K isoforms began to emerge (Hill et al. 2000; Roche et al. 1994, 1998; Vanhaesebroeck et al. 1999). Isoform-specific functions were exemplified by mice with inactivated p110 γ (Hirsch et al. 2000; Li et al. 2000; Sasaki et al. 2000) or p110 δ (Clayton et al. 2002; Jou et al. 2002; Okkenhaug et al. 2002), PI3K isoforms that are preferentially expressed in leukocytes. These mice are viable and fertile but show largely non-overlapping immune phenotypes. The phenotypes of these genetically modified mice identified p110 γ and p110 δ as targets in immunity and inflammation (Rommel et al. 2007; Ruckle et al. 2006; Soond et al. 2010).

Another area of isoform-specific function and possible therapeutic intervention is represented by the role of p110 β in platelet biology and thrombosis (Jackson et al. 2005). The p110 β isoform plays a key role in regulating the formation and stability of integrin/adhesion bonds, necessary for shear activation of platelets (Jackson et al. 2005). An isoform-selective p110 β inhibitor eliminates occlusive thrombus formation but does not prolong bleeding time in vivo (Jackson et al. 2005). These studies defined p110 β as a new target for antithrombotic therapy.

3 The Development of PI3K Inhibitors for Human Disease Starts to Inform Basic Science

In 2003, the first isoform-selective inhibitor, IC87114, which has high selectivity for p110 δ , was published (Sadhu et al. 2003). Over the last decade, ever increasing efforts were made to create both isoform-selective and pan-PI3K inhibitors for therapeutic use, efforts aided by the first crystal structure of a PI3K, that of p110 γ (Walker et al. 1999).

Isoform-selective inhibitors for p110 δ (CAL101/hematologic malignancies) and p110 β (AZD6482/thrombosis) have recently entered early clinical evaluation. Compounds that are effective against all class I PI3K isoforms, including sometimes mTOR, are currently being advanced into cancer patients with solid tumours. PI3K inhibitors have not yet been tested in allergy, inflammation and autoimmunity.

Several PI3K drug candidates have started to raise questions that impact on basic research, especially in the regulation of cell survival by PI3K. Indeed, inhibition of class I PI3K activity with pan-class I PI3K inhibitor compounds does not efficiently induce apoptosis, but rather lead to a G0/G1 cell cycle arrest (Dan et al. 2009; Fan et al. 2007; Guillard et al. 2009; Raynaud et al. 2007). In other words, inhibition of class I PI3K activity appears to be better at slowing down cell proliferation than at killing cells. This observation is reminiscent of what has been found in flies and worms, where inactivation of class I PI3K activity inhibits cell growth but does not induce cell death (Leever et al. 1996; Morris et al. 1996). Mammalian cells have recently been shown to be able to survive and proliferate normally with extremely reduced levels of class I PI3K activity (Foukas et al. 2010).

Looking back, it is clear that the effect on cell survival has been most prominently associated with PI3K action. It is becoming increasingly clear that while PI3K and Akt are effective modulators of anti-apoptotic signalling, in many systems, they are neither necessary nor sufficient to protect against cell death (reviewed in Vanhaesebroeck et al. 2001). These data suggest that the role of PI3K, and especially of Akt, in the control of cell survival and apoptosis may have been overestimated.

It is possible that the apoptosis-inducing activity of the pan-PI3K inhibitor LY294002, seen in some but not all cells, may be due to off-target effects. It is even more likely that cellular stress may have played a role in the outcome of some of the early studies on LY294002, for example, when tested on explanted cells such as neurons which are undergoing tissue culture stress (Yao and Cooper 1995). An option for increasing therapeutic effectiveness of PI3K inhibitors in cancer could be to broaden the PI3K target spectrum to include class II and class III PI3Ks whose potential role in cancer is largely unexplored. It might also be of interest to target PI3K-C2 α . Indeed, in a recent study, RNAi targeted to this isoform of PI3K led to cell death in half of the panel of cancer cells tested (Elis et al. 2008). PI3K-C2 α is relatively resistant to LY294002 (Domin et al. 1997; Virbasius et al. 1996) and might not have been inhibited by the doses of LY294002 that allowed cells to survive in the presence of this compound. The class III PI3K, vps34, may also be an important cancer target, given that it has been implicated in autophagy, a response to which cells under stress can resort to overcome adverse conditions.

From *in vitro* studies in large panels of cancer cell lines, it is becoming clear that there is no significant correlation between PTEN status and growth inhibition by class I PI3K inhibitors (Edgar et al. 2010; O'Brien et al. 2010). It is possible that in cancer patients, however, PTEN status could still be a predictor of responsiveness to PI3K pathway inhibition (Cloughesy et al. 2008). Interestingly, cells can be sensitive to the growth-inhibitory effect of class I PI3K inhibitors without having mutations in *PTEN* or *PIK3CA*, indicating that the PI3K pathway in these cells could be switched on by alternative mechanisms, such as amplification of HER2 (O'Brien et al. 2010), expression of oncogenic Ras or other mechanisms. In a panel of breast cancer cell lines, the presence of *PIK3CA* mutations seems to provide some enhanced sensitivity to class I PI3K inhibitors (O'Brien et al. 2010). However,

while this difference is statistically significant, it is small in absolute terms (less than 2-fold overall) and it remains to be seen if such small differences will make an impact in a clinical context. Taken together, these observations indicate that patient selection solely on the basis of *PIK3CA/PTEN* mutational status may not be as straightforward as originally hoped for and that more work is needed to define the molecular parameters that predict cancer cell sensitivity to PI3K inhibition, efforts which are under way (O'Brien et al. 2010).

New evidence also shows that in cancer cell lines, there is no good correlation between the presence of *PIK3CA* mutations and the steady state or growth factor-stimulated activity of PI3K and Akt (Morrow et al. 2005; Stemke-Hale et al. 2008; Vasudevan et al. 2009). This is in contrast to engineered cell model systems where gain-of-function mutations in *PIK3CA* are linked to increased PI3K signalling. It is likely that in cancer cells, other signalling networks come into play and that regulatory feedback loops affect the status of the PI3K activities. Interestingly, some cells with mutant *PIK3CA* show a dependency on the PDK1 and SGK3 protein kinases (Vasudevan et al. 2009), and it will be important to determine the genes and signalling pathways that might modulate the sensitivity of PI3K mutant cells to PI3K inhibitors.

If (class I) PI3K inhibition alone does not induce cancer cell death, the question arises what are the cancer-cell intrinsic effects of such inhibition that could be exploited for therapy. A cancer-specific role of PI3K signalling in intracellular nutrient sensing and control of metabolic pathways needs to be considered (Coloff and Rathmell 2006; Foukas et al. 2006; Jones and Thompson 2009; Plas and Thompson 2005). Such a role is also supported by the phenotypes of PI3K inactivation in flies and worms (Leever et al. 1996; Morris et al. 1996). Inhibition of PI3K in vivo has been documented to have a major impact on glucose uptake in tumour cells, as measured by ¹⁸F-fluoro deoxyglucose PET scans (Engelman et al. 2008). Other areas of cell-intrinsic impact of PI3K inhibition such as cell migration, invasion and metastasis also need to be examined.

It is most likely that class I PI3K inhibitors will be clinically effective only in combination with other interventions, such as targeted therapies against the EGF-R or MAPK pathways (Engelman et al. 2008; Faber et al. 2009; Sos et al. 2009), or more generic approaches such as chemo- and radiotherapy. One of the challenges for the future will be to delineate cancer types that might benefit from such combined therapies. An early example of such effective combination strategies is emerging in breast cancer where PI3K inhibitors can overcome resistance to EGF-R-directed therapy (Sergina et al. 2007).

It is important to keep in mind that most of the data on the impact of PI3K inhibition in cancer come from studies with cultured cell lines and xenografts. These conditions may affect the requirement for PI3K which may then differ significantly from the roles of PI3K in an autochthonous tumour growing in vivo. Indeed, the impact of PI3K inhibition on the stroma, including immune cells, fibroblasts and endothelial cells, could be substantial but remains largely unexplored. A role of PI3K in developmental angiogenesis has recently been established (Graupera et al. 2008), but the functions of PI3K in tumour angiogenesis are not

defined. An indirect role of PI3K blockade may also underpin the promising results of the phase I trials with the p110 δ inhibitor CAL-101, which induced disease stabilisation in a substantial number of patients with B-cell lymphoma (Flinn et al. 2009). The direct impact of p110 δ -centred inhibitors on the proliferation and survival of haematological cancer cells is modest, and it is possible that indirect actions of PI3K inhibitors come to play in this clinical setting.

4 Some Outstanding Questions in PI3K Biology and Signalling

While Akt has been the most studied target of PI3K, many questions on its regulation and function remain unanswered. Indeed, we still do not have a full understanding of its activation by PDK1 and mTORC2, of its inactivation and of the many feedback loops that control this kinase. We are largely ignorant of the mechanisms by which Akt regulates its cellular location and affects its many targets, notably those in the nucleus. We also have little definitive understanding of the specific, non-redundant functions of the three Akt isoforms. As aptly captured by Brian Hemmings when reviewing the field ten years after the molecular cloning of Akt, this is still “a hard Akt to follow” (Brazil and Hemmings 2001). It will also be important to re-evaluate the pro-survival and growth-promoting role of Akt and to define the signalling context that would make it a potentially exploitable therapeutic target.

PI3K effectors other than Akt also deserve more attention and scrutiny. Indeed, other than Akt, PI3K regulates other tyrosine kinases (such as Btk) and affects adaptor proteins (such as Gab2) and a plethora of GEFs and GAPs for monomeric GTPases of the Rac, Ras and Arf families (Vanhaesebroeck et al. 2001). The regulation of these GEFs and GAPs is complex and difficult to track experimentally, but some of these proteins could play important roles in PI3K signalling pathways. This is illustrated by P-REX2a, which activates the small GTPase Rac and is regulated by both PIP₃ and the G _{$\beta\gamma$} subunits of heterotrimeric G proteins, and which has recently been shown to interact with PTEN, inhibiting PTEN function (Fine et al. 2009).

The roles of the PI3K isoforms in human disease need to be further delineated. In a non-cancer context, class I PI3K isoforms have highly non-redundant functions, but it is not clear at this point how such specificity is achieved, as all PI3K isoforms activate Akt indiscriminately. It is possible that PI3K isoforms produce PIP₃ in different cellular compartments, and they could also differentially regulate small GTPases such as RhoA (Papakonstanti et al. 2007, 2008). In cancer, some of this non-redundancy is lost, possibly because the pathways upstream of the PI3K isoforms have been deregulated (Vanhaesebroeck et al. 2010).

Powerful tools to address some of these questions now available. These include isoform-specific inhibitors for p110 β , p110 γ and p110 δ as well as an array of mutant and transgenic mice. The differential roles of p110 isoforms in cancer remain an important topic. It is not clear why the gene encoding p110 α is so

selectively mutated in cancer. These mutations increase the activity of p110 α by enhanced association with the plasma membrane (Gymnopoulos et al. 2007; Mandelker et al. 2009), or by release from a p85-mediated inhibition (Miled et al. 2007), but the detailed molecular mechanisms of increased downstream signalling remain to be determined. There is suggestive evidence that different mutations can have a differential biological output such as in breast cancer cells, where the E545K mutation of *PIK3CA* appears to be associated with an enhanced metastatic phenotype compared to the H1047R mutation (Pang et al. 2009).

Thus far, the focus of the field has been on class I PI3Ks and their action through the PH-domain-mediated binding of key effectors to PIP₃ and PI(3,4)P₂. Relatively little attention has been paid to class II and III PI3Ks, their physiological roles and possible involvement in disease. These PI3Ks operate through PI3P and its effector proteins which bind this lipid with their PX or FYVE domains. While PH domains are more abundant than PX and FYVE domains, only a very small subset of PH domains binds PIP₃ or PI(3,4)P₂ (Lemmon 2008). In contrast, all PX and FYVE domains bind to PI3P. Therefore, PI3P has many more effectors than PIP₃ and PI(3,4)P₂. These effectors are very diverse and include p40 and p47 subunits of NADPH oxidase and proteins with sorting and scaffolding functions in membrane transport such as early endosome antigen-1 (EEA1), Hrs/vps27, ESCRT components, Alfy, kinesins and sorting nexin family members. PI3P-binding proteins also include the lipid kinase Fab1/PIKfyve (which converts PI3P to PI(3,5)P₂), the protein kinase SGK3 and additional GAPs (such as RGS-PX1) (reviewed in Birkeland and Stenmark 2004; Di Paolo and De Camilli 2006; Hurley 2006; Lemmon 2008; Vanhaesebroeck et al. 2010).

A key question is whether PI3P is involved in acute signalling and to what extent it influences signalling by extracellular agonists. Class II PI3K isoforms have been reported to generate PI3P in an agonist-dependent manner (reviewed in Falasca and Maffucci 2009; Vanhaesebroeck et al. 2010) and vps34 has been shown to control amino acid-dependent activation of S6 kinase-1 through unknown intermediates (Byfield et al. 2005; Nobukuni et al. 2005). At present there are no small molecule inhibitors of class II and III PI3Ks in the public domain (Shuttleworth et al. 2009). The importance of PI3P in disease is underscored by the observation that germline inactivation of PI3P-phosphatases of the myotubularin family in humans can lead to neuropathies and myopathy (Nicot and Laporte 2008).

Last but not least, we know very little about the production of the PI3K lipids themselves, their levels in disease, their subcellular localisation and their dynamic interconversion to other phosphoinositides. The frequent loss of the tumour suppressor PTEN in cancer demonstrates the importance of 3-phosphoinositide turnover. More recent observations assign important roles to 5-phosphatases of PIP₃, including IPP5E, whose inactivation is involved in ciliopathies (Bielas et al. 2009; Jacoby et al. 2009), and SHIP2, which has been implicated in insulin signalling and glucose homeostasis (Ooms et al. 2009). INPP4 is a 4-phosphatase of PI(3,4)P₂; its INPP4B isoform is a tumour suppressor that inhibits PI3K signalling (Gewinner et al. 2009). PI3P turnover is regulated by myotubularin phosphatases, some of which have been implicated in myopathies

and neuropathies (Nicot and Laporte 2008). These data show that it will be essential to monitor the levels and species of phosphoinositides in disease, in combination with proteomic and lipidomic profiling. Although it is now possible to monitor the subcellular distribution of 3-phosphoinositides with labelled lipid-binding domains, no progress has been made in the quantification of 3-phosphoinositides. Indeed, over the last decade, the entire field has almost exclusively relied on proxy readouts such as the phosphorylation of Akt. The disconnects between PI3K pathway activation and Akt phosphorylation that starts to surface (Vasudevan et al. 2009) make it imperative to develop new methods for monitoring 3-phosphoinositides in cells.

5 Concluding Remarks

Remarkable progress has been made over the last two decades in our knowledge of PI3K biology and signalling. PI3Ks have been identified as powerful signalling enzymes that respond to diverse upstream inputs and feed into complex downstream networks. Class I PI3Ks generate the tightly regulated second messenger PIP₃ signalling platform. At the level of cellular signalling, the four PI3K isoforms of class I, despite their identical lipid kinase activities, carry out largely non-redundant tasks, and recent evidence suggests that different isoforms can cooperate in achieving specific effects. The molecular basis for these distinctions and complementations is not understood. The extent to which different isoforms can substitute for each other is also not known.

High points in PI3K studies include genetically engineered mice, high resolution crystal structures, biochemical and cellular high throughput assays, cell-based and in vivo imaging assays, human genetics and isoform-selective inhibitors. There is an active debate in the field about selectively targeting single isoforms of PI3K versus a broader, pan-PI3K directed approach. First generation drugs against class I PI3K isoforms have entered clinical testing. Several other drugs targeting alternative components of the PI3K signalling network are at a similar stage of development. Despite many open questions, there is hope that an understanding of the genetic signatures that mark a role for PI3K in disease will translate into therapeutic benefits. First generation drugs are often “learning tools” that will be outperformed by better drugs and knowledge. Clinical experience, basic science and drug development are poised to interdigitate and to complement each other as the PI3K field evolves from a cellular signalling specialty to an area of broad medical significance and impact.

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References

- Alessi DR, Andjelkovic M, Caudwell B, Cron P, Morrice N, Cohen P, Hemmings BA (1996) Mechanism of activation of protein kinase B by insulin and IGF 1. *EMBO J* 15:6541-6551
- Alessi DR, Deak M, Casamayor A, Caudwell FB, Morrice N, Norman DG, Gaffney P, Reese CB, MacDougall CN, Harbison D, Ashworth A, Bownes M (1997a) 3 Phosphoinositide dependent protein kinase 1 (PDK1): structural and functional homology with the *Drosophila* DSTPK61 kinase. *Curr Biol* 7:776-789
- Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PR, Reese CB, Cohen P (1997b) Characterization of a 3 phosphoinositide dependent protein kinase which phosphorylates and activates protein kinase B. *Curr Biol* 7:261-269
- Andjelkovic M, Alessi DR, Meier R, Fernandez A, Lamb NJ, Frech M, Cron P, Cohen P, Lucocq JM, Hemmings BA (1997) Role of translocation in the activation and function of protein kinase B. *J Biol Chem* 272:31515-31524
- Arcaro A, Wymann MP (1993) Wortmannin is a potent phosphatidylinositol 3 kinase inhibitor: the role of phosphatidylinositol 3,4,5 trisphosphate in neutrophil responses. *Biochem J* 296 (Pt 2):297-301
- Arcaro A, Volinia S, Zvelebil MJ, Stein R, Watton SJ, Layton MJ, Gout I, Ahmadi K, Downward J, Waterfield MD (1998) Human phosphoinositide 3 kinase C2beta, the role of calcium and the C2 domain in enzyme activity. *J Biol Chem* 273:33082-33090
- Auger KR, Serunian LA, Soltoff SP, Libby P, Cantley LC (1989) PDGF dependent tyrosine phosphorylation stimulates production of novel polyphosphoinositides in intact cells. *Cell* 57:167-175
- Bellacosa A, Testa JR, Staal SP, Tsichlis PN (1991) A retroviral oncogene, akt, encoding a serine threonine kinase containing an SH2 like region. *Science* 254:274-277
- Bielas SL, Silhavy JL, Brancati F, Kisseleva MV, Al Gazali L, Sztrihai L, Bayoumi RA, Zaki MS, Abdel Aleem A, Rosti RO, Kayserili H, Swistun D, Scott LC, Bertini E, Boltshauser E, Fazzi E, Travaglini L, Field SJ, Gayral S, Jacoby M, Schurmans S, Dallapiccola B, Majerus PW, Valente EM, Gleeson JG (2009) Mutations in INPP5E, encoding inositol polyphosphate 5 phosphatase E, link phosphatidylinositol signaling to the ciliopathies. *Nat Genet* 41:1032-1036
- Birkeland HC, Stenmark H (2004) Protein targeting to endosomes and phagosomes via FYVE and PX domains. *Curr Top Microbiol Immunol* 282:89-115
- Brazil DP, Hemmings BA (2001) Ten years of protein kinase B signalling: a hard Akt to follow. *Trends Biochem Sci* 26:657-664
- Brown RA, Ho LK, Weber Hall SJ, Shipley JM, Fry MJ (1997) Identification and cDNA cloning of a novel mammalian C2 domain containing phosphoinositide 3 kinase, HsC2 PI3K. *Biochem Biophys Res Commun* 233:537-544
- Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis J, Greenberg ME (1999) Akt promotes cell survival by phosphorylating and inhibiting a Fork head transcription factor. *Cell* 96:857-868
- Burgering BM, Coffey PJ (1995) Protein kinase B (c Akt) in phosphatidylinositol 3 OH kinase signal transduction. *Nature* 376:599-602
- Byfield MP, Murray JT, Backer JM (2005) hVps34 is a nutrient regulated lipid kinase required for activation of p70 S6 kinase. *J Biol Chem* 280:33076-33082
- Campbell IG, Russell SE, Choong DY, Montgomery KG, Ciavarella ML, Hooi CS, Cristiano BE, Pearson RB, Phillips WA (2004) Mutation of the PIK3CA gene in ovarian and breast cancer. *Cancer Res* 64:7678-7681
- Carpenter CL, Duckworth BC, Auger KR, Cohen B, Schaffhausen BS, Cantley LC (1990) Purification and characterization of phosphoinositide 3 kinase from rat liver. *J Biol Chem* 265:19704-19711
- Chang HW, Aoki M, Fruman D, Auger KR, Bellacosa A, Tsichlis PN, Cantley LC, Roberts TM, Vogt PK (1997) Transformation of chicken cells by the gene encoding the catalytic subunit of PI 3 kinase. *Science* 276:1848-1850

- Chantry D, Vojtek A, Kashishian A, Holtzman DA, Wood C, Gray PW, Cooper JA, Hoekstra MF (1997) p110delta, a novel phosphatidylinositol 3 kinase catalytic subunit that associates with p85 and is expressed predominantly in leukocytes. *J Biol Chem* 272:19236-19241
- Cheever ML, Sato TK, de Beer T, Kutateladze TG, Emr SD, Overduin M (2001) Phox domain interaction with PtdIns(3)P targets the Vam7 t SNARE to vacuole membranes. *Nat Cell Biol* 3:613-618
- Clayton E, Bardi G, Bell SE, Chantry D, Downes CP, Gray A, Humphries LA, Rawlings D, Reynolds H, Vigorito E, Turner M (2002) A crucial role for the p110delta subunit of phosphatidylinositol 3 kinase in B cell development and activation. *J Exp Med* 196:753-763
- Cloughesy TF, Yoshimoto K, Nghiemphu P, Brown K, Dang J, Zhu S, Hsueh T, Chen Y, Wang W, Youngkin D, Liao L, Martin N, Becker D, Bergsneider M, Lai A, Green R, Oglesby T, Koletto M, Trent J, Horvath S, Mischel PS, Mellinghoff IK, Sawyers CL (2008) Antitumor activity of rapamycin in a Phase I trial for patients with recurrent PTEN deficient glioblastoma. *PLoS Med* 5(1):e8
- Coffer PJ, Woodgett JR (1991) Molecular cloning and characterisation of a novel putative protein serine kinase related to the cAMP dependent and protein kinase C families. *Eur J Biochem* 201:475-481
- Coloff JL, Rathmell JC (2006) Metabolic regulation of Akt: roles reversed. *J Cell Biol* 175:845-847
- Courtneidge SA, Heber A (1987) An 81 kd protein complexed with middle T antigen and pp 60csrc: a possible phosphatidylinositol kinase. *Cell* 50:1031-1037
- Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA (1995) Inhibition of glycogen synthase kinase 3 by insulin mediated by protein kinase B. *Nature* 378:785-789
- Dan S, Yoshimi H, Okamura M, Mukai Y, Yamori T (2009) Inhibition of PI3K by ZSTK474 suppressed tumor growth not via apoptosis but G0/G1 arrest. *Biochem Biophys Res Commun* 379:104-109
- Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg ME (1997) Akt phosphorylation of BAD couples survival signals to the cell intrinsic death machinery. *Cell* 91:231-241
- del Peso L, Gonzalez Garcia M, Page C, Herrera R, Nunez G (1997) Interleukin 3 induced phosphorylation of BAD through the protein kinase Akt. *Science* 278:687-689
- Di Paolo G, De Camilli P (2006) Phosphoinositides in cell regulation and membrane dynamics. *Nature* 443:651-657
- Domin J, Pages F, Volinia S, Rittenhouse SE, Zvebil MJ, Stein RC, Waterfield MD (1997) Cloning of a human phosphoinositide 3 kinase with a C2 domain that displays reduced sensitivity to the inhibitor wortmannin. *Biochem J* 326(Pt 1):139-147
- Edgar KA, Wallin JJ, Berry M, Lee LB, Prior WW, Sampath D, Friedman LS, Belvin M (2010) Isoform specific phosphoinositide 3 kinase inhibitors exert distinct effects in solid tumors. *Cancer Res* 70:1164-1172
- Elis W, Triantafellow E, Wolters NM, Sian KR, Caponigro G, Borawski J, Gaither LA, Murphy LO, Finan PM, Mackeigan JP (2008) Down regulation of class II phosphoinositide 3 kinase alpha expression below a critical threshold induces apoptotic cell death. *Mol Cancer Res* 6:614-623
- Ellson CD, Gobert Gosse S, Anderson KE, Davidson K, Erdjument-Bromage H, Tempst P, Thuring JW, Cooper MA, Lim ZY, Holmes AB, Gaffney PR, Coadwell J, Chilvers ER, Hawkins PT, Stephens LR (2001) PtdIns(3)P regulates the neutrophil oxidase complex by binding to the PX domain of p40(phox). *Nat Cell Biol* 3:679-682
- Engelman JA, Chen L, Tan X, Crosby K, Guimaraes AR, Upadhyay R, Maira M, McNamara K, Perera SA, Song Y, Chirieac LR, Kaur R, Lightbown A, Simendinger J, Li T, Padera RF, Garcia Echeverria C, Weissleder R, Mahmood U, Cantley LC, Wong KK (2008) Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. *Nat Med* 14:1351-1356
- Escobedo JA, Navankasattusas S, Kavanaugh WM, Milfay D, Fried VA, Williams LT (1991) cDNA cloning of a novel 85 kd protein that has SH2 domains and regulates binding of PI3 kinase to the PDGF beta receptor. *Cell* 65:75-82

- Faber AC, Li D, Song Y, Liang MC, Yeap BY, Bronson RT, Lifshits E, Chen Z, Maira SM, Garcia Echeverria C, Wong KK, Engelman JA (2009) Differential induction of apoptosis in HER2 and EGFR addicted cancers following PI3K inhibition. *Proc Natl Acad Sci USA* 106:19503-19508
- Falasca M, Maffucci T (2009) Rethinking phosphatidylinositol 3 monophosphate. *Biochim Biophys Acta* 1793:1795-1803
- Fan QW, Cheng CK, Nicolaides TP, Hackett CS, Knight ZA, Shokat KM, Weiss WA (2007) A dual phosphoinositide 3 kinase alpha/mTOR inhibitor cooperates with blockade of epidermal growth factor receptor in PTEN mutant glioma. *Cancer Res* 67:7960-7965
- Fine B, Hodakoski C, Koujak S, Su T, Saal LH, Maurer M, Hopkins B, Keniry M, Sulis ML, Mense S, Hibshoosh H, Parsons R (2009) Activation of the PI3K pathway in cancer through inhibition of PTEN by exchange factor P REX2a. *Science* 325:1261-1265
- Flinn IA, Byrd JC, Furman RR, Brown JR, Benson DM, Coutre SE, Kahl BS, Smith BD, Wagner Johnston ND, Spurgeon SE, Giese NA, Yu AS (2009) Evidence of clinical activity in a phase 1 study of CAL 101, an oral PI10 δ isoform selective inhibitor of phosphatidylinositol 3 kinase, in patients with relapsed or refractory B Cell malignancies. *ASH 2009 Abstract*
- Foukas LC, Claret M, Pearce W, Okkenhaug K, Meek S, Peskett E, Sancho S, Smith AJ, Withers DJ, Vanhaesebroeck B (2006) Critical role for the p110 α phosphoinositide 3 OH kinase in growth and metabolic regulation. *Nature* 441:366-370
- Foukas LC, Berenjano IM, Gray A, Khwaja A, Vanhaesebroeck B (2010) Activity of any class IA PI 3 kinase isoform can sustain cell proliferation and survival. *Proc Natl Acad Sci USA* (in press)
- Franke TF, Yang SI, Chan TO, Datta K, Kazlauskas A, Morrison DK, Kaplan DR, Tsichlis PN (1995) The protein kinase encoded by the Akt proto-oncogene is a target of the PDGF activated phosphatidylinositol 3 kinase. *Cell* 81:727-736
- Frech M, Andjelkovic M, Ingley E, Reddy KK, Falck JR, Hemmings BA (1997) High affinity binding of inositol phosphates and phosphoinositides to the pleckstrin homology domain of RAC/protein kinase B and their influence on kinase activity. *J Biol Chem* 272:8474-8481
- Fry MJ, Panayotou G, Dhand R, Ruiz Larrea F, Gout I, Nguyen O, Courtneidge SA, Waterfield MD (1992) Purification and characterization of a phosphatidylinositol 3 kinase complex from bovine brain by using phosphopeptide affinity columns. *Biochem J* 288(Pt 2):383-393
- Gaullier JM, Simonsen A, D'Arrigo A, Bremnes B, Stenmark H, Aasland R (1998) FYVE fingers bind PtdIns(3)P. *Nature* 394:432-433
- Gewinner C, Wang ZC, Richardson A, Teruya-Feldstein J, Etemadmoghadam D, Bowtell D, Barretina J, Lin WM, Rameh L, Salmena L, Pandolfi PP, Cantley LC (2009) Evidence that inositol polyphosphate 4 phosphatase type II is a tumor suppressor that inhibits PI3K signaling. *Cancer Cell* 16:115-125
- Graupera M, Guillermet-Guibert J, Foukas LC, Phng LK, Cain RJ, Salpekar A, Pearce W, Meek S, Millan J, Cutillas PR, Smith AJ, Ridley AJ, Ruhrberg C, Gerhardt H, Vanhaesebroeck B (2008) Angiogenesis selectively requires the p110 α isoform of PI3K to control endothelial cell migration. *Nature* 453:662-666
- Guillard S, Clarke PA, Te Poele R, Mohri Z, Bjerke L, Valenti M, Raynaud F, Eccles SA, Workman P (2009) Molecular pharmacology of phosphatidylinositol 3 kinase inhibition in human glioma. *Cell Cycle* 8:443-453
- Gymnopoulos M, Elsliger MA, Vogt PK (2007) Rare cancer specific mutations in PIK3CA show gain of function. *Proc Natl Acad Sci USA* 104:5569-5574
- Harlan JE, Hajduk PJ, Yoon HS, Fesik SW (1994) Pleckstrin homology domains bind to phosphatidylinositol 4,5 bisphosphate. *Nature* 371:168-170
- Haslam RJ, Koide HB, Hemmings BA (1993) Pleckstrin domain homology. *Nature* 363:309-310
- Hawkins PT, Jackson TR, Stephens LR (1992) Platelet derived growth factor stimulates synthesis of PtdIns(3,4,5)P₃ by activating a PtdIns(4,5)P₂ 3 OH kinase. *Nature* 358:157-159
- Herman PK, Emr SD (1990) Characterization of VPS34, a gene required for vacuolar protein sorting and vacuole segregation in *Saccharomyces cerevisiae*. *Mol Cell Biol* 10:6742-6754

- Hiles ID, Otsu M, Volinia S, Fry MJ, Gout I, Dhand R, Panayotou G, Ruiz Larrea F, Thompson A, Totty NF et al (1992) Phosphatidylinositol 3 kinase: structure and expression of the 110 kd catalytic subunit. *Cell* 70:419 429
- Hill K, Welti S, Yu J, Murray JT, Yip SC, Condeelis JS, Segall JE, Backer JM (2000) Specific requirement for the p85 p110alpha phosphatidylinositol 3 kinase during epidermal growth factor stimulated actin nucleation in breast cancer cells. *J Biol Chem* 275:3741 3744
- Hirsch E, Katanaev VL, Garlanda C, Azzolino O, Pirola L, Silengo L, Sozzani S, Mantovani A, Altruda F, Wymann MP (2000) Central role for G protein coupled phosphoinositide 3 kinase gamma in inflammation. *Science* 287:1049 1053
- Hu P, Mondino A, Skolnik EY, Schlessinger J (1993) Cloning of a novel, ubiquitously expressed human phosphatidylinositol 3 kinase and identification of its binding site on p85. *Mol Cell Biol* 13:7677 7688
- Hurley JH (2006) Membrane binding domains. *Biochim Biophys Acta* 1761:805 811
- Isakoff SJ, Cardozo T, Andreev J, Li Z, Ferguson KM, Abagyan R, Lemmon MA, Aronheim A, Skolnik EY (1998) Identification and analysis of PH domain containing targets of phosphatidylinositol 3 kinase using a novel in vivo assay in yeast. *EMBO J* 17:5374 5387
- Jackson TR, Stephens LR, Hawkins PT (1992) Receptor specificity of growth factor stimulated synthesis of 3 phosphorylated inositol lipids in Swiss 3T3 cells. *J Biol Chem* 267:16627 16636
- Jackson SP, Schoenwaelder SM, Goncalves I, Nesbitt WS, Yap CL, Wright CE, Kenche V, Anderson KE, Dopheide SM, Yuan Y, Sturgeon SA, Prabakaran H, Thompson PE, Smith GD, Shepherd PR, Daniele N, Kulkarni S, Abbott B, Saylik D, Jones C, Lu L, Giuliano S, Hughan SC, Angus JA, Robertson AD, Salem HH (2005) PI 3 kinase p110beta: a new target for antithrombotic therapy. *Nat Med* 11:507 514
- Jacoby M, Cox JJ, Gayral S, Hampshire DJ, Ayub M, Blockmans M, Pernot E, Kisseleva MV, Compere P, Schiffmann SN, Gergely F, Riley JH, Perez Morga D, Woods CG, Schurmans S (2009) INPP5E mutations cause primary cilium signaling defects, ciliary instability and ciliopathies in human and mouse. *Nat Genet* 41:1027 1031
- Jaiswal BS, Janakiraman V, Kljavin NM, Chaudhuri S, Stern HM, Wang W, Kan Z, Dbouk HA, Peters BA, Waring P, Dela Vega T, Kenski DM, Bowman KK, Lorenzo M, Li H, Wu J, Modrusan Z, Stinson J, Eby M, Yue P, Kaminker JS, de Sauvage FJ, Backer JM, Seshagiri S (2009) Somatic mutations in p85alpha promote tumorigenesis through class IA PI3K activation. *Cancer Cell* 16:463 474
- Jones RG, Thompson CB (2009) Tumor suppressors and cell metabolism: a recipe for cancer growth. *Genes Dev* 23:537 548
- Jones PF, Jakubowicz T, Pitossi FJ, Maurer F, Hemmings BA (1991) Molecular cloning and identification of a serine/threonine protein kinase of the second messenger subfamily. *Proc Natl Acad Sci USA* 88:4171 4175
- Jou ST, Carpino N, Takahashi Y, Piekorz R, Chao JR, Wang D, Ihle JN (2002) Essential, nonredundant role for the phosphoinositide 3 kinase p110delta in signaling by the B cell receptor complex. *Mol Cell Biol* 22:8580 8591
- Kanai F, Liu H, Field SJ, Akbary H, Matsuo T, Brown GE, Cantley LC, Yaffe MB (2001) The PX domains of p47phox and p40phox bind to lipid products of PI(3)K. *Nat Cell Biol* 3:675 678
- Kaplan DR, Whitman M, Schaffhausen B, Pallas DC, White M, Cantley L, Roberts TM (1987) Common elements in growth factor stimulation and oncogenic transformation: 85 kd phosphoprotein and phosphatidylinositol kinase activity. *Cell* 50:1021 1029
- Klarlund JK, Guilherme A, Holik JJ, Virbasius JV, Chawla A, Czech MP (1997) Signaling by phosphoinositide 3,4,5 trisphosphate through proteins containing pleckstrin and Sec7 homology domains. *Science* 275:1927 1930
- Kops GJ, de Rooter ND, De Vries Smits AM, Powell DR, Bos JL, Burgering BM (1999) Direct control of the Forkhead transcription factor AFX by protein kinase B. *Nature* 398:630 634

- Krugmann S, Anderson KE, Ridley SH, Risso N, McGregor A, Coadwell J, Davidson K, Eguinoa A, Ellson CD, Lipp P, Manifava M, Ktistakis N, Painter G, Thuring JW, Cooper MA, Lim ZY, Holmes AB, Dove SK, Michell RH, Grewal A, Nazarian A, Erdjument Bromage H, Tempst P, Stephens LR, Hawkins PT (2002) Identification of ARAP3, a novel PI3K effector regulating both Arf and Rho GTPases, by selective capture on phosphoinositide affinity matrices. *Mol Cell* 9:95-108
- Leevers SJ, Weinkove D, MacDougall LK, Hafen E, Waterfield MD (1996) The Drosophila phosphoinositide 3 kinase Dp110 promotes cell growth. *EMBO J* 15:6584-6594
- Lemmon MA (2008) Membrane recognition by phospholipid binding domains. *Nat Rev Mol Cell Biol* 9:99-111
- Li Z, Jiang H, Xie W, Zhang Z, Smrcka AV, Wu D (2000) Roles of PLC beta2 and beta3 and PI3Kgamma in chemoattractant mediated signal transduction. *Science* 287:1046-1049
- Macara IG, Marinetti GV, Balduzzi PC (1984) Transforming protein of avian sarcoma virus UR2 is associated with phosphatidylinositol kinase activity: possible role in tumorigenesis. *Proc Natl Acad Sci USA* 81:2728-2732
- MacDougall LK, Domin J, Waterfield MD (1995) A family of phosphoinositide 3 kinases in Drosophila identifies a new mediator of signal transduction. *Curr Biol* 5:1404-1415
- Maehama T, Dixon JE (1998) The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5 trisphosphate. *J Biol Chem* 273: 13375-13378
- Mandelker D, Gabelli SB, Schmidt Kittler O, Zhu J, Cheong I, Huang CH, Kinzler KW, Vogelstein B, Amzel LM (2009) A frequent kinase domain mutation that changes the interaction between PI3Kalpha and the membrane. *Proc Natl Acad Sci USA* 106:16996-17001
- Mayer BJ, Ren R, Clark KL, Baltimore D (1993) A putative modular domain present in diverse signaling proteins. *Cell* 73:629-630
- Miled N, Yan Y, Hon WC, Perisic O, Zvelebil M, Inbar Y, Schneidman Duhovny D, Wolfson HJ, Backer JM, Williams RL (2007) Mechanism of two classes of cancer mutations in the phosphoinositide 3 kinase catalytic subunit. *Science* 317:239-242
- Misawa H, Ohtsubo M, Copeland NG, Gilbert DJ, Jenkins NA, Yoshimura A (1998) Cloning and characterization of a novel class II phosphoinositide 3 kinase containing C2 domain. *Biochem Biophys Res Commun* 244:531-539
- Morgan SJ, Smith AD, Parker PJ (1990) Purification and characterization of bovine brain type I phosphatidylinositol kinase. *Eur J Biochem* 191:761-767
- Morris JZ, Tissenbaum HA, Ruvkun G (1996) A phosphatidylinositol 3 OH kinase family member regulating longevity and diapause in *Caenorhabditis elegans*. *Nature* 382:536-539
- Morrow CJ, Gray A, Dive C (2005) Comparison of phosphatidylinositol 3 kinase signalling within a panel of human colorectal cancer cell lines with mutant or wild type PIK3CA. *FEBS Lett* 579:5123-5128
- Mu FT, Callaghan JM, Steele Mortimer O, Stenmark H, Parton RG, Campbell PL, McCluskey J, Yeo JP, Tock EP, Toh BH (1995) EEA1, an early endosome associated protein. EEA1 is a conserved alpha helical peripheral membrane protein flanked by cysteine "fingers" and contains a calmodulin binding IQ motif. *J Biol Chem* 270:13503-13511
- Nicot AS, Laporte J (2008) Endosomal phosphoinositides and human diseases. *Traffic* 9: 1240-1249
- Nobukuni T, Joaquin M, Roccio M, Dann SG, Kim SY, Gulati P, Byfield MP, Backer JM, Natt F, Bos JL, Zwartkruis FJ, Thomas G (2005) Amino acids mediate mTOR/raptor signaling through activation of class 3 phosphatidylinositol 3OH kinase. *Proc Natl Acad Sci USA* 102:14238-14243
- O'Brien C, Wallin JJ, Sampath D, Guhathakurta D, Savage H, Punnoose EA, Guan J, Berry L, Prior WW, Amler LC, Belvin M, Friedman L, Lackner M (2010) Predictive biomarkers of sensitivity to the phosphatidylinositol 3' kinase inhibitor GDC 0941 in breast cancer preclinical models. *Clin Cancer Res* [Epub ahead of print]
- Ogg S, Paradis S, Gottlieb S, Patterson GI, Lee L, Tissenbaum HA, Ruvkun G (1997) The Fork head transcription factor DAF-16 transduces insulin like metabolic and longevity signals in *C. elegans*. *Nature* 389:994-999

- Okada T, Sakuma L, Fukui Y, Hazeki O, Ui M (1994) Blockage of chemotactic peptide induced stimulation of neutrophils by wortmannin as a result of selective inhibition of phosphatidylinositol 3 kinase. *J Biol Chem* 269:3563 3567
- Okkenhaug K, Bilancio A, Farjot G, Priddle H, Sancho S, Peskett E, Pearce W, Meek SE, Salpekar A, Waterfield MD, Smith AJ, Vanhaesebroeck B (2002) Impaired B and T cell antigen receptor signaling in p110delta PI 3 kinase mutant mice. *Science* 297:1031 1034
- Ono F, Nakagawa T, Saito S, Owada Y, Sakagami H, Goto K, Suzuki M, Matsuno S, Kondo H (1998) A novel class II phosphoinositide 3 kinase predominantly expressed in the liver and its enhanced expression during liver regeneration. *J Biol Chem* 273:7731 7736
- Ooms LM, Horan KA, Rahman P, Seaton G, Gurung R, Kethesparan DS, Mitchell CA (2009) The role of the inositol polyphosphate 5 phosphatases in cellular function and human disease. *Biochem J* 419:29 49
- Otsu M, Hiles I, Gout I, Fry MJ, Ruiz Larrea F, Panayotou G, Thompson A, Dhand R, Hsuan J, Totty N et al (1991) Characterization of two 85 kd proteins that associate with receptor tyrosine kinases, middle T/pp 60c src complexes, and PI3 kinase. *Cell* 65:91 104
- Pang H, Flinn R, Patsialou A, Wyckoff J, Roussos ET, Wu H, Pozzuto M, Goswami S, Condeelis JS, Bresnick AR, Segall JE, Backer JM (2009) Differential enhancement of breast cancer cell motility and metastasis by helical and kinase domain mutations of class IA phosphoinositide 3 kinase. *Cancer Res* 69:8868 8876
- Papakonstanti EA, Ridley AJ, Vanhaesebroeck B (2007) The p110delta isoform of PI 3 kinase negatively controls RhoA and PTEN. *EMBO J* 26:3050 3061
- Papakonstanti EA, Zwaenepoel O, Bilancio A, Burns E, Nock GE, Houseman B, Shokat K, Ridley AJ, Vanhaesebroeck B (2008) Distinct roles of class IA PI3K isoforms in primary and immortalized macrophages. *J Cell Sci* 121:4124 4133
- Paradis S, Ruvkun G (1998) *Caenorhabditis elegans* Akt/PKB transduces insulin receptor like signals from AGE 1 PI3 kinase to the DAF 16 transcription factor. *Genes Dev* 12:2488 2498
- Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, Olivi A, McLendon R, Rasheed BA, Keir S, Nikolskaya T, Nikolsky Y, Busam DA, Tekleab H, Diaz LA Jr, Hartigan J, Smith DR, Strausberg RL, Marie SK, Shinjo SM, Yan H, Riggins GJ, Bigner DD, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW (2008) An integrated genomic analysis of human glioblastoma multiforme. *Science* 321:1807 1812
- Philp AJ, Campbell IG, Leet C, Vincan E, Rockman SP, Whitehead RH, Thomas RJ, Phillips WA (2001) The phosphatidylinositol 3' kinase p85alpha gene is an oncogene in human ovarian and colon tumors. *Cancer Res* 61:7426 7429
- Plas DR, Thompson CB (2005) Akt dependent transformation: there is more to growth than just surviving. *Oncogene* 24:7435 7442
- Powis G, Bonjouklian R, Berggren MM, Gallegos A, Abraham R, Ashendel C, Zalkow L, Matter WF, Dodge J, Grindey G et al (1994) Wortmannin, a potent and selective inhibitor of phosphatidylinositol 3 kinase. *Cancer Res* 54:2419 2423
- Raynaud FI, Eccles S, Clarke PA, Hayes A, Nutley B, Alix S, Henley A, Di Stefano F, Ahmad Z, Guillard S, Bjerke LM, Kelland L, Valenti M, Patterson L, Gowan S, de Haven BA, Hayakawa M, Kaizawa H, Koizumi T, Ohishi T, Patel S, Saghir N, Parker P, Waterfield M, Workman P (2007) Pharmacologic characterization of a potent inhibitor of class I phosphatidylinositol 3 kinases. *Cancer Res* 67:5840 5850
- Roche S, Koegl M, Courtneidge SA (1994) The phosphatidylinositol 3 kinase alpha is required for DNA synthesis induced by some, but not all, growth factors. *Proc Natl Acad Sci USA* 91:9185 9189
- Roche S, Downward J, Raynal P, Courtneidge SA (1998) A function for phosphatidylinositol 3 kinase beta (p85alpha p110beta) in fibroblasts during mitogenesis: requirement for insulin and lysophosphatidic acid mediated signal transduction. *Mol Cell Biol* 18:7119 7129
- Rodriguez Viciano P, Warne PH, Dhand R, Vanhaesebroeck B, Gout I, Fry MJ, Waterfield MD, Downward J (1994) Phosphatidylinositol 3 OH kinase as a direct target of Ras. *Nature* 370:527 532

- Rommel C, Camps M, Ji H (2007) PI3K delta and PI3K gamma: partners in crime in inflammation in rheumatoid arthritis and beyond? *Nat Rev Immunol* 7:191 201
- Ruckle T, Schwarz MK, Rommel C (2006) PI3Kgamma inhibition: towards an 'aspirin of the 21st century'? *Nat Rev Drug Discov* 5:903 918
- Sadhu C, Masinovsky B, Dick K, Sowell CG, Staunton DE (2003) Essential role of phosphoinositide 3 kinase delta in neutrophil directional movement. *J Immunol* 170:2647 2654
- Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell SM, Riggins GJ, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Velculescu VE (2004) High frequency of mutations of the PIK3CA gene in human cancers. *Science* 304:554
- Sarbassov DD, Guertin DA, Ali SM, Sabatini DM (2005) Phosphorylation and regulation of Akt/PKB by the rictor mTOR complex. *Science* 307:1098 1101
- Sasaki T, Irie Sasaki J, Jones RG, Oliveira dos Santos AJ, Stanford WL, Bolon B, Wakeham A, Itie A, Bouchard D, Koziaradzki I, Joza N, Mak TW, Ohashi PS, Suzuki A, Penninger JM (2000) Function of PI3Kgamma in thymocyte development, T cell activation, and neutrophil migration. *Science* 287:1040 1046
- Schu PV, Takegawa K, Fry MJ, Stack JH, Waterfield MD, Emr SD (1993) Phosphatidylinositol 3 kinase encoded by yeast VPS34 gene essential for protein sorting. *Science* 260:88 91
- Sergina NV, Rausch M, Wang D, Blair J, Hann B, Shokat KM, Moasser MM (2007) Escape from HER family tyrosine kinase inhibitor therapy by the kinase inactive HER3. *Nature* 445:437 441
- Shibasaki F, Homma Y, Takenawa T (1991) Two types of phosphatidylinositol 3 kinase from bovine thymus. Monomer and heterodimer form. *J Biol Chem* 266:8108 8114
- Shuttleworth S, Silva F, Tomassi C, Cecil A, Hill T, Rogers H, Townsend P (2009) Progress in the design and development of phosphoinositide 3 kinase (PI3K) inhibitors for the treatment of chronic diseases. *Prog Med Chem* 48:81 131
- Sjolander A, Yamamoto K, Huber BE, Lapetina EG (1991) Association of p21ras with phosphatidylinositol 3 kinase. *Proc Natl Acad Sci USA* 88:7908 7912
- Skolnik EY, Margolis B, Mohammadi M, Lowenstein E, Fischer R, Drepps A, Ullrich A, Schlessinger J (1991) Cloning of PI3 kinase associated p85 utilizing a novel method for expression/cloning of target proteins for receptor tyrosine kinases. *Cell* 65:83 90
- Song X, Xu W, Zhang A, Huang G, Liang X, Virbasius JV, Czech MP, Zhou GW (2001) Phox homology domains specifically bind phosphatidylinositol phosphates. *Biochemistry* 40:8940 8944
- Soond DR, Bjorgo E, Moltu K, Dale VQ, Patton DT, Torgersen KM, Galleway F, Twomey B, Clark J, Gaston JH, Tasken K, Bunyard P, Okkenhaug K (2010) PI3K p110{delta} regulates T cell cytokine production during primary and secondary immune responses in mice and humans. *Blood* 115:2203 2213
- Sos ML, Fischer S, Ullrich R, Peifer M, Heuckmann JM, Koker M, Heynck S, Stuckrath I, Weiss J, Fischer F, Michel K, Goel A, Regales L, Politi KA, Perera S, Getlik M, Heukamp LC, Ansen S, Zander T, Beroukhim R, Kashkar H, Shokat KM, Sellers WR, Rauh D, Orr C, Hoeflich KP, Friedman L, Wong KK, Pao W, Thomas RK (2009) Identifying genotype dependent efficacy of single and combined PI3K and MAPK pathway inhibition in cancer. *Proc Natl Acad Sci USA* 106:18351 18356
- Stemke Hale K, Gonzalez Angulo AM, Lluch A, Neve RM, Kuo WL, Davies M, Carey M, Hu Z, Guan Y, Sahin A, Symmans WF, Pusztai L, Nolden LK, Horlings H, Berns K, Hung MC, van de Vijver MJ, Valero V, Gray JW, Bernards R, Mills GB, Hennessy BT (2008) An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. *Cancer Res* 68:6084 6091
- Stenmark H, Aasland R, Toh BH, D'Arrigo A (1996) Endosomal localization of the autoantigen EEA1 is mediated by a zinc binding FYVE finger. *J Biol Chem* 271:24048 24054
- Stephens L, Smrcka A, Cooke FT, Jackson TR, Sternweis PC, Hawkins PT (1994) A novel phosphoinositide 3 kinase activity in myeloid derived cells is activated by G protein beta gamma subunits. *Cell* 77:83 93

- Stephens LR, Eguinoa A, Erdjument Bromage H, Lui M, Cooke F, Coadwell J, Smrcka AS, Thelen M, Cadwallader K, Tempst P, Hawkins PT (1997) The G beta gamma sensitivity of a PI3K is dependent upon a tightly associated adaptor, p101. *Cell* 89:105-114
- Stephens L, Anderson K, Stokoe D, Erdjument Bromage H, Painter GF, Holmes AB, Gaffney PR, Reese CB, McCormick F, Tempst P, Coadwell J, Hawkins PT (1998) Protein kinase B kinases that mediate phosphatidylinositol 3,4,5 trisphosphate dependent activation of protein kinase B. *Science* 279:710-714
- Stocker H, Andjelkovic M, Oldham S, Laffargue M, Wymann MP, Hemmings BA, Hafen E (2002) Living with lethal PIP3 levels: viability of flies lacking PTEN restored by a PH domain mutation in Akt/PKB. *Science* 295:2088-2091
- Stokoe D, Stephens LR, Copeland T, Gaffney PR, Reese CB, Painter GF, Holmes AB, McCormick F, Hawkins PT (1997) Dual role of phosphatidylinositol 3,4,5 trisphosphate in the activation of protein kinase B. *Science* 277:567-570
- Stoyanov B, Volinia S, Hanck T, Rubio I, Loubtchenkov M, Malek D, Stoyanova S, Vanhaesebroeck B, Dhand R, Nurnberg B et al (1995) Cloning and characterization of a G protein activated human phosphoinositide 3 kinase. *Science* 269:690-693
- Sugimoto Y, Whitman M, Cantley LC, Erikson RL (1984) Evidence that the Rous sarcoma virus transforming gene product phosphorylates phosphatidylinositol and diacylglycerol. *Proc Natl Acad Sci USA* 81:2117-2121
- TGCA (2008) Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 455:1061-1068
- Thomas RK, Baker AC, DeBiasi RM, Winckler W, Laframboise T, Lin WM, Wang M, Feng W, Zander T, MacConaill L, Lee JC, Nicoletti R, Hatton C, Goyette M, Girard L, Majumdar K, Ziaugra L, Wong KK, Gabriel S, Beroukhi R, Peyton M, Barretina J, Dutt A, Emery C, Greulich H, Shah K, Sasaki H, Gazdar A, Minna J, Armstrong SA, Mellinghoff IK, Hodi FS, Dranoff G, Mischel PS, Cloughesy TF, Nelson SF, Liau LM, Mertz K, Rubin MA, Moch H, Loda M, Catalona W, Fletcher J, Signoretti S, Kaye F, Anderson KC, Demetri GD, Dummer R, Wagner S, Herlyn M, Sellers WR, Meyerson M, Garraway LA (2007) High throughput oncogene mutation profiling in human cancer. *Nat Genet* 39:347-351
- Traynor Kaplan AE, Harris AL, Thompson BL, Taylor P, Sklar LA (1988) An inositol tetrakisphosphate containing phospholipid in activated neutrophils. *Nature* 334:353-356
- Traynor Kaplan AE, Thompson BL, Harris AL, Taylor P, Omann GM, Sklar LA (1989) Transient increase in phosphatidylinositol 3,4 bisphosphate and phosphatidylinositol trisphosphate during activation of human neutrophils. *J Biol Chem* 264:15668-15673
- Vanhaesebroeck B, Leever SJ, Panayotou G, Waterfield MD (1997a) Phosphoinositide 3 kinases: a conserved family of signal transducers. *Trends Biochem Sci* 22:267-272
- Vanhaesebroeck B, Welham MJ, Kotani K, Stein R, Warne PH, Zvelebil MJ, Higashi K, Volinia S, Downward J, Waterfield MD (1997b) P110delta, a novel phosphoinositide 3 kinase in leukocytes. *Proc Natl Acad Sci USA* 94:4330-4335
- Vanhaesebroeck B, Jones GE, Allen WE, Zicha D, Hooshmand Rad R, Sawyer C, Wells C, Waterfield MD, Ridley AJ (1999) Distinct PI(3)Ks mediate mitogenic signalling and cell migration in macrophages. *Nat Cell Biol* 1:69-71
- Vanhaesebroeck B, Leever SJ, Ahmadi K, Timms J, Katso R, Driscoll PC, Woscholski R, Parker PJ, Waterfield MD (2001) Synthesis and function of 3 phosphorylated inositol lipids. *Annu Rev Biochem* 70:535-602
- Vanhaesebroeck B, Guillermet Guibert J, Graupera M, Bilanges B (2010) The emerging mechanisms of isoform specific PI3K signalling. *Nat Rev Mol Cell Biol* (in press)
- Vasudevan KM, Barbie DA, Davies MA, Rabinovsky R, McNear CJ, Kim JJ, Hennessy BT, Tseng H, Pochanard P, Kim SY, Dunn IF, Schinzel AC, Sandy P, Hoersch S, Sheng Q, Gupta PB, Boehm JS, Reiling JH, Silver S, Lu Y, Stenke Hale K, Dutta B, Joy C, Sahin AA, Gonzalez Angulo AM, Lluch A, Rameh LE, Jacks T, Root DE, Lander ES, Mills GB, Hahn WC, Sellers WR, Garraway LA (2009) AKT independent signaling downstream of oncogenic PIK3CA mutations in human cancer. *Cancer Cell* 16:21-32

- Virbasius JV, Guilherme A, Czech MP (1996) Mouse p170 is a novel phosphatidylinositol 3 kinase containing a C2 domain. *J Biol Chem* 271:13304-13307
- Vlahos CJ, Matter WF, Hui KY, Brown RF (1994) A specific inhibitor of phosphatidylinositol 3 kinase, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002). *J Biol Chem* 269:5241-5248
- Walker EH, Perisic O, Ried C, Stephens L, Williams RL (1999) Structural insights into phosphoinositide 3-kinase catalysis and signalling. *Nature* 402:313-320
- Welch HC, Coadwell WJ, Ellson CD, Ferguson GJ, Andrews SR, Erdjument-Bromage H, Tempst P, Hawkins PT, Stephens LR (2002) PDK1, a PtdIns(3,4,5)P₃ and Gbetagamma-regulated guanine nucleotide exchange factor for Rac. *Cell* 108:809-821
- Whitman M, Kaplan DR, Schaffhausen B, Cantley L, Roberts TM (1985) Association of phosphatidylinositol kinase activity with polyoma middle T competent for transformation. *Nature* 315:239-242
- Whitman M, Downes CP, Keeler M, Keller T, Cantley L (1988) Type I phosphatidylinositol kinase makes a novel inositol phospholipid, phosphatidylinositol 3-phosphate. *Nature* 332:644-646
- Wood LD, Parsons DW, Jones S, Lin J, Sjoblom T, Leary RJ, Shen D, Boca SM, Barber T, Ptak J, Silliman N, Szabo S, Dezso Z, Ustyanksky V, Nikolskaya T, Nikolsky Y, Karchin R, Wilson PA, Kaminker JS, Zhang Z, Croshaw R, Willis J, Dawson D, Shipitsin M, Willson JK, Sukumar S, Polyak K, Park BH, Pethiyagoda CL, Pant PV, Ballinger DG, Sparks AB, Hartigan J, Smith DR, Suh E, Papadopoulos N, Buckhaults P, Markowitz SD, Parmigiani G, Kinzler KW, Velculescu VE, Vogelstein B (2007) The genomic landscapes of human breast and colorectal cancers. *Science* 318:1108-1113
- Xu Y, Hortsman H, Seet L, Wong SH, Hong W (2001) SNX3 regulates endosomal function through its PX domain-mediated interaction with PtdIns(3)P. *Nat Cell Biol* 3:658-666
- Yano H, Nakanishi S, Kimura K, Hanai N, Saitoh Y, Fukui Y, Nonomura Y, Matsuda Y (1993) Inhibition of histamine secretion by wortmannin through the blockade of phosphatidylinositol 3-kinase in RBL 2H3 cells. *J Biol Chem* 268:25846-25856
- Yao R, Cooper GM (1995) Requirement for phosphatidylinositol 3-kinase in the prevention of apoptosis by nerve growth factor. *Science* 267:2003-2006
- Zhou K, Takegawa K, Emr SD, Firtel RA (1995) A phosphatidylinositol (PI) kinase gene family in *Dictyostelium discoideum*: biological roles of putative mammalian p110 and yeast Vps34p PI 3-kinase homologs during growth and development. *Mol Cell Biol* 15:5645-5656
- Zvebil MJ, MacDougall L, Leever S, Volinia S, Vanhaesebroeck B, Gout I, Panayotou G, Domin J, Stein R, Pages F et al (1996) Structural and functional diversity of phosphoinositide 3-kinases. *Philos Trans R Soc Lond B Biol Sci* 351:217-223

Oncogenic Mutations of PIK3CA in Human Cancers

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Abstract The involvement of the PIK3CA gene product p110 α , the catalytic subunit of phosphatidylinositol 3-kinase (PI3K), in human cancer has been suggested for over 15 years, and support for this proposal had been provided by both genetic and functional studies, including most recently the discovery of common activating missense mutations of PIK3CA in a wide variety of common human tumor types. This chapter will focus on the discovery of these mutations and describes their relevance to a wide range of common human tumor types.

Of note, the identification and functional analysis of the PIK3CA gene are reviewed in other chapters in this book. However, a brief mention will be made here of its general properties as background to our focus on the discovery of its cancer-specific mutations.

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1 Introduction

Phosphoinositide kinases (PIKs) are lipid kinases that phosphorylate the inositol ring of phosphoinositides, thus acting as signal transducers. Depending on the phosphorylation site on the carbohydrate, PIKs are categorized into three families: phosphoinositide 3-kinases (PI3Ks), phosphoinositide 4-kinases (PIP4Ks) and phosphoinositide 5-kinases (PIP5Ks). PI3Ks are further grouped into classes I, II or III, depending on their subunit structure, their regulation and their substrate selectivity. Class I PI3Ks, are composed of two subgroups, IA and IB (Vanhaesebroeck and Waterfield 1999). The class IA PI3K subgroup consists of three catalytic subunits: p110 α , p110 β , and p110 δ that form heterodimers with one of five regulatory domains: p85 α , p85 β , p85 γ , p50 α , and p55 α . These PI3Ks are activated by cell-surface receptor tyrosine kinases. PI3Ks IA and IB catalyze the formation of PtdIns (3,4,5)P₃ (PIP3), a process that is reversed by the action of the lipid phosphatase, PTEN. PIP3 serves as an anchor for Pleckstrin homology (PH) domain-containing proteins such as the serine/threonine kinases AKT1, AKT2, and AKT3, which, once localized to the membrane, get activated by 3-phosphoinositide dependent protein kinase-1 (PDK1). AKT has numerous protein targets, including mTor, Bad, Caspase 9, Tuberin, GSK3 β , and a subset of forkhead transcription factors. The biological consequences of AKT activation are broad and can be subdivided into regulation of cell proliferation, survival and motility (Cantley 2002; Katso et al. 2001; Vivanco and Sawyers 2002).

2 Links Between the PI3K Pathway and Cancer

The role of PI3Ks and specifically p110 α in cancer was implicated when its kinase activity was shown to be associated with viral oncoproteins (Cantley et al. 1991). This was further substantiated by the discovery that some avian and murine retroviruses encode oncogenic derivatives of the cellular *PIK3CA* and *Akt* genes, respectively (Bellacosa et al. 1991; Chang et al. 1997; Staal 1987).

Furthermore, PTEN (phosphatase and tensin homolog), which reverses the reaction catalyzed by PI3Ks by dephosphorylating 3-position on inositol head groups, was found to be a tumor suppressor gene commonly mutated in human tumors (Li et al. 1997; Steck et al. 1997) resulting in constitutive activation of the PI3K pathway.

Other investigations showed the amplification of genomic regions containing AKT or PIK3CA genes (Actor et al. 2002; Bastian et al. 1998; Bellacosa et al. 1995; Cheng et al. 1992, 1996; Knobbe and Reifemberger 2003; Shayesteh et al. 1999; Staal 1987; Thompson et al. 1995) in various cancer types with the implication that PI3K was functioning as an oncogene. In addition, mutations in p85, the regulatory subunit of PI3K, in ovarian and colon tumors have been reported (Philp et al. 2001). Furthermore, the fact that the downstream targets of AKT, such as the forkhead

transcription factors FKHR and FKHRL1 have been shown to be translocated in several tumor types (Barr et al. 1993) provides strong evidence that this pathway plays a major role in neoplasia.

3 High Throughput Sequencing of Gene Families in Human Cancer

These discoveries suggested that PI3K might be genetically altered in human cancer. One way to definitely implicate a gene in human cancer is to discover tumor-specific mutations in the gene and to evaluate the functional effects of those mutations. This type of mutation search involves direct sequencing of gene(s) in a panel of tumors to identify variations from a reference sequence. The patient's constitutional DNA is then analyzed to determine whether the variant arose specifically within the tumor, i.e., whether the change is somatic.

In the past, technical hurdles have limited the ability to perform mutational analysis of candidate genes in a high throughput fashion. Several important advances have aided the development of high throughput approaches for DNA sequencing and mutation detection in human cancer. The first has been the collection and isolation of high-quality tumor tissue for these analyses, either through generation of early passage tumor cell lines or through selective capture or microdissection of neoplastic tissue. This has permitted the sensitive detection of somatic mutations that would otherwise have been masked by contaminating normal tissue. The second advance has been the delineation of the sequence of the human genome coupled with progress in automated methods for large-scale sequence analysis of specific loci. These methods have been optimized to provide rapid and robust sequence analysis of nearly all exonic regions in the human genome (Jones et al. 2008; Parsons et al. 2008; Sjoblom et al. 2006; Wood et al. 2007). Finally, several methods for automated mutation detection have been developed and applied for analysis of somatic alterations in cancer (Bardelli et al. 2003; Stephens et al. 2006). By direct comparison of sequence chromatograms from tumor and normal tissues, these methods have allowed the sensitive identification of most types of somatic sequence alterations. The combination of these advances has now created an opportunity for systematically identifying somatic mutations in human cancers and evaluating the roles of such mutated genes in tumorigenesis.

4 PIK3CA is Somatically Mutated in Colorectal Cancer

The clear link between the PI3K pathway and cancer noted above stimulated a study to determine whether PI3K genes are genetically altered in human tumors. To do this, a high throughput sequencing approach was used to sequence all of the

PI3K genes in a panel of 35 colorectal cancers and corresponding normal tissues. Sequencing of the exons encoding the kinase domain of all 16 members of the PI3K family showed that PIK3CA was the only gene to harbor somatic (i.e., tumor specific) mutations. Sequencing the rest of the gene in 199 additional colorectal cancers revealed that PIK3CA is somatically mutated in 32% of cases (Samuels et al. 2004). All but three of the alterations were heterozygous and no truncating or nonsense alterations were observed, which is consistent with the mutational signature of oncogenes. To determine at what stage of colorectal cancer PIK3CA mutations occur, 76 premalignant colorectal tumors were also examined. This identified only two mutations, both in advanced adenomas, suggesting that PIK3CA mutations arise late in tumorigenesis, just before or concurrent with invasion.

Importantly, over 80% of the somatic missense mutations were found in the kinase and helical domains of the PIK3CA subunit. This discovery of “hotspot” mutations is reminiscent of alterations in other oncogenes such as KRAS and BRAF (Bos et al. 1987; Davies et al. 2002; Rajagopalan et al. 2002). Further evaluation of PIK3CA mutational status in colon cancer has since been performed by several other groups and is described in more detail later.

Indeed, this discovery was surprising because despite extensive characterization of this pathway at the biochemical and biological levels, PIK3CA was not known to be mutated in human cancer.

5 PIK3CA is Mutated in a Wide Variety of Human Tumor Types

The initial discovery of PIK3CA mutations in colon cancer led to the examination of PIK3CA mutations in additional cancer types. These data and clinicopathological correlations for a subset of the most common tumor types or those with common mutations of PIK3CA are described below. Though amplifications of PIK3CA have been identified in numerous tumor types as well, the description below focuses virtually exclusively on studies that have evaluated mutations of PIK3CA. Of note, the descriptions below include only published studies. However, the Sanger Institute Cancer Genome Project has sequenced PIK3CA in a large number of additional samples. These data are continuously updated, publically available, and can be accessed at <http://www.sanger.ac.uk/genetics/CGP/cosmic>.

Breast cancer. In the paper describing the initial discovery of PIK3CA mutations in cancer, PIK3CA mutations were also identified in 1/12 breast cancers (Samuels et al. 2004). However, due to the relatively small number of samples studied and the fact that, despite years of effort, a commonly mutated breast cancer oncogene had not yet been discovered, several groups immediately initiated more comprehensive mutational analyses of PIK3CA in breast cancer (Bachman et al. 2004; Campbell et al. 2004; Lee et al. 2005; Levine et al. 2005; Saal et al. 2005; Wu et al. 2005b).

These efforts were immediately rewarded with very large number of mutations, making it immediately clear that PIK3CA was the most commonly mutated oncogene yet discovered in breast cancer. It is now appreciated that mutations of PIK3CA are found in 25-40% of all human breast cancers. This discovery was particularly gratifying since it had long been known that the PI3K pathway was often activated in breast cancer via phosphorylation of Akt (Page et al. 2000), yet in the absence of frequent PTEN mutations, the mechanism for the activation of Akt remained elusive. Therefore, the presence of PIK3CA mutations provided a molecular explanation for this long-standing conundrum.

Since the initial discovery of PIK3CA mutations in breast cancer, substantial effort has been expended in an attempt to correlate PIK3CA mutations in breast cancer with clinicopathological parameters such as estrogen receptor (ER)/progesterone receptor (PR) positivity, the presence of lymph node metastases, and response to therapy. These relationships are summarized below; however, it is worth noting that there are conflicting data on many of these proposed relationships.

Saal et al. were the first group to propose definitive clinicopathological correlates to PIK3CA mutations in breast cancer (Saal et al. 2005). Their data indicated that PIK3CA mutations were most often present in tumors with intact, expressed PTEN genes; in tumors that had metastasized to lymph nodes; and in tumors with expression of the ER, PR, and ERBB2. Data from Stemke-Hale et al. also demonstrated that PIK3CA mutations were more common in hormone receptor positive and HER2-positive breast cancers (Stemke-Hale et al. 2008). Li et al. confirmed and extended several of these relationships, demonstrating that PIK3CA mutation correlated with worse survival and that mutations were more commonly found in larger tumors with ER and PR expression (Li et al. 2006). Lerma et al. and Lai et al. also confirmed the association between PIK3CA mutation and poorer survival (Lai et al. 2008; Lerma et al. 2008). However, these data correlating PIK3CA mutation with poorer survival are controversial, as Barbareschi et al. suggested that whereas exon 9 mutations are associated with poor prognosis, exon 20 mutations are associated with better prognosis (Barbareschi et al. 2007). Furthermore, Maruyama et al. and Perez-Tenorio suggested that PIK3CA mutations are actually associated with better survival (Maruyama et al. 2007; Perez-Tenorio et al. 2007).

Buttita et al. provided another potentially important clinicopathological correlate that PIK3CA mutations were more commonly found in lobular breast cancers than in ductal breast cancers (Buttita et al. 2006). Barbareschi et al. found a similar correlation, but suggested that it was specific to samples with mutations in exon 9 (Barbareschi et al. 2007).

PIK3CA mutational status has also been correlated with response to therapy in breast cancer. For example, Berns et al. have suggested that oncogenic mutations of PIK3CA may render breast cancers more resistant to treatment with the antibody-based therapeutic trastuzumab (Berns et al. 2007), and Eichhorn et al. have shown that mutational activation of PIK3CA similarly render cells more resistant to the anti-HER2 agent Lapatinib (Eichhorn et al. 2008). In contrast, Liedtke et al.

demonstrated no relationship between PIK3CA mutational status and sensitivity to standard regimens of anthracycline and paclitaxel-based chemotherapy (Liedtke et al. 2008).

Colon cancer. The initial paper describing the novel discovery of PIK3CA mutations reported the sequence of the entire coding region of the gene in 234 colon tumors and identified a mutation frequency of 32% (Samuels et al. 2004). This remains the most comprehensive mutational analysis of PIK3CA mutations in colon cancer because of the large number of samples studied and the fact that all 20 exons were sequenced in every sample. Interestingly, in this study mutations were twice as common in tumors with microsatellite instability (RER+) than in tumors that were proficient for DNA repair (RER-).

There is some disagreement in the literature as to whether PIK3CA mutational status correlates with RER status. Whereas Abubaker et al. confirmed the work of Samuels et al. by demonstrating a clear relationship between PIK3CA mutation and microsatellite instability (Abubaker et al. 2008a), Velho et al. disagreed, suggesting that PIK3CA mutations occur at roughly equivalent frequencies in RER+ and RER- tumors (Velho et al. 2005). However, Velho et al. reported an unusually low PIK3CA mutation frequency in their samples (14%).

In addition to RER status, several studies have correlated PIK3CA mutation status to other pathological parameters. For example, Mikami et al. performed an interesting study demonstrating that while PIK3CA mutations were frequently found in the common “protruded-type” of colon cancer, they were very uncommon in the rare “flat-type” colon cancers (Mikami et al. 2006). Miyaki et al. asked whether PIK3CA mutations were found at different frequencies in sporadic colon cancers and those from patients with inherited colon cancer predisposition (Miyaki et al. 2007). They concluded that PIK3CA mutations occurred at similar frequency in sporadic and inherited tumors, but that mutations in patients with inherited predisposition occurred predominantly in the kinase domain whereas mutations in sporadic cases occurred predominantly in the helical domain. This group also confirmed the work of Samuels et al. demonstrating that preinvasive colon tumors generally harbor wild-type PIK3CA genes. Finally, Benvenuti et al. showed that PIK3CA mutations are more prevalent in colorectal cancers from women than from men (Benvenuti et al. 2008).

Several groups have also attempted to correlate PIK3CA mutation status with survival and response to therapy. Several groups have clearly shown that PIK3CA mutation is correlated with poor prognosis, even in patients whose tumor had been completely resected (Barault et al. 2008; Kato et al. 2007; Ogino et al. 2009). Ogino et al. further demonstrated that this effect was dependent on K-Ras mutational status; the presence of PIK3CA mutation conferred no significant effect on mortality among patients with tumors harboring K-Ras mutations. As such, they suggested that clinical trials of PI3K inhibitors may need to be stratified by K-Ras mutational status to accurately assess the efficacy of the inhibitors. Finally, both Jhawer et al. and Sartore-Bianchi et al. showed that colon cancer cells with mutant PIK3CA genes tended to be resistant to therapeutic anti-EGFR antibodies (Jhawer et al. 2008; Sartore-Bianchi et al. 2009).

Endometrial cancer. Endometrial cancer has proven to be one of the most interesting tumor types for the identification and study of PIK3CA mutations, both because of the high frequency of mutations and the novel finding that PIK3CA mutations are often coincident with PTEN mutations in this tumor type. Oda et al. reported a high frequency of mutations of PIK3CA in uterine endometrioid cancer (36%), which is all the more remarkable because they sequenced only the exons containing mutational hotspots, so the actual mutation frequency is likely to be somewhat higher (Oda et al. 2005). And, despite initial reports that PIK3CA activation and PTEN inactivation were mutually exclusive, this group identified coincident mutations of PIK3CA and PTEN in 26% of samples. Remarkably, PIK3CA mutations were actually more common in tumors with mutant PTEN genes than in tumors with wild-type PTEN genes. This study was the first study to suggest that hyperactivation of PI3K signaling by coincident mutations in two members of the pathway could be conducive to tumorigenesis. This was especially intriguing because of the widespread belief that hyperactivation of oncogenic signaling pathways such as PI3K can thwart cancer pathogenesis by inducing p53-dependent senescence (Chen et al. 2005; Kim et al. 2007). These data reporting coincident mutations in PIK3CA and PTEN were then supported by three additional groups (Hayes et al. 2006; Kang et al. 2008; Velasco et al. 2006). Of note, one group has reported a somewhat lower frequency of PIK3CA mutation in endometrial cancer (10%) (Miyake et al. 2008).

In contrast to the data with PTEN, mutations in PIK3CA do appear to be mutually exclusive with mutations of K-Ras in endometrial cancer (Kang et al. 2008; Velasco et al. 2006). However, it is worth noting that this reported mutual exclusivity with oncogenic K-Ras is somewhat controversial (Ollikainen et al. 2007).

Catusas has demonstrated that the presence of PIK3CA mutations is correlated with various clinicopathological factors such as invasion of the myometrium, high grade tumors, deeply invasive tumors that exhibit lymphovascular invasion (Catusas et al. 2008). Most recently, PIK3CA mutations have also been demonstrated in 15% of uterine serous carcinoma, a less common form of endometrial cancer (Hayes et al. 2009).

Brain tumors. In the initial report, Samuels et al. sequenced PIK3CA in 15 glioblastoma multiformes (GBMs) and identified four mutations, a 27% mutation frequency. This was particularly exciting to brain tumor researchers since it implicated PI3K activation in the majority of malignant gliomas, as PTEN was already known to be mutated in a substantial fraction of malignant gliomas. This report was immediately followed up by Broderick et al. who sequenced the catalytic and helical domain of PIK3CA in a large number of different brain tumor types and identified mutations in 14% of anaplastic oligodendrogliomas, 5% of GBMs, 5% of medulloblastomas, and 3% of anaplastic astrocytomas. No mutations were identified in low grade astrocytomas or ependymomas (Broderick et al. 2004).

Hartman et al., Knobbe et al., and Kita et al., also sequenced PIK3CA in GBMs and all reported a mutation rate of ~5% (Hartmann et al. 2005; Kita et al. 2007; Knobbe et al. 2005). Gallia et al. identified mutations of PIK3CA in 15% of GBMs,

and demonstrated that the frequency of mutation was roughly equivalent among cell lines, xenografts, and primary tumors (Gallia et al. 2006). They further demonstrated an equivalent frequency of mutation between pediatric and adult GBMs. Hartmann et al. have sequenced PIK3CA in oligodendrogliomas and identified a mutation frequency of ~2% (Hartmann et al. 2006).

In contrast, Mueller et al. sequenced PIK3CA in 30 primary GBMs but were unable to identify any mutations (Mueller et al. 2005), and suggested that PIK3CA mutations could be more common in GBM cell lines than in primary GBMs. However, this group studied a relatively small number of samples that were intentionally biased toward samples with wild-type PTEN genes. In light of subsequent studies in endometrial cancer demonstrating that PIK3CA mutations can actually occur preferentially in tumors with mutant PTEN genes, it is possible that the sample set used by these investigators could have led to a reduced apparent frequency of PIK3CA mutations in GBM. Finally, Pang et al. have sequenced PIK3CA in meningiomas and identified a low mutation frequency of 1% (Pang et al. 2006).

Skin cancer. Perhaps one of the most interesting and surprising recent findings in the PIK3CA field was the recent report of common hotspot mutations of PIK3CA in two benign skin lesions—epidermal nevi and seborrheic keratoses (SK), two noninvasive keratinocyte-derived skin tumors. Epidermal nevi are congenital lesions that are either present at birth or develop during early childhood, whereas SK are similar lesions that are associated with the aging process. Hafner et al. sequenced the hotspot exons of PIK3CA in these tumors and demonstrated that 27% of EN and 16% of SK harbor PIK3CA mutations (Hafner et al. 2007, 2008), and further demonstrated that PIK3CA mutations are present in solar lentigo, thought to be a precursor lesion for SK (Hafner et al. 2009). These intriguing findings challenge the idea that PIK3CA mutations are associated with tumor cell invasion (Samuels et al. 2004). Additionally, they are reminiscent of previous findings in colon cancer demonstrating that if K-Ras mutations occur in the early stages of tumorigenesis, they lead to the formation of a benign lesion known as an aberrant crypt focus instead of frank cancer (Jen et al. 1994). The finding of PIK3CA mutations in these benign skin tumors also raises the possibility that mutational activation of PIK3CA may be causing oncogene-induced senescence in human keratinocytes, as mutational activation of PIK3CA genes has been shown to lead to senescent-like features in several human cell types (Kim et al. 2007).

In contrast to the frequent mutations of PIK3CA in these benign skin tumors, mutations of PIK3CA are relatively rare in malignant melanoma (~3%), which was surprising given the prominent role of PTEN inactivation in this tumor type (Omholt et al. 2006).

Ovarian cancer. Even before the identification of PIK3CA mutations, it was widely appreciated that amplification and overexpression of PIK3CA was found in a substantial number of ovarian cancers (Shayesteh et al. 1999; Zhang et al. 2003). As such, after the initial report of PIK3CA mutations, several groups immediately sequenced the gene in ovarian cancer and identified a mutation frequency of 4–12% (Campbell et al. 2004; Levine et al. 2005; Wang et al. 2005). Campbell et al. and

Wang et al. also clearly demonstrated a substantial histological subtype bias, in that mutations were much more common in the relatively rare endometrioid, clear cell, and mucinous types; whereas they were fairly rare in the most common serous type tumors. The fact that these mutations were rare in serous tumors was also confirmed by Nakayama et al. (2006). Most recently, Kolasa et al. demonstrated that mutant PIK3CA correlates with low FIGO stage, lower tumor grade, and early age at diagnosis (Kolasa et al. 2009).

Gastric cancer. Even before the work of Samuels et al. PIK3CA had been implicated in the pathogenesis of gastric cancer by the identification of genomic amplifications (Byun et al. 2003). Then, in their initial study, Samuels et al. identified PIK3CA mutations in 25% gastric cancers (3/12) (Samuels et al. 2004). Subsequent studies reported somewhat lower mutation frequencies of 4 and 11% (Li et al. 2005; Velho et al. 2005). However, little additional work has been performed on clinicopathological correlates of PIK3CA in this tumor type.

Lung cancer. Amplifications of PIK3CA in lung cancer were reported by Massion et al. (2004). That same year, Samuels et al. reported a low (4%) frequency of PIK3CA mutations in lung cancer (Samuels et al. 2004). Kawano et al. then confirmed this low frequency in a larger sample set (Kawano et al. 2006). Of note, they demonstrated that PIK3CA mutation occurs more commonly in squamous cell carcinoma (7%) than in adenocarcinoma (2%). Okudela et al. similarly identified mutations of PIK3CA in 4% of lung cancers (Okudela et al. 2007). Finally, in the largest lung cancer study performed to date, Yamamoto et al. studied >700 lung cancer samples and identified PIK3CA mutations in 2% of all major histology types (Yamamoto et al. 2008).

Interestingly, Kawano et al. were the first (and as yet only) to demonstrate that mutant alleles of PIK3CA are occasionally amplified in cancer cells (Kawano et al. 2007). This finding is strikingly reminiscent of the well-described amplification of mutant alleles of EGFR that also occurs in lung cancer (Sharma et al. 2007).

Thyroid cancer. There is some disagreement in the literature regarding the role of PIK3CA mutation in the pathogenesis of thyroid cancer, perhaps in part due to the wide variety of different pathological types of this disease. However, when taken together, the studies suggest that PIK3CA mutations are more important in the pathogenesis of anaplastic thyroid cancer and follicular thyroid cancer than in the pathogenesis of papillary carcinoma of the thyroid.

In the initial, very large study, Garcia-Rostan identified PIK3CA mutations in 16% of anaplastic thyroid carcinomas, 8% of follicular thyroid carcinomas, and 2% of papillary thyroid carcinomas (Garcia-Rostan et al. 2005). However, that same year Wu et al. suggested that PIK3CA mutations rarely occurred in these tumor types (Wu et al. 2005a). In a subsequent study, Wang et al. reported PIK3CA mutations in 13% of follicular thyroid carcinomas and 1% of papillary thyroid carcinomas (Wang et al. 2007). Abubaker et al. then specifically focused on papillary thyroid carcinoma and identified mutations in 2% of 499 cases studied (Abubaker et al. 2008b). Similarly, Santarpia et al. focused exclusively on anaplastic thyroid cancer and identified mutations in 14% of 36 cases (Santarpia et al. 2008).

Head and neck cancer. A large number of studies have examined the role of PIK3CA mutation in the pathogenesis of head and neck cancer. Qiu et al. evaluated PIK3CA mutational status in 38 squamous cell carcinomas and identified four samples with mutations (11%). Interestingly, in three of the four cases with mutations were derived from pharyngeal cancer samples (Qiu et al. 2006). Later, this group used a more sensitive mutation detection method that made it possible to identify mutations in tumors of mixed origin and found the mutation rate to be significantly higher than previously reported (21%) (Qiu et al. 2008). Kozaki et al. sequenced PIK3CA in oral squamous cell carcinomas and identified mutations in 21% of cell lines and 17% of primary tumors (Kozaki et al. 2006). Yan Yan et al. were unable to identify mutations of PIK3CA in pharyngeal cancer samples (Or et al. 2006). Liu et al. identified mutations of PIK3CA in 4% of nasopharyngeal carcinomas (Liu et al. 2007). Fenic et al. were unable to identify any mutations of PIK3CA in a series of 33 squamous cell carcinomas (Fenic et al. 2007). Chou et al. identified PIK3CA mutations in 10% of nasopharyngeal carcinomas, and demonstrated that there was no significant relationship to clinicopathological characteristics of the tumors (Chou et al. 2008). Murugan et al. identified mutations in 30% of head and neck cancer cell lines, 11% in primary tumors from patients in India, and no tumors from Vietnam (Murugan et al. 2008).

Cervical cancer. It was appreciated that amplification and overexpression of PIK3CA played an important role in the pathogenesis of cervical cancer, even before the identification of PIK3CA mutations (Ma et al. 2000). Then, Miyake et al. identified mutations of PIK3CA in 3 of 22 cases (14%) (Miyake et al. 2008) and Cui et al. identified mutations in 15/184 invasive cervical carcinomas (8%) (Cui et al. 2009).

Pancreatic cancer. Schonleben et al. identified PIK3CA in 11% of intraductal papillary mucinous carcinoma of the pancreas (Schonleben et al. 2006).

Esophageal cancer. Phillips et al. identified PIK3CA mutations in 12% of squamous cell carcinomas of the esophagus, and 6% of adenocarcinomas of the esophagus (Phillips et al. 2006). Mori et al. identified mutations in 2/88 (2%) of esophageal squamous cell carcinomas (Mori et al. 2008). In contrast, Akagi et al. did not identify any PIK3CA mutations in esophageal squamous cell carcinoma (Akagi et al. 2009).

Liver/biliary tract cancer. Riener et al. evaluated both liver and biliary tract tumors for PIK3CA mutations and identified mutations in 1/45 cholangiocarcinomas (2%), 1/23 gallbladder carcinomas (4%), and 1/50 hepatocellular carcinomas (2%) (Riener et al. 2008). Tanaka et al. were unable to identify any mutations of PIK3CA in 47 hepatocellular cancers from Japanese patients (Tanaka et al. 2006).

Pituitary tumors. Lin et. evaluated 353 pituitary tumors and identified PIK3CA mutations in 8/91 invasive pituitary tumors (9%) but not in any of 262 noninvasive pituitary tumors (Lin et al. 2009). Of note, these data are consistent with the idea initially expressed by Samuels et al. that mutation of PIK3CA correlates with invasiveness.

Urological tumors. Andersson et al. identified mutations of PIK3CA in 8/28 penile tumors (29%) (Andersson et al. 2008). Lopez-Knowles identified PIK3CA mutations in 11/87 (13%) of bladder cancers (Lopez-Knowles et al. 2006).

Leukemia/lymphoma. PIK3CA mutations were identified in 17/215 diffuse large B cell lymphomas (Abubaker et al. 2007). Muller et al. did not identify any mutations of PIK3CA in acute myeloid leukemia, myelodysplastic syndromes, or non-Hodgkin lymphomas (Muller et al. 2007). Similarly, Hummerdal was unable to identify PIK3CA in acute myeloid leukemia (Hummerdal et al. 2006).

Neuroblastoma. Dam et al. were unable to identify PIK3CA mutations in any of 69 neuroblastomas (Dam et al. 2006).

These mutation frequencies indicate that PIK3CA is one of the two most commonly mutated genes identified in human cancers (the other being KRAS). Taken together, the genetic alterations described above suggested that mutant PIK3CA is an oncogene for the following reasons: (a) the high mutation frequency, (b) the vast majority of mutations are heterozygous missense changes, (c) many of the mutations affect highly conserved residues, and (d) more than 80% of the mutations in PIK3CA cluster in two regions, within the helical (exon 9) and catalytic (exon 20) domains. These characteristics suggested that these genetic alterations may be kinase activating, similar to oncogenic mutations found in other oncogenes such as BRAF (Davies et al. 2002; Rajagopalan et al. 2002). Indeed, follow-up functional analyses of the PIK3CA mutations confirmed them to be constitutively kinase activating and oncogenic (Bader et al. 2006; Kang et al. 2005; Samuels et al. 2004, 2005; Zhao and Vogt 2008).

6 Somatic Mutations in the PI3K Pathway Typically Occur in a Mutually Exclusive Fashion

The significance of the PI3K pathway in human cancer was further emphasized by additional mutational analyses of other genes involved in PI3K signaling. If two genes are mutated in a mutually exclusive fashion in a single tumor type, it is likely that they provide the same selective pressure for clonal expansion. This concept has been demonstrated by the mutual exclusivity found between APC and beta-catenin mutations (Morin et al. 1997; Sparks et al. 1998) or KRAS and BRAF mutations (Davies et al. 2002; Rajagopalan et al. 2002). The work of Saal et al. indicates that this notion is also true for the PI3K pathway (Saal et al. 2005). In this study, the PIK3CA mutation status of a panel of breast tumors which had lost PTEN expression was compared with a matched control set that had retained PTEN expression. A highly significant association between PIK3CA mutations and retention of wild-type PTEN protein expression was observed. However, conflicting data on this point has also been published (Saal et al. 2007).

Another genetic study aimed at testing the involvement of additional members of the PI3K pathway in colorectal cancer evaluated 146 colorectal cancers for somatic mutations in this pathway. Somatic mutations were identified in PDK1 (3/146), p21-activated kinase 4 (PAK4) (2/146), AKT2 (2/146), insulin-related receptor INSR (1/146), v-Erb-B erythroblastic leukemia viral oncogene homolog ERBB4 (1/146), PTEN (7/146), as well as amplification of the insulin receptor

substrate IRS2 (3/146). When these same tumor panel was analyzed for PIK3CA mutations, 37 mutations were found. Thus, a total of 58 alterations were found in the PI3K pathway, yet only two of the tumors had alterations in two genes in the PI3K pathway, making this mutual exclusivity statistically significant ($p < 0.02$, chi-square test) (Parsons et al. 2005).

Similarly, AKT1 was found to be somatically mutated in 8% breast, 6% colorectal and 2% ovarian cancers (Carpten et al. 2007). The AKT1 mutation was found to be mutually exclusive of PIK3CA and complete loss of PTEN protein expression. Although the sample size was insufficient to document statistical significance, the lack of coincidence of these mutations indicates that the AKT1 mutation was sufficient for pathological activation of the PI3K/AKT pathway (Carpten et al. 2007). A similar study by Bleeker et al. showed that AKT1 was mutated in 5% breast 1.1% colorectal and 0.6% lung cancers. Within the neoplasms of breast origin, the AKT1 mutation was mutually exclusive with respect to the PIK3CA mutations (Bleeker et al. 2008).

Strikingly, whole exome sequencing studies (Parsons et al. 2008; Wood et al. 2007) have also highlighted the importance of the PI3K pathway in cancer, as a large portion of the genes found to be somatically mutated are involved in PI3K signaling. In the breast cancer study, these genes included PIK3CA and previously unreported mutations in PIK3R1, PIK3R4, and RPS6KA3. In colorectal cancer the PI3K pathway components found to be mutated differed from those in breast, with mutations found in IRS2, IRS4, PIK3R5, PRKCZ, PTEN, RHEB, and RPS6KB1 in addition to PIK3CA (Wood et al. 2007). Similarly, in the whole genome study of glioblastoma tumors the PI3K genes PIK3CA, PIK3R1, PTEN, and IRS1 were found to be altered in 50% of tumors and in all cases, mutations within each tumor affected only a single member of the pathway in a mutually exclusive manner ($p < 0.05$) (Parsons et al. 2008). The fact that all but one of the cancers with mutations in members of the PI3K pathway did not have alterations in other members of the same pathway again suggests that such alterations are functionally equivalent in tumorigenesis.

However, as described in greater detail above, similar analyses of endometrial cancer presented a different suggestion. As PTEN mutations occur at high frequency in endometrial carcinoma, primary endometrial carcinomas were screened for mutations in the helical and catalytic domains of PIK3CA and 36% of tumors had mutations in this gene and coexistence of PIK3CA/PTEN mutations were observed at high frequency (26%). Interestingly, PIK3CA mutations were more common in tumors with PTEN mutations (46%) compared with those without PTEN mutations (24%). Thus, a combination of PIK3CA/PTEN alterations might play a role in development of certain tumors (Oda et al. 2005).

The emphasis of the importance of the PI3K pathway in cancer development fits with the conclusions of recent mutation analyses in colorectal, breast, pancreatic and glioblastoma cancers that have revealed two unifying features: (1) there are a few major gene alterations that occur in the majority of cancers and a much larger number of genes that are mutated at relatively low frequency. (2) While the number of cancer causing genes has become larger and each cancer type has specific

genomic alterations, the altered genes affect a limited number of cellular signaling pathways (Cancer Genome Atlas Research Network 2008; Jones et al. 2008; Parsons et al. 2008; Sjoblom et al. 2006; Wood et al. 2007).

In the case of colon cancer, it appears that the molecular explanation for activation of PI3K signaling is generally now understood. However, there are many other tumor types in which the gene causing PI3K activation has not yet been discovered. For example, several studies have indicated that approximately sixty percent of melanomas contain activated AKT (most likely the Akt3 isoform) (Stahl et al. 2004; Bastian et al. 1998). There are several known genetic events that explain a subset of these cases. For example, somatic inactivation of PTEN is found in 10–30% of melanomas (Birck et al. 2000; Chudnovsky et al. 2005; Guldberg et al. 1997; Robertson et al. 1998; Tsao et al. 1998; Zhou et al. 2000), and mutational activation of PIK3CA is found in another ~3% (Board et al. 2008; Omholt et al. 2006). Amplification of the Akt3 locus itself is also likely to be responsible for an additional small fraction of cases with Akt activation (Bastian et al. 1998; Thompson et al. 1995). However, this leaves >20% of melanoma cases with unexplained activation of Akt. As such, it is likely that one or more other members of the PI3K pathway suffer somatic mutations in this disease process and are responsible for AKT activation in the remaining tumors. A mutational analysis of additional genes that lie in these two pathways may identify novel somatic mutations in melanoma.

These types of additional sequencing studies are particularly exciting since they have the potential to uncover additional mutations affecting the PI3K pathway and provide a strong rationale for development of new therapeutic and diagnostic approaches for the already sizable number of individuals who have mutations in the PI3K pathway.

In addition to providing substantial insight into the basic biological mechanisms that drive human cancer pathogenesis, the discovery of activating mutations in PIK3CA has caused academic and industrial groups to redouble their efforts to develop and test pharmacological inhibitors of PI3K, since mutations in the PI3K signaling pathway are now known to occur at a frequency double what was previously predicted. Numerous academic and industrial groups are currently developing and testing novel pharmacological inhibitors of PI3K enzymes, and this is discussed in much detail in other sections of this book. However, the discovery of oncogenic mutations in PIK3CA itself has provided substantial impetus to these efforts since it has dramatically emphasized the important role of PI3K in cancer pathogenesis and made it possible to quickly and easily identify tumors with activation of PI3K signaling by virtue of mutations in PIK3CA.

7 Conclusion

By combining the large amount of sequencing data over the past 5 years, we find that PIK3CA is one of the most commonly mutated oncogenes in human cancers. In all the tumor types examined to date, mutations cluster within two hotspots. It is

now evident that cancers of the endometrium, breast, and colon, as well as benign tumors of the skin are among the tumor types with the highest frequencies of PIK3CA mutations. There is some inconsistency in the literature regarding the frequency of PIK3CA mutations in individual tumor types; however, these discrepancies are likely due to a number of factors including the specific exons that were sequenced, geographical variation, sample source preservation and methods used for DNA isolation. However, despite these discrepancies, the high frequency of PIK3CA mutation and the discovery of hotspot mutations have important clinical implications for diagnosis, prognosis and therapy. Targeting this mutant protein with novel therapeutics could have a substantial impact on eliminating the morbidity and mortality of human cancer.

References

- Abubaker J, Bavi PP, Al Harbi S, Siraj AK, Al Dayel F, Uddin S, Al Kuraya K (2007) PIK3CA mutations are mutually exclusive with PTEN loss in diffuse large B cell lymphoma. *Leukemia* 21:2368–2370
- Abubaker J, Bavi P, Al Harbi S, Ibrahim M, Siraj AK, Al Sanea N, Abduljabbar A, Ashari LH, Alhomoud S, Al Dayel F et al (2008a) Clinicopathological analysis of colorectal cancers with PIK3CA mutations in Middle Eastern population. *Oncogene* 27:3539–3545
- Abubaker J, Jehan Z, Bavi P, Sultana M, Al Harbi S, Ibrahim M, Al Nuaim A, Ahmed M, Amin T, Al Fehaily M et al (2008b) Clinicopathological analysis of papillary thyroid cancer with PIK3CA alterations in a Middle Eastern population. *J Clin Endocrinol Metab* 93:611–618
- Actor B, Cobbers JM, Buschges R, Wolter M, Knobbe CB, Lichter P, Reifenberger G, Weber RG (2002) Comprehensive analysis of genomic alterations in gliosarcoma and its two tissue components. *Genes Chromosomes Cancer* 34:416–427
- Akagi I, Miyashita M, Makino H, Nomura T, Hagiwara N, Takahashi K, Cho K, Mishima T, Ishibashi O, Ushijima T et al (2009) Overexpression of PIK3CA is associated with lymph node metastasis in esophageal squamous cell carcinoma. *Int J Oncol* 34:767–775
- Andersson P, Kolaric A, Windahl T, Kirrander P, Soderkvist P, Karlsson MG (2008) PIK3CA, HRAS and KRAS gene mutations in human penile cancer. *J Urol* 179:2030–2034
- Bachman KE, Argani P, Samuels Y, Silliman N, Ptak J, Szabo S, Konishi H, Karakas B, Blair BG, Lin C et al (2004) The PIK3CA gene is mutated with high frequency in human breast cancers. *Cancer Biol Ther* 3:772–775
- Bader AG, Kang S, Vogt PK (2006) Cancer specific mutations in PIK3CA are oncogenic *in vivo*. *Proc Natl Acad Sci USA* 103:1475–1479
- Barault L, Veyrie N, Jooste V, Lecorre D, Chapusot C, Ferraz JM, Lievre A, Cortet M, Bouvier AM, Rat P et al (2008) Mutations in the RAS MAPK, PI(3)K (phosphatidylinositol 3 OH kinase) signaling network correlate with poor survival in a population based series of colon cancers. *Int J Cancer* 122:2255–2259
- Barbareschi M, Buttitta F, Felicioni L, Cotrupi S, Barassi F, Del Grammastro M, Ferro A, Dalla Palma P, Galligioni E, Marchetti A (2007) Different prognostic roles of mutations in the helical and kinase domains of the PIK3CA gene in breast carcinomas. *Clin Cancer Res* 13:6064–6069
- Bardelli A, Parsons DW, Silliman N, Ptak J, Szabo S, Saha S, Markowitz S, Willson JK, Parmigiani G, Kinzler KW et al (2003) Mutational analysis of the tyrosine kinome in colorectal cancers. *Science* 300:949
- Barr FG, Galili N, Holick J, Biegel JA, Rovera G, Emanuel BS (1993) Rearrangement of the PAX3 paired box gene in the paediatric solid tumour alveolar rhabdomyosarcoma. *Nat Genet* 3:113–117

- Bastian BC, LeBoit PE, Hamm H, Brocker EB, Pinkel D (1998) Chromosomal gains and losses in primary cutaneous melanomas detected by comparative genomic hybridization. *Cancer Res* 58:2170 2175
- Bellacosa A, Testa JR, Staal SP, Tschlis PN (1991) A retroviral oncogene, akt, encoding a serine threonine kinase containing an SH2 like region. *Science* 254:274 277
- Bellacosa A, de Feo D, Godwin AK, Bell DW, Cheng JQ, Altomare DA, Wan M, Dubeau L, Scambia G, Masciullo V et al (1995) Molecular alterations of the AKT2 oncogene in ovarian and breast carcinomas. *Int J Cancer* 64:280 285
- Benvenuti S, Frattini M, Arena S, Zanon C, Cappelletti V, Coradini D, Daidone MG, Pilotti S, Pierotti MA, Bardelli A (2008) PIK3CA cancer mutations display gender and tissue specificity patterns. *Hum Mutat* 29:284 288
- Berns K, Horlings HM, Hennessy BT, Madiredjo M, Hijmans EM, Beelen K, Linn SC, Gonzalez Angulo AM, Stemke Hale K, Hauptmann M et al (2007) A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. *Cancer Cell* 12:395 402
- Birch A, Ahrenkiel V, Zeuthen J, Hou Jensen K, Guldberg P (2000) Mutation and allelic loss of the PTEN/MMAC1 gene in primary and metastatic melanoma biopsies. *J Invest Dermatol* 114:277 280
- Bleeker FE, Felicioni L, Buttitta F, Lamba S, Cardone L, Rodolfo M, Scarpa A, Leenstra S, Frattini M, Barbareschi M et al (2008) AKT1(E17K) in human solid tumours. *Oncogene* 27:5648 5650
- Board RE, Thelwell NJ, Ravetto PF, Little S, Ranson M, Dive C, Hughes A, Whitcombe D (2008) Multiplexed assays for detection of mutations in PIK3CA. *Clin Chem* 54:757 760
- Bos JL, Fearon ER, Hamilton SR, Verlaan de Vries M, van Boom JH, van der Eb AJ, Vogelstein B (1987) Prevalence of ras gene mutations in human colorectal cancers. *Nature* 327:293 297
- Broderick DK, Di C, Parrett TJ, Samuels YR, Cummins JM, McLendon RE, Fuets DW, Velculescu VE, Bigner DD, Yan H (2004) Mutations of PIK3CA in anaplastic oligodendrogliomas, high grade astrocytomas, and medulloblastomas. *Cancer Res* 64:5048 5050
- Buttitta F, Felicioni L, Barassi F, Martella C, Paolizzi D, Fresu G, Salvatore S, Cuccurullo F, Mezzetti A, Campani D, Marchetti A (2006) PIK3CA mutation and histological type in breast carcinoma: high frequency of mutations in lobular carcinoma. *J Pathol* 208:350 355
- Byun DS, Cho K, Ryu BK, Lee MG, Park JI, Chae KS, Kim HJ, Chi SG (2003) Frequent monoallelic deletion of PTEN and its reciprocal association with PIK3CA amplification in gastric carcinoma. *Int J Cancer* 104:318 327
- Campbell IG, Russell SE, Choong DY, Montgomery KG, Ciavarella ML, Hooi CS, Cristiano BE, Pearson RB, Phillips WA (2004) Mutation of the PIK3CA gene in ovarian and breast cancer. *Cancer Res* 64:7678 7681
- Cancer Genome Atlas Research Network (2008) Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 455:1061 1068
- Cantley LC (2002) The phosphoinositide 3 kinase pathway. *Science* 296:1655 1657
- Cantley LC, Auger KR, Carpenter C, Duckworth B, Graziani A, Kapeller R, Soltoff S (1991) Oncogenes and signal transduction. *Cell* 64:281 302
- Carpten JD, Faber AL, Horn C, Donoho GP, Briggs SL, Robbins CM, Hostetter G, Boguslawski S, Moses TY, Savage S et al (2007) A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. *Nature* 448:439 444
- Catasus L, Gallardo A, Cuatrecasas M, Prat J (2008) PIK3CA mutations in the kinase domain (exon 20) of uterine endometrial adenocarcinomas are associated with adverse prognostic parameters. *Mod Pathol* 21:131 139
- Chang HW, Aoki M, Fruman D, Auger KR, Bellacosa A, Tschlis PN, Cantley LC, Roberts TM, Vogt PK (1997) Transformation of chicken cells by the gene encoding the catalytic subunit of PI 3 kinase. *Science* 276:1848 1850
- Chen Z, Trotman LC, Shaffer D, Lin HK, Dotan ZA, Niki M, Koutcher JA, Scher HI, Ludwig T, Gerald W et al (2005) Crucial role of p53 dependent cellular senescence in suppression of Pten deficient tumorigenesis. *Nature* 436:725 730

- Cheng JQ, Godwin AK, Bellacosa A, Taguchi T, Franke TF, Hamilton TC, Tsichlis PN, Testa JR (1992) AKT2, a putative oncogene encoding a member of a subfamily of protein serine/threonine kinases, is amplified in human ovarian carcinomas. *Proc Natl Acad Sci USA* 89:9267-9271
- Cheng JQ, Ruggeri B, Klein WM, Sonoda G, Altomare DA, Watson DK, Testa JR (1996) Amplification of AKT2 in human pancreatic cells and inhibition of AKT2 expression and tumorigenicity by antisense RNA. *Proc Natl Acad Sci USA* 93:3636-3641
- Chou CC, Chou MJ, Tzen CY (2008) PIK3CA mutation occurs in nasopharyngeal carcinoma but does not significantly influence the disease specific survival. *Med Oncol* 26(3):322-326
- Chudnovsky Y, Khavari PA, Adams AE (2005) Melanoma genetics and the development of rational therapeutics. *J Clin Invest* 115:813-824
- Cui B, Zheng B, Zhang X, Stendahl U, Andersson S, Wallin KL (2009) Mutation of PIK3CA: possible risk factor for cervical carcinogenesis in older women. *Int J Oncol* 34:409-416
- Dam V, Morgan BT, Mazanek P, Hogarty MD (2006) Mutations in PIK3CA are infrequent in neuroblastoma. *BMC Cancer* 6:177
- Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W et al (2002) Mutations of the BRAF gene in human cancer. *Nature* 417(6892):949-954
- Eichhorn PJ, Gili M, Scaltriti M, Serra V, Guzman M, Nijkamp W, Beijersbergen RL, Valero V, Seoane J, Bernards R, Baselga J (2008) Phosphatidylinositol 3 kinase hyperactivation results in lapatinib resistance that is reversed by the mTOR/phosphatidylinositol 3 kinase inhibitor NVP-BEZ235. *Cancer Res* 68:9221-9230
- Fenic I, Steger K, Gruber C, Arens C, Woenckhaus J (2007) Analysis of PIK3CA and Akt/protein kinase B in head and neck squamous cell carcinoma. *Oncol Rep* 18:253-259
- Gallia GL, Rand V, Siu IM, Eberhart CG, James CD, Marie SK, Oba Shinjo SM, Carlotti CG, Caballero OL, Simpson AJ et al (2006) PIK3CA gene mutations in pediatric and adult glioblastoma multiforme. *Mol Cancer Res* 4:709-714
- Garcia Rostan G, Costa AM, Pereira Castro I, Salvatore G, Hernandez R, Hermsem MJ, Herrero A, Fusco A, Cameselle Teijeiro J, Santoro M (2005) Mutation of the PIK3CA gene in anaplastic thyroid cancer. *Cancer Res* 65:10199-10207
- Guldberg P, thor Straten P, Birck A, Ahrenkiel V, Kirkin AF, Zeuthen J (1997) Disruption of the MMAC1/PTEN gene by deletion or mutation is a frequent event in malignant melanoma. *Cancer Res* 57:3660-3663
- Hafner C, Lopez Knowles E, Luis NM, Toll A, Baselga E, Fernandez Casado A, Hernandez S, Ribe A, Mentzel T, Stoehr R et al (2007) Oncogenic PIK3CA mutations occur in epidermal nevi and seborrheic keratoses with a characteristic mutation pattern. *Proc Natl Acad Sci USA* 104:13450-13454
- Hafner C, Vogt T, Landthaler M, Musebeck J (2008) Somatic FGFR3 and PIK3CA mutations are present in familial seborrheic keratoses. *Br J Dermatol* 159:214-217
- Hafner C, Stoehr R, van Oers JM, Zwarthoff EC, Hofstaedter F, Landthaler M, Hartmann A, Vogt T (2009) FGFR3 and PIK3CA mutations are involved in the molecular pathogenesis of solar lentigo. *Br J Dermatol* 160:546-551
- Hartmann C, Bartels G, Gehlhaar C, Holtkamp N, von Deimling A (2005) PIK3CA mutations in glioblastoma multiforme. *Acta Neuropathol* 109:639-642
- Hartmann C, Devermann L, Gehlhaar C, Holtkamp N, von Deimling A (2006) PIK3CA mutations in oligodendroglial tumours. *Neuropathol Appl Neurobiol* 32:209-212
- Hayes MP, Wang H, Espinal Witter R, Douglas W, Solomon GJ, Baker SJ, Ellenson LH (2006) PIK3CA and PTEN mutations in uterine endometrioid carcinoma and complex atypical hyperplasia. *Clin Cancer Res* 12:5932-5935
- Hayes MP, Douglas W, Ellenson LH (2009) Molecular alterations of EGFR and PIK3CA in uterine serous carcinoma. *Gynecol Oncol* 113(3):370-373
- Hummerdal P, Andersson P, Willander K, Linderholm M, Soderkvist P, Jonsson JI (2006) Absence of hot spot mutations of the PIK3CA gene in acute myeloid leukaemia. *Eur J Haematol* 77:86-87

- Jen J, Powell SM, Papadopoulos N, Smith KJ, Hamilton SR, Vogelstein B, Kinzler KW (1994) Molecular determinants of dysplasia in colorectal lesions. *Cancer Res* 54:5523-5526
- Jhawer M, Goel S, Wilson AJ, Montagna C, Ling YH, Byun DS, Nasser S, Arango D, Shin J, Klampfer L et al (2008) PIK3CA mutation/PTEN expression status predicts response of colon cancer cells to the epidermal growth factor receptor inhibitor cetuximab. *Cancer Res* 68:1953-1961
- Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A et al (2008) Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* 321:1801-1806
- Kang S, Bader AG, Vogt PK (2005) Phosphatidylinositol 3 kinase mutations identified in human cancer are oncogenic. *Proc Natl Acad Sci USA* 102:802-807
- Kang S, Seo SS, Chang HJ, Yoo CW, Park SY, Dong SM (2008) Mutual exclusiveness between PIK3CA and KRAS mutations in endometrial carcinoma. *Int J Gynecol Cancer* 18:1339-1343
- Kato S, Iida S, Higuchi T, Ishikawa T, Takagi Y, Yasuno M, Enomoto M, Uetake H, Sugihara K (2007) PIK3CA mutation is predictive of poor survival in patients with colorectal cancer. *Int J Cancer* 121:1771-1778
- Katso R, Okkenhaug K, Ahmadi K, White S, Timms J, Waterfield MD (2001) Cellular function of phosphoinositide 3 kinases: implications for development, homeostasis, and cancer. *Annu Rev Cell Dev Biol* 17:615-675
- Kawano O, Sasaki H, Endo K, Suzuki E, Haneda H, Yukiue H, Kobayashi Y, Yano M, Fujii Y (2006) PIK3CA mutation status in Japanese lung cancer patients. *Lung Cancer* 54:209-215
- Kawano O, Sasaki H, Okuda K, Yukiue H, Yokoyama T, Yano M, Fujii Y (2007) PIK3CA gene amplification in Japanese non small cell lung cancer. *Lung Cancer* 58:159-160
- Kim JS, Lee C, Bonifant CL, Ressom H, Waldman T (2007) Activation of p53 dependent growth suppression in human cells by mutations in PTEN or PIK3CA. *Mol Cell Biol* 27:662-677
- Kita D, Yonekawa Y, Weller M, Ohgaki H (2007) PIK3CA alterations in primary (de novo) and secondary glioblastomas. *Acta Neuropathol* 113:295-302
- Knobbe CB, Reifenberger G (2003) Genetic alterations and aberrant expression of genes related to the phosphatidylinositol 3' kinase/protein kinase B (Akt) signal transduction pathway in glioblastomas. *Brain Pathol* 13:507-518
- Knobbe CB, Trampe Kieslich A, Reifenberger G (2005) Genetic alteration and expression of the phosphoinositide 3 kinase/Akt pathway genes PIK3CA and PIKE in human glioblastomas. *Neuropathol Appl Neurobiol* 31:486-490
- Kolasa IK, Rembiszewska A, Felisiak A, Ziolkowska Seta I, Murawska M, Moes J, Timorek A, Dansonka Mieszkowska A, Kupryjanczyk J (2009) PIK3CA amplification associates with resistance to chemotherapy in ovarian cancer patients. *Cancer Biol Ther* 8:21-26
- Kozaki K, Imoto I, Pimkhaokham A, Hasegawa S, Tsuda H, Omura K, Inazawa J (2006) PIK3CA mutation is an oncogenic aberration at advanced stages of oral squamous cell carcinoma. *Cancer Sci* 97:1351-1358
- Lai YL, Mau BL, Cheng WH, Chen HM, Chiu HH, Tzen CY (2008) PIK3CA exon 20 mutation is independently associated with a poor prognosis in breast cancer patients. *Ann Surg Oncol* 15:1064-1069
- Lee JW, Soung YH, Kim SY, Lee HW, Park WS, Nam SW, Kim SH, Lee JY, Yoo NJ, Lee SH (2005) PIK3CA gene is frequently mutated in breast carcinomas and hepatocellular carcinomas. *Oncogene* 24:1477-1480
- Leira E, Catusus L, Gallardo A, Peiro G, Alonso C, Aranda I, Barnadas A, Prat J (2008) Exon 20 PIK3CA mutations decreases survival in aggressive (HER 2 positive) breast carcinomas. *Virchows Arch* 453:133-139
- Levine DA, Bogomolny F, Yee CJ, Lash A, Barakat RR, Borgen PI, Boyd J (2005) Frequent mutation of the PIK3CA gene in ovarian and breast cancers. *Clin Cancer Res* 11:2875-2878
- Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliareis C, Rodgers L, McCombie R et al (1997) PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* 275:1943-1947

- Li VS, Wong CW, Chan TL, Chan AS, Zhao W, Chu KM, So S, Chen X, Yuen ST, Leung SY (2005) Mutations of PIK3CA in gastric adenocarcinoma. *BMC Cancer* 5:29
- Li SY, Rong M, Grieu F, Iacopetta B (2006) PIK3CA mutations in breast cancer are associated with poor outcome. *Breast Cancer Res Treat* 96:91-95
- Liedtke C, Cardone L, Tordai A, Yan K, Gomez HL, Figureoa LJ, Hubbard RE, Valero V, Souchon EA, Symmans WF et al (2008) PIK3CA activating mutations and chemotherapy sensitivity in stage II-III breast cancer. *Breast Cancer Res* 10:R27
- Lin Y, Jiang X, Shen Y, Li M, Ma H, Xing M, Lu Y (2009) Frequent mutations and amplifications of the PIK3CA gene in pituitary tumors. *Endocr Relat Cancer* 16:301-310
- Liu P, Li DJ, Qin HD, Zhang RH, Chen LZ, Zeng YX (2007) Screening for mutations in the hotspot mutation regions of PIK3CA gene in nasopharyngeal carcinoma. *Ai Zheng* 26:15-20
- Lopez Knowles E, Hernandez S, Malats N, Kogevinas M, Lloreta J, Carrato A, Tardon A, Serra C, Real FX (2006) PIK3CA mutations are an early genetic alteration associated with FGFR3 mutations in superficial papillary bladder tumors. *Cancer Res* 66:7401-7404
- Ma YY, Wei SJ, Lin YC, Lung JC, Chang TC, Whang Peng J, Liu JM, Yang DM, Yang WK, Shen CY (2000) PIK3CA as an oncogene in cervical cancer. *Oncogene* 19:2739-2744
- Maruyama N, Miyoshi Y, Taguchi T, Tamaki Y, Monden M, Noguchi S (2007) Clinicopathologic analysis of breast cancers with PIK3CA mutations in Japanese women. *Clin Cancer Res* 13:408-414
- Massion PP, Taflan PM, Shyr Y, Rahman SM, Yildiz P, Shakhthour B, Edgerton ME, Ninan M, Andersen JJ, Gonzalez AL (2004) Early involvement of the phosphatidylinositol 3 kinase/Akt pathway in lung cancer progression. *Am J Respir Crit Care Med* 170:1088-1094
- Mikami M, Noshio K, Yamamoto H, Takahashi T, Maehata T, Taniguchi H, Adachi Y, Imamura A, Fujita M, Hosokawa M et al (2006) Mutational analysis of beta catenin and the RAS/RAF signalling pathway in early flat type colorectal tumours. *Eur J Cancer* 42:3065-3072
- Miyake T, Yoshino K, Enomoto T, Takata T, Ugaki H, Kim A, Fujiwara K, Miyatake T, Fujita M, Kimura T (2008) PIK3CA gene mutations and amplifications in uterine cancers, identified by methods that avoid confounding by PIK3CA pseudogene sequences. *Cancer Lett* 261:120-126
- Miyaki M, Iijima T, Yamaguchi T, Takahashi K, Matsumoto H, Yasutome M, Funata N, Mori T (2007) Mutations of the PIK3CA gene in hereditary colorectal cancers. *Int J Cancer* 121:1627-1630
- Mori R, Ishiguro H, Kimura M, Mitsui A, Sasaki H, Tomoda K, Mori Y, Ogawa R, Katada T, Kawano O et al (2008) PIK3CA mutation status in Japanese esophageal squamous cell carcinoma. *J Surg Res* 145:320-326
- Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein B, Kinzler KW (1997) Activation of beta catenin/Tcf signaling in colon cancer by mutations in beta catenin or APC. *Science* 275:1787-1790
- Mueller W, Mizoguchi M, Silen E, D'Amore K, Nutt CL, Louis DN (2005) Mutations of the PIK3CA gene are rare in human glioblastoma. *Acta Neuropathol* 109:654-655
- Muller CI, Miller CW, Hofmann WK, Gross ME, Walsh CS, Kawamata N, Luong QT, Koeffler HP (2007) Rare mutations of the PIK3CA gene in malignancies of the hematopoietic system as well as endometrium, ovary, prostate and osteosarcomas, and discovery of a PIK3CA pseudogene. *Leuk Res* 31:27-32
- Murugan AK, Hong NT, Fukui Y, Munirajan AK, Tsuchida N (2008) Oncogenic mutations of the PIK3CA gene in head and neck squamous cell carcinomas. *Int J Oncol* 32:101-111
- Nakayama K, Nakayama N, Kurman RJ, Cope L, Pohl G, Samuels Y, Velculescu VE, Wang TL, Shih Ie M (2006) Sequence mutations and amplification of PIK3CA and AKT2 genes in purified ovarian serous neoplasms. *Cancer Biol Ther* 5:779-785
- Oda K, Stokoe D, Taketani Y, McCormick F (2005) High frequency of coexistent mutations of PIK3CA and PTEN genes in endometrial carcinoma. *Cancer Res* 65:10669-10673
- Ogino S, Noshio K, Kirkner GJ, Shima K, Irahara N, Kure S, Chan AT, Engelman JA, Kraft P, Cantley LC et al (2009) PIK3CA mutation is associated with poor prognosis among patients with curatively resected colon cancer. *J Clin Oncol* 27:1477-1484

- Okudela K, Suzuki M, Kageyama S, Bunai T, Nagura K, Igarashi H, Takamochi K, Suzuki K, Yamada T, Niwa H et al (2007) PIK3CA mutation and amplification in human lung cancer. *Pathol Int* 57:664 671
- Ollikainen M, Gylling A, Puputti M, Nupponen NN, Abdel Rahman WM, Butzow R, Peltomaki P (2007) Patterns of PIK3CA alterations in familial colorectal and endometrial carcinoma. *Int J Cancer* 121:915 920
- Omholt K, Krockel D, Ringborg U, Hansson J (2006) Mutations of PIK3CA are rare in cutaneous melanoma. *Melanoma Res* 16:197 200
- Or YY, Hui AB, To KF, Lam CN, Lo KW (2006) PIK3CA mutations in nasopharyngeal carcinoma. *Int J Cancer* 118:1065 1067
- Page C, Huang M, Jin X, Cho K, Lilja J, Reynolds RK, Lin J (2000) Elevated phosphorylation of AKT and Stat3 in prostate, breast, and cervical cancer cells. *Int J Oncol* 17:23 28
- Pang JC, Chung NY, Chan NH, Poon WS, Thomas T, Ng HK (2006) Rare mutation of PIK3CA in meningiomas. *Acta Neuropathol* 111:284 285
- Parsons DW, Wang TL, Samuels Y, Bardelli A, Cummins JM, DeLong L, Silliman N, Ptak J, Szabo S, Willson JK et al (2005) Colorectal cancer: mutations in a signalling pathway. *Nature* 436:792
- Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL et al (2008) An integrated genomic analysis of human glioblastoma multiforme. *Science* 321:1807 1812
- Perez Tenorio G, Alkhorri L, Olsson B, Waltersson MA, Nordenskjold B, Rutqvist LE, Skoog L, Stal O (2007) PIK3CA mutations and PTEN loss correlate with similar prognostic factors and are not mutually exclusive in breast cancer. *Clin Cancer Res* 13:3577 3584
- Phillips WA, Russell SE, Ciavarella ML, Choong DY, Montgomery KG, Smith K, Pearson RB, Thomas RJ, Campbell IG (2006) Mutation analysis of PIK3CA and PIK3CB in esophageal cancer and Barrett's esophagus. *Int J Cancer* 118:2644 2646
- Philp AJ, Campbell IG, Leet C, Vincan E, Rockman SP, Whitehead RH, Thomas RJ, Phillips WA (2001) The phosphatidylinositol 3' kinase p85alpha gene is an oncogene in human ovarian and colon tumors. *Cancer Res* 61:7426 7429
- Qiu W, Schonleben F, Li X, Ho DJ, Close LG, Manolidis S, Bennett BP, Su GH (2006) PIK3CA mutations in head and neck squamous cell carcinoma. *Clin Cancer Res* 12:1441 1446
- Qiu W, Tong GX, Manolidis S, Close LG, Assaad AM, Su GH (2008) Novel mutant enriched sequencing identified high frequency of PIK3CA mutations in pharyngeal cancer. *Int J Cancer* 122:1189 1194
- Rajagopalan H, Bardelli A, Lengauer C, Kinzler KW, Vogelstein B, Velculescu VE (2002) Tumorigenesis: RAF/RAS oncogenes and mismatch repair status. *Nature* 418:934
- Riener MO, Bawohl M, Clavien PA, Jochum W (2008) Rare PIK3CA hotspot mutations in carcinomas of the biliary tract. *Genes Chromosomes Cancer* 47:363 367
- Robertson GP, Fumari FB, Miele ME, Glendening MJ, Welch DR, Fountain JW, Lugo TG, Huang HJ, Cavenee WK (1998) In vitro loss of heterozygosity targets the PTEN/MMAC1 gene in melanoma. *Proc Natl Acad Sci USA* 95:9418 9423
- Saal LH, Holm K, Maurer M, Memeo L, Su T, Wang X, Yu JS, Malmstrom PO, Mansukhani M, Enoksson J et al (2005) PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. *Cancer Res* 65:2554 2559
- Saal LH, Johansson P, Holm K, Gruvberger Saal SK, She QB, Maurer M, Koujak S, Ferrando AA, Malmstrom P, Memeo L et al (2007) Poor prognosis in carcinoma is associated with a gene expression signature of aberrant PTEN tumor suppressor pathway activity. *Proc Natl Acad Sci USA* 104:7564 7569
- Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell SM, Riggins GJ et al (2004) High frequency of mutations of the PIK3CA gene in human cancers. *Science* 304:554

- Samuels Y, Diaz LA Jr, Schmidt Kitterl O, Cummins JM, Delong L, Cheong I, Rago C, Huso DL, Lengauer C, Kinzler KW et al (2005) Mutant PIK3CA promotes cell growth and invasion of human cancer cells. *Cancer Cell* 7:561 573
- Santaripia L, El Naggar AK, Cote GJ, Myers JN, Sherman SI (2008) Phosphatidylinositol 3 kinase/akt and ras/raf mitogen activated protein kinase pathway mutations in anaplastic thyroid cancer. *J Clin Endocrinol Metab* 93:278 284
- Sartore Bianchi A, Martini M, Molinari F, Veronese S, Nichelatti M, Artale S, Di Nicolantonio F, Saletti P, De Dosso S, Mazzucchelli L et al (2009) PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR targeted monoclonal antibodies. *Cancer Res* 69:1851 1857
- Schonleben F, Qiu W, Ciau NT, Ho DJ, Li X, Allendorf JD, Remotti HE, Su GH (2006) PIK3CA mutations in intraductal papillary mucinous neoplasm/carcinoma of the pancreas. *Clin Cancer Res* 12:3851 3855
- Sharma SV, Bell DW, Settleman J, Haber DA (2007) Epidermal growth factor receptor mutations in lung cancer. *Nat Rev Cancer* 7:169 181
- Shayesteh L, Lu Y, Kuo WL, Baldocchi R, Godfrey T, Collins C, Pinkel D, Powell B, Mills GB, Gray JW (1999) PIK3CA is implicated as an oncogene in ovarian cancer. *Nat Genet* 21:99 102
- Sjjoblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, Mandelker D, Leary RJ, Ptak J, Silliman N et al (2006) The consensus coding sequences of human breast and colorectal cancers. *Science* 314:268 274
- Sparks AB, Morin PJ, Vogelstein B, Kinzler KW (1998) Mutational Analysis of the APC/b catenin/Tcf Pathway in Colorectal Cancer. *Cancer Res* 58:1130 1134
- Staal SP (1987) Molecular cloning of the akt oncogene and its human homologues AKT1 and AKT2: amplification of AKT1 in a primary human gastric adenocarcinoma. *Proc Natl Acad Sci USA* 84:5034 5037
- Stahl JM, Sharma A, Cheung M, Zimmerman M, Cheng JQ, Bosenberg MW, Kester M, Sandirasegarane L, Robertson GP (2004) Deregulated Akt3 activity promotes development of malignant melanoma. *Cancer Res* 64:7002 7010
- Steck PA, Pershouse MA, Jasser SA, Yung WK, Lin H, Ligon AH, Langford LA, Baumgard ML, Hattier T, Davis T et al (1997) Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. *Nat Genet* 15:356 362
- Stemke Hale K, Gonzalez Angulo AM, Lluch A, Neve RM, Kuo WL, Davies M, Carey M, Hu Z, Guan Y, Sahin A et al (2008) An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. *Cancer Res* 68:6084 6091
- Stephens M, Sloan JS, Robertson PD, Scheet P, Nickerson DA (2006) Automating sequence based detection and genotyping of SNPs from diploid samples. *Nat Genet* 38:375 381
- Tanaka Y, Kanai F, Tada M, Asaoka Y, Guleng B, Jazag A, Ohta M, Ikenoue T, Tateishi K, Obi S et al (2006) Absence of PIK3CA hotspot mutations in hepatocellular carcinoma in Japanese patients. *Oncogene* 25:2950 2952
- Thompson FH, Emerson J, Olson S, Weinstein R, Leavitt SA, Leong SP, Emerson S, Trent JM, Nelson MA, Salmon SE et al (1995) Cytogenetics of 158 patients with regional or disseminated melanoma. Subset analysis of near diploid and simple karyotypes. *Cancer Genet Cytogenet* 83:93 104
- Tsao H, Zhang X, Benoit E, Haluska FG (1998) Identification of PTEN/MMAC1 alterations in uncultured melanomas and melanoma cell lines. *Oncogene* 16:3397 3402
- Vanhaesebroeck B, Waterfield MD (1999) Signaling by distinct classes of phosphoinositide 3 kinases. *Exp Cell Res* 253:239 254
- Velasco A, Bussaglia E, Pallares J, Dolcet X, Llobet D, Encinas M, Llecha N, Palacios J, Prat J, Matias Guiu X (2006) PIK3CA gene mutations in endometrial carcinoma: correlation with PTEN and K RAS alterations. *Hum Pathol* 37:1465 1472
- Velho S, Oliveira C, Ferreira A, Ferreira AC, Suriano G, Schwartz S Jr, Duval A, Carneiro F, Machado JC, Hamelin R, Seruca R (2005) The prevalence of PIK3CA mutations in gastric and colon cancer. *Eur J Cancer* 41:1649 1654

- Vivanco I, Sawyers CL (2002) The phosphatidylinositol 3 Kinase AKT pathway in human cancer. *Nat Rev Cancer* 2:489 501
- Wang Y, Helland A, Holm R, Kristensen GB, Borresen Dale AL (2005) PIK3CA mutations in advanced ovarian carcinomas. *Hum Mutat* 25:322
- Wang Y, Hou P, Yu H, Wang W, Ji M, Zhao S, Yan S, Sun X, Liu D, Shi B et al (2007) High prevalence and mutual exclusivity of genetic alterations in the phosphatidylinositol 3 kinase/akt pathway in thyroid tumors. *J Clin Endocrinol Metab* 92:2387 2390
- Wood LD, Parsons DW, Jones S, Lin J, Sjoblom T, Leary RJ, Shen D, Boca SM, Barber T, Ptak J et al (2007) The genomic landscapes of human breast and colorectal cancers. *Science* 318 (5853):1108 1113
- Wu G, Mambo E, Guo Z, Hu S, Huang X, Gollin SM, Trink B, Ladenson PW, Sidransky D, Xing M (2005a) Uncommon mutation, but common amplifications, of the PIK3CA gene in thyroid tumors. *J Clin Endocrinol Metab* 90:4688 4693
- Wu G, Xing M, Mambo E, Huang X, Liu J, Guo Z, Chatterjee A, Goldenberg D, Gollin SM, Sukumar S et al (2005b) Somatic mutation and gain of copy number of PIK3CA in human breast cancer. *Breast Cancer Res* 7:R609 R616
- Yamamoto H, Shigematsu H, Nomura M, Lockwood WW, Sato M, Okumura N, Soh J, Suzuki M, Wistuba II, Fong KM et al (2008) PIK3CA mutations and copy number gains in human lung cancers. *Cancer Res* 68:6913 6921
- Zhang L, Yang N, Katsaros D, Huang W, Park JW, Fracchioli S, Vezzani C, Rigault de la Longrais IA, Yao W, Rubin SC, Coukos G (2003) The oncogene phosphatidylinositol 3' kinase catalytic subunit alpha promotes angiogenesis via vascular endothelial growth factor in ovarian carcinoma. *Cancer Res* 63:4225 4231
- Zhao L, Vogt PK (2008) Helical domain and kinase domain mutations in p110alpha of phosphatidylinositol 3 kinase induce gain of function by different mechanisms. *Proc Natl Acad Sci USA* 105:2652 2657
- Zhou XP, Gimm O, Hampel H, Niemann T, Walker MJ, Eng C (2000) Epigenetic PTEN silencing in malignant melanomas without PTEN mutation. *Am J Pathol* 157:1123 1128

Structural Effects of Oncogenic PI3K α Mutations

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Abstract Physiological activation of PI3K α is brought about by the release of the inhibition by p85 when the nSH2 binds the phosphorylated tyrosine of activated receptors or their substrates. Oncogenic mutations of PI3K α result in a constitutively activated enzyme that triggers downstream pathways that increase tumor aggressiveness and survival. Structural information suggests that some mutations also activate the enzyme by releasing p85 inhibition. Other mutations work by different mechanisms. For example, the most common mutation, His1047Arg, causes a conformational change that increases membrane association resulting in greater accessibility to the substrate, an integral membrane component. These effects are examples of the subtle structural changes that result in increased activity.

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The structures of these and other mutants are providing the basis for the design of isozyme-specific, mutation-specific inhibitors for individualized cancer therapies.

1 Introduction

In 2004, Samuels et al. reported the results of the analysis of the sequences of all known lipid kinase genes in 35 colorectal cancers. *PIK3CA*, which encodes the p110 α catalytic subunit of PI3K α , was the only gene that showed somatic (i.e., tumor-specific) mutations (Samuels et al. 2004). Extension of this analysis to *PIK3CA* in 199 additional colorectal cancers revealed mutations in 32% of the tumors (74 out of 199 tumors). In contrast, in 76 premalignant colorectal tumors only two mutations were found; both these tumors were very advanced tubulovillous adenomas. These observations were interpreted as indicating that *PIK3CA* mutations generally arise late in tumorigenesis, just before or coincident with invasion. In the same study, mutations *PIK3CA* mutations were also identified in several other tumor types, including those of the breast, brain, stomach, and lung. Subsequent studies have identified similar mutations in these and many other tumor types (Parsons et al. 2008; T.C.G.A.R. 2008). Over 1,500 such mutations are now recorded in a cancer mutation database (Wellcome Trust Sanger Institute 2009).

The *PIK3CA* gene codes for p110 α , the major catalytic subunit of PI3K α . This subunit is composed of five domains: an adaptor-binding domain (ABD), a Ras-binding domain (RBD), a C2 domain, a helical domain, and a kinase domain (Cantley 2002; Vanhaesebroeck and Waterfield 1999; Huang et al. 2007; Amzel et al. 2008). The complete enzyme also contains one of several regulatory subunits of similar size and function. The regulatory subunits, such as p85 α , also contain five domains: an SH3 domain, a GAP domain, an N-terminal SH2 (nSH2) domain, an inter-SH2 domain (iSH2), and a C-terminal SH2 domain (cSH2) (Escobedo et al. 1991; Otsu et al. 1991). The iSH2 domain is responsible, in part, for binding the catalytic domain, while the nSH2 and cSH2 domains mediate the interactions between PI3K α and the tyrosine kinase receptors that activate it.

Of the five p110 α domains, mutations were initially found in the ABD, C2, helical domain, and the kinase domain. No mutations were found in the Ras binding domain. A large percentage of mutations ($\sim 75\%$) occurred in two clusters – one in the helical domain and the other in the kinase domain (Samuels et al. 2004). Mutations have since been identified in 140 of the 1068 residues of *PIK3CA*. While some of these mutations are rare, others are commonly observed. The residues with the highest number of observed mutations, so-called “hotspots” are His1047 in the kinase domain (470 occurrences) and Glu545, Glu542 and Gln546 (362, 193, and 51 reported occurrences, respectively).

Samuels et al. also showed that the H1047R mutation resulted in a small but significant increase in enzymatic activity with respect to the wild type (Samuels et al. 2004). Subsequently, it was determined that two additional high frequency mutations, Glu542Lys and Glu545Lys, also increased enzymatic activity (Carson et al. 2008).

The increase in activity produced by the mutations was similar in magnitude to the increase that is observed when PI3K α is activated by its physiological effectors, including phosphorylated tyrosine kinase receptors and their substrates (Yu et al. 1998a). These data were interpreted as indicating that the observed mutations of p110 α result in a constitutively activated enzyme, which in turn regulates cellular pathways that contribute to several aspects of tumor growth. These considerations made PI3K α an ideal target for the development of chemotherapeutic agents.

PI3K α is only one of several PI3K enzymes that are central to numerous aspects of metabolism and cell growth. Because of the potential toxicity associated with such pleiotropic enzymes, chemotherapeutic agents that target PI3K α must be at least isoform-specific, preferentially inhibiting the PI3K α isoform over the others. The ideal agent would of course be one that inhibited mutant PI3K α but not normal (wild type or wt) forms of the enzyme. Structural information is crucial for this type of development.

The structure of a major portion of the complete PI3K α , determined by X-ray diffraction, provided the first look at the overall subunit organization of the enzyme as well as the positions of mutations within the three-dimensional structure (Huang et al. 2007). This structure contains many of the portions of the enzyme most important for the regulation of enzymatic activity and for understanding the effects of common mutations: the complete p110 α and two domains of the regulatory subunit p85, viz., nSH2 and iSH2. Of the four regions of p85, iSH2 is absolutely required for binding to p110 and nSH2 is the domain most directly involved in PI3K regulation (Yu et al. 1998b). The iSH2 domain is a long coiled-coil that protects the large subunit from degradation by cellular proteases (Fu et al. 2004). The nSH2 domain has an inhibitory effect on the kinase activity, but this inhibition is relieved when the domain binds to a phosphorylated tyrosine from an activated upstream protein (Yu et al. 1998a). In this regard, physiological activation of PI3K α is, in reality, removal of the inhibition by nSH2.

In addition, the same portion of the oncogenic H1047R mutant of PI3K α was determined as the free enzyme and in complex with the covalent inhibitor wortmannin (Mandelker et al. 2009).

2 Description of the Structure

The p110 α /niSH2 complex (Huang et al. 2007) is a large sail-boat shaped molecule with a narrow cross section (~ 80 Å, Fig. 1) (Huang et al. 2007). The base is approximately 100 Å and its height 100 Å. The p110 portion forms the “sail” while the iSH2 of p85 constitutes the “hull” (Fig. 1a). Four of the p110 domains are arranged roughly along the top edge of p85, with the RBD at the top of the molecule. The base of the p110 subunit has the ABD at one end and the kinase domain at the other but permits a direct interaction between these two domains. The helical and C2 domain are closer to the center of the structure, at one side of the kinase domain along the narrow thickness of the subunit.

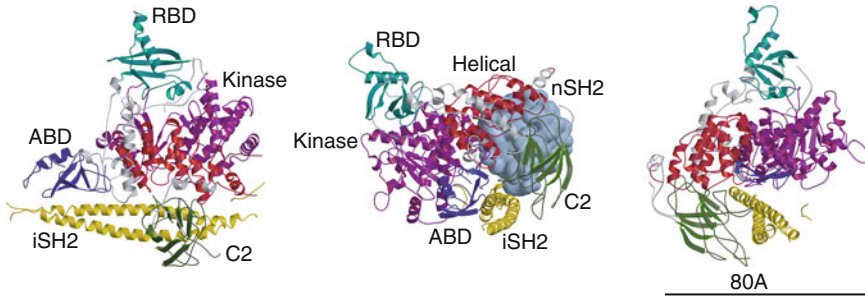


Fig. 1 Structure of the p110 α /niSH2 heterodimer. (a) Ribbon diagram of the p110 α /niSH2 heterodimer. For p110 α , ABD is colored navy blue, RBD is turquoise, C2 is green, helical is red and kinase is purple. p85 α iSH2 is yellow; all linkers are colored gray. (b) View of the p110 α /niSH2 heterodimer highlighting the iSH2 ABD and iSH2 C2 contacts. The nSH2 domain is modeled in a light blue surface. In this orientation of the p110 α /niSH2 heterodimer, the kinase, C2 and iSH2 domains are in contact with the membrane. (c) View of p110 α /niSH2 at 90° from (a) that highlights its shape and dimensions

The ABD (residues 1–108) and the RBD (residues 191–291) domains interact with the kinase domain over a large surface area. They both fold with α/β topologies and are connected to each other by an 81-residue linker (109–190) that contains two helices.

A short helix and a long coil (residues 292–329) connects the RBD to the C2 domain (residues 330–480), which folds as a β -sandwich composed of 2 four-stranded antiparallel β -sheets. C2 interacts not only with the helical and kinase p110 domains but also with the iSH2 domain of p85: H-bonds between Asp560 and Asn564 of iSH2 to Asn345 of C2 form the major interaction sites. The topology of the C2 domain of the p110 domains in class I PI3K enzymes appears to be highly conserved despite the fact that this domain has lower sequence homology than the other four domains (27% identity between α and γ). The differences between the C2 domains of p110 α and p110 γ are concentrated in the loops connecting the β -strands and include insertions/deletions: the loop spanning residues 406–424, for example, is ten residues shorter in p110 α than in p110 γ (Fig. 2). Differences in conformation between the loops of the two isoforms may be a consequence of the interaction of the C2 domain of p110 α with the iSH2 domain of the regulatory subunit p85 (p85 is not the regulatory subunit of p110 γ). Importantly, the conformation that the loops adopt in p110 γ makes it impossible for the iSH2 coiled-coil to fit in a position equivalent to that of the p110 α /niSH2 complex.

The helical domain is connected through a linker (residues 481–524) to the C2 domain and through another linker (residues 525–696) to the kinase domain (Fig. 1). The kinase domain (residues 696–1068) folds as an α/β structure composed of two subdomains separated by a cleft that harbors the catalytic site of the enzyme, in an arrangement reminiscent of other kinases. This similarity allows assignment of the catalytic and the activation loops of p110 α to residues 912–920 and 933–957. The structure of this domain is conserved among Class I PI3Ks (rmsd between

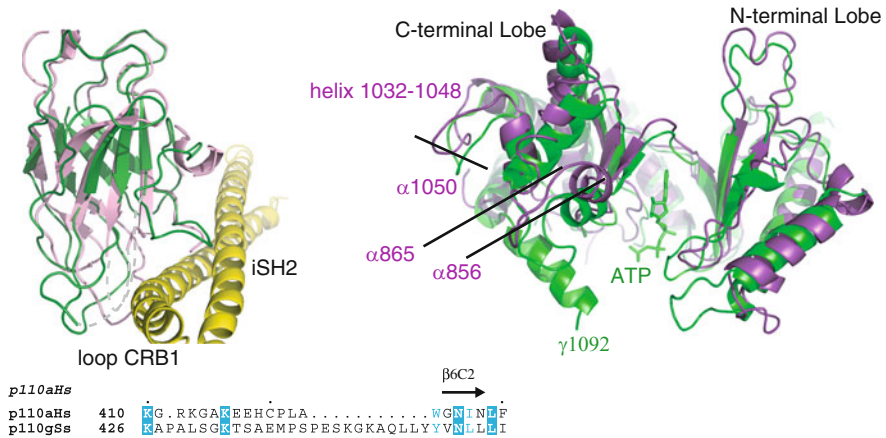


Fig. 2 Structural alignment and overlap of human p110 α /niSH2 heterodimer (PDB id 2RD0) and wild boar p110 γ (PDB id 1E8X) in relationship to the iSH2 domain of p110 α . (a) Ribbon diagram of the p110 α /niSH2 C2 (*green*) and p110 γ C2 (*lavender*) domains showing the differences in loops and CRB2. (b) Structural overlap of the kinase domains of structure 2RD0 (*purple*) and 1E8X (*green*). Helices that show the largest differences as well as the ATP are shown. Observed C terminal residues in both structures are also shown (1050 and 1092)

p110 α and p110 γ for 288 C α atoms is 1.8 Å), especially for residues surrounding the binding pocket. The largest differences occur in the helices spanning residues 856–865, a region that lines the ATP binding site, as well as in residues 1032–1048 (rmsd 3.2 Å), a region that contains two positions that are mutated with high frequency in cancers (Fig. 2b).

The ATP binding site was identified by aligning the structure of the kinase domain of p110 α with that of the same domain in the structure of the complex of p110 γ with ATP. The high degree of similarity between the two structures in this region allowed an unambiguous localization of the ATP.

3 Association with the Lipid Membrane

Although PI3Ks are not integral membrane proteins, their substrate, PIP₂ is mainly found as a plasma membrane component. To gain access to their substrates, PI3Ks must be recruited to the plasma membrane. The C2 domain within the p110 sequence is thought to provide a locus for the association of PI3Ks with membranes. In PI3K α , iSH2 binds between the C2 domain and the rest of the p110 in such a way that if C2 and the kinase domain interact with the membrane, iSH2 must also interact (Fig. 3). In this arrangement, iSH2 would provide a large contact surface lined with positively charged residues lysines 447, 448, 480, 530, 532, 551, and 561, and arginines 461, 465, 472, 480, 523, 534, 543, and 544 (Fig. 3c). Residues 723–729 and 863–867 of the kinase domain, which includes positively

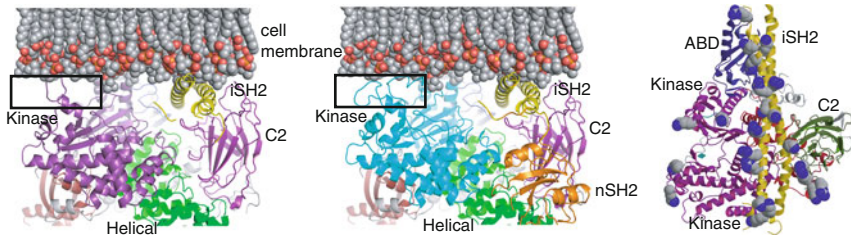


Fig. 3 Model of the association of the p110 α /niSH2 heterodimer with the lipid membrane. (a) Model of the lipid membrane with a ribbon diagram of the p110 α /niSH2 structure. A *black box* highlights the loops that move in the mutant structure. (b) Model of the lipid membrane with a ribbon diagram of the p110 α H1047R/niSH2. A *black box* highlights the loops that change conformation (residues 864-874 and 1050-1062). (c) Face of the p110 α /niSH2 heterodimer that interacts with the membrane; the iSH2 is shown as *yellow ribbons*. Positively charged residues, such as lysines and arginines, are shown in *black* as ball and stick representations

charged residues Lys723, Lys729, Lys863, and Lys867 complete this surface. Arg349, Lys410, Arg412, Lys413, and Lys416 of the C2 domain of p110 may also interact with the membrane.

4 Cancer-Specific Mutations

The cancer-associated mutations that have been identified in the ABD, C2, helical, and kinase domains of p110 α were believed to act through unrelated mechanisms (Zhao and Vogt 2008a,b) but these hypotheses were difficult to interpret in the absence of structural information. The structure of the p110 α /niSH2 suggests specific mechanisms through which these mutations increase kinase activity (Huang et al. 2007, 2008; Vogt et al. 2007).

As the ABD domain was known to interact with p85, ABD mutations Arg38Cys, Arg38His, and Arg88Gln were initially thought to disrupt the interaction between ABD and iSH2. However, the structure of the complex between ABD and iSH2 showed that these mutations are not located at the interface between the two domains (Miled et al. 2007). In the structure of the p110 α /niSH2 heterodimer, Arg38 and Arg88 are located at a contact surface between the ABD and the kinase domains, at hydrogen bonding distance (<3.2 Å) of Gln738, Asp743, and Asp746 of the N-terminal lobe of the kinase domain (Fig. 4a, b). Thus, mutations of Arg38 and Arg88 are likely to disrupt these interactions, resulting in a conformational change of the kinase domain that alters enzymatic activity.

Before the determination of the structure of the p110 α /niSH2 complex, the C2 domain was considered to be the main locus of interaction of PI3K with the membrane. Not surprisingly, mutations in the C2 domain were thought to change the affinity of p110 α for the lipid membrane (Vogt et al. 2007). In the structure of the complex, however, Asn345, which is mutated to Lys in some cancers, is within

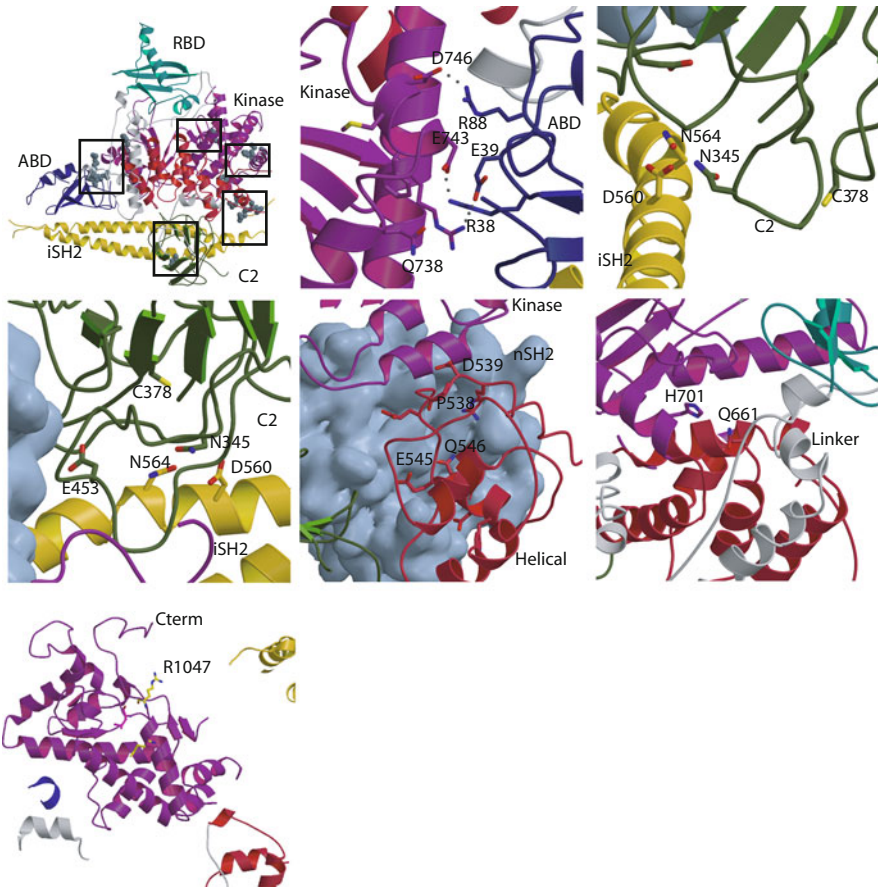


Fig. 4 Somatic mutations of p110 α identified in human cancers localize to domain interfaces. (a) Location of representative mutations within p110 α and niSH2. Amino acids mutated in cancers are shown as CPK models and framed with a *black box*. (b) ABD Arg38 and Arg88 mutations at the interface of the ABD and kinase domains. (c) C2 Asn345 mutation at the interface with iSH2. The C378R mutation is also shown. (d) C2 E453N mutation at the interface of C2 with iSH2 on one side and modeled nSH2 on the other side. (e) Mutations in the helical domain (Glu542, Glu545, and Gln 546) are located at the interface with nSH2 (*light blue surface*). (f) Helical Gln661 mutation is located across from kinase domain residue His701, which also is independently mutated. (g) Kinase Met1043 and His1047 located near the C terminal end of the protein, shown in relationship to the helical domain (*red*), the iSH2 domain (*yellow*) as observed in the p110 α H1047R/niSH2 structure

hydrogen bonding distance (2.8 Å and 3.0 Å) of Asn564 and Asp560 of iSH2, suggesting that mutation of Asn345 would disrupt the interaction of the C2 domain with iSH2 (Fig. 4a, c). This mutation may alter the regulatory effect of p85 on p110 α rather than disrupt the interaction between p110 α with the membrane. Another mutation identified in cancers, Glu453Gln, is also located at the interface between C2 and iSH2 (Fig. 4d). A recently identified mutation (Cys378Arg) most

likely increases the positive charge of the surface proposed to interact with the membrane, as it is contiguous with the surface of the iSH2 domain (Fig. 4c, d).

The two residues of the helical domain that are most frequently mutated in cancers are Glu542 and Glu545 (Fig. 4e). In the majority of cases, these two residues are mutated to Lys, causing a charge reversal. These residues as well as the less frequently mutated Gln546 are located on an exposed region of the helical domain (Fig. 4e). Biochemical studies suggested that they interact with Lys379 and Arg340 of the p85 nSH2 domain and that this interaction inhibited the activity of the catalytic subunit (Miled et al. 2007). Though nSH2 was included in the wild type p110 α /niSH2 protein complex, it was not highly ordered in the crystal. However, in the crystal structure of the same construct carrying the His1047Arg mutation, the nSH2 domain was clearly visible (Mandelker et al. 2009). In this structure, the nSH2 is located close to the interface between the kinase and the helical domain, in a manner that allows it to interact with both domains as well with the C2 domain of p110 α . Biochemical experiments showed that mutations at residues 542, 545, and 546 abrogate the inhibitory effect of nSH2 (Miled et al. 2007). The structure suggests a mechanism through which the mutations may have this effect: they could modify the interaction of nSH2 with the helical and the kinase domains in a manner similar to that achieved by binding of the phosphotyrosine residue of physiological activators to nSH2. Other examples of somatic mutations at the interface between domains are provided by those at His701 of the kinase domain and Gln661 at the helical domain (Fig. 4f).

His1047 in the kinase domain is another hot spot for somatic mutations in cancer and is associated with an unfavorable clinical prognosis breast cancers (Lai et al. 2008; Lerma et al. 2008; Kalinsky et al. 2009). It is interesting that His1047 is mutated to Arg in the majority of cases, yet arginine is normally present at the homologous position in human p110 γ (Fig. 4g). In the structure of the p110 α /niSH2 p85 complex of the wild type enzyme, His1047 is located within a helix of the C-terminal lobe of the kinase domain and is close to the C-terminal end of the activation loop, making a hydrogen bond with the main chain carbonyl of Leu956 within the activation loop. In the structure of the His1047Arg mutant with and without wortmannin (Mandelker et al. 2009) (Fig. 5), the orientation of the arginine side chain of residue 1047 is perpendicular to that of the histidine residue in the wild type. In this orientation, Arg1047 occupies a crevice in the kinase domain and points toward the membrane (Fig. 4g). Additional effects of this mutation include ordering of the C-terminal residues of p110 α 1050–1062, which were disordered in the wild type (Fig. 3a). The new orientation of this loop places some of its residues directly on the surface that was proposed to interact with the membrane (Fig. 3b). The conformation of another loop (residues 864–874) that also interacts with the membrane assumes different conformations in the wild type and the mutant (Fig. 3a, b). Taken together, these changes suggest that the gain of function that results from the His1047Arg is a result of stronger interaction of the mutant with the membrane that, in turn, provides increased accessibility to the PIP₂ substrate. This mode of increasing enzymatic activity is compatible with the observation that binding to a cognate phosphorylated peptide further increases the activity of this

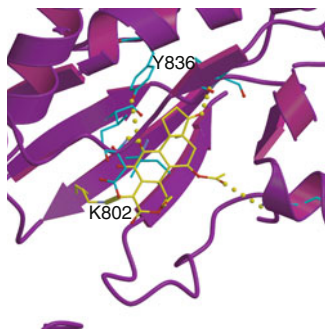


Fig. 5 Wortmannin bound to the p110 α H1047R/nSH2. The kinase domain is shown as *purple ribbons* with the wortmannin carbons in *yellow*. The covalent bond between K802 and wortmannin is shown as a *thicker line* to distinguish it from other bonds. Residues at hydrogen bonding distance Q859, Y836, V851 are shown in turquoise

mutant (Carson et al. 2008). Another less frequently observed mutation, Met1043Ile, is located on the same helix and may exert its effect through changes in the activation loop (Fig. 4g).

In addition to mutations in p110 α , mutations in p85 α have been observed, particularly in brain tumors (Parsons et al. 2008; T.C.G.A.R. 2008). Residues Asn564 and Asp560 of iSH2, which make H-bonds with Asn345, were found to be mutated. These iSH2 mutations probably affect PI3K activity by the same mechanism proposed above for the Asn345 mutations, i.e., by disrupting the interaction between the iSH2 and C2 domains.

Most other mutations in p85 α have involved truncations or deletions starting at or near residue 571 (Fig. 6). In particular, a truncation mutant known as p65, that lacks all amino acids C-terminal to residue 571 leads to constitutively activated PI3K α activity (Jimenez et al. 1998). It is possible, that residues 581–593 constrain the location of the inhibitory nSH2 domain and that the deletion removes this constraint. Based on the crystal structure of p110/nSH2, however, an alternative possibility appears more likely: truncation at residue 571 might destabilize the iSH2 coiled-coil around residues 560 and 564 that make an important contact with Asn345 of the C2 domain (Fig. 6). Thus, the effect of this truncation may also be equivalent to that of the Asn345 mutation discussed above.

Another intriguing mutation identified in p85 α is Gly376Arg. Residue 376 is within the nSH2 domain in a tightly packed volume at the interface between nSH2 and the C2 domain of p110 α . It forms a close contact with Glu365 of C2 and substitution of the glycine at residue 376 with a bulky positively charged arginine residue would disrupt this contact.

In sum, most of the p85 α mutations described to date, whether they be subtle point mutations or large deletions, appear to disrupt the interaction between nSH2 and iSH2 and the C2 domain of p110 α , thereby relieving inhibition of the kinase domain by nSH2. Others, such as His1047Arg may increase interaction with the plasma membrane.

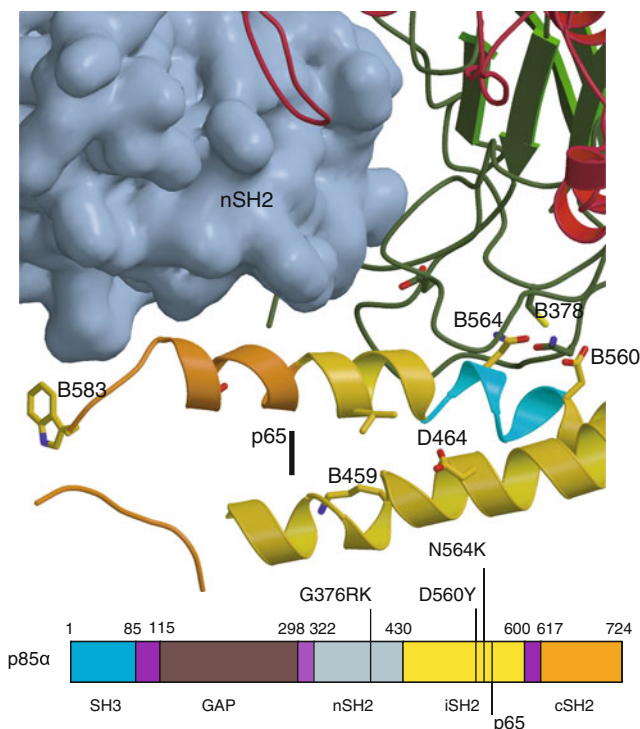


Fig. 6 Somatic mutations of p85 α identified in human cancers. (a) Modeled structure of nSH2 domain shown as a surface in relation to C2, iSH2 and helical domains. Single mutations observed in the iSH2 are shown as stick and ball representations (Lys459, Asp464, Glu560, Asn564, Trp583); the indel mutation is shown in turquoise (DKRMNS560del) and p65 (deletion from 571) is shown in orange. (b) Location of mutations with respect to the indicated domains with p85 α

5 Summary and Conclusions

PI3K α is mutated in many cancers and biochemical analyses have shown that they often result in constitutively activated enzymes. Some mutants, such as His1047Arg, can be further activated by tyrosine phosphorylated peptides derived from their physiological effectors. The increased PI3K α activity of the mutants activate downstream processes that control cell growth, survival, apoptosis, differentiation, motility, migration, and adhesion. Structural information on PI3K α has provided an initial look at possible mechanisms through which the oncogenic mutations may result in enzyme activation. Most commonly, mutations occur at the interfaces between p110 α domains or between p110 α and p85 domains, disrupting the negative regulatory influences that results from the interfacial contacts. Other mutations, such as His1047Arg, may affect the activity of PI3K α by changing the interaction of the protein with the membrane.

References

- Amzel LM et al (2008) Structural comparisons of class I phosphoinositide 3 kinases. *Nat Rev Cancer* 8:665–669
- Cantley LC (2002) The phosphoinositide 3 kinase pathway. *Science* 296(5573):1655–1657
- Carson JD et al (2008) Effects of oncogenic p110 α subunit mutations on the lipid kinase activity of phosphoinositide 3 kinase. *Biochem J* 409(2):519–524
- Escobedo JA et al (1991) cDNA cloning of a novel 85 kd protein that has SH2 domains and regulates binding of PI3 kinase to the PDGF beta receptor. *Cell* 65(1):75–82
- Fu Z et al (2004) The iSH2 domain of PI 3 kinase is a rigid tether for p110 and not a conformational switch. *Arch Biochem Biophys* 432(2):244–251
- Huang CH et al (2007) The structure of a human p110 α /p85 α complex elucidates the effects of oncogenic PI3K α mutations. *Science* 318(5857):1744–1748
- Huang CH et al (2008) Insights into the oncogenic effects of PIK3CA mutations from the structure of p110 α /p85 α . *Cell Cycle* 7:1151–1156
- Jimenez C et al (1998) Identification and characterization of a new oncogene derived from the regulatory subunit of phosphoinositide 3 kinase. *EMBO J* 17(3):743–753
- Kalinsky K et al (2009) PIK3CA mutation associates with improved outcome in breast cancer. *Clin Cancer Res* 15(16):5049–5059
- Lai YL et al (2008) PIK3CA exon 20 mutation is independently associated with a poor prognosis in breast cancer patients. *Ann Surg Oncol* 15(4):1064–1069
- Jerma E et al (2008) Exon 20 PIK3CA mutations decreases survival in aggressive (HER 2 positive) breast carcinomas. *Virchows Arch* 453(2):133–139
- Mandelker D et al (2009) A frequent kinase domain mutation that changes the interaction between PI3K α and the membrane. *PNAS* 8(9):665–669
- Miled N et al (2007) Mechanism of two classes of cancer mutations in the phosphoinositide 3 kinase catalytic subunit. *Science* 317(5835):239–242
- Otsu M et al (1991) Characterization of two 85 kd proteins that associate with receptor tyrosine kinases, middle T/pp 60c src complexes, and PI3 kinase. *Cell* 65(1):91–104
- Parsons DW et al (2008) An integrated genomic analysis of human glioblastoma multiforme. *Science* 321(5897):1807–1812
- Samuels Y et al (2004) High frequency of mutations of the PIK3CA gene in human cancers. *Science* 304(5670):554
- T.C.G.A.R. (2008) Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 455(7216):1061–1068
- Vanhaesebroeck B, Waterfield MD (1999) Signaling by distinct classes of phosphoinositide 3 kinases. *Exp Cell Res* 253(1):239–254
- Vogt PK et al (2007) Cancer specific mutations in phosphatidylinositol 3 kinase. *Trends Biochem Sci* 32(7):342–349
- Wellcome Trust Sanger Institute (2009) Catalogue of Somatic Cancer Mutations
- Yu J et al (1998a) Regulation of the p85/p110 phosphatidylinositol 3' kinase: stabilization and inhibition of the p110 α catalytic subunit by the p85 regulatory subunit. *Mol Cell Biol* 18(3):1379–1387
- Yu J, Wjasow C, Backer JM (1998b) Regulation of the p85/p110 α phosphatidylinositol 3' kinase. Distinct roles for the n terminal and c terminal SH2 domains. *J Biol Chem* 273(46): 30199–30203
- Zhao L, Vogt PK (2008a) Class I PI3K in oncogenic cellular transformation. *Oncogene* 27(41):5486–5496
- Zhao L, Vogt PK (2008b) Helical domain and kinase domain mutations in p110 α of phosphatidylinositol 3 kinase induce gain of function by different mechanisms. *Proc Natl Acad Sci USA* 105(7):2652–2657

Comparing the Roles of the p110 α and p110 β Isoforms of PI3K in Signaling and Cancer

Nina Ilić and Thomas M. Roberts

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Abstract Phosphatidylinositol-3-kinases (PI3K) are a family of enzymes that act downstream of cell surface receptors leading to activation of multiple signaling pathways regulating cellular growth, proliferation, motility, and survival. To date, most research efforts have focused on a group of PI3K-family enzymes termed class I, of which the most studied member is PI3K α . PI3K α is an oncogene frequently mutated in human cancer, as is the chief negative regulator of the pathway,

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the tumor suppressor PTEN. Recently, it has been suggested that tumors deficient for PTEN might depend on the function of another class I member, PI3K β , to sustain their transformed phenotype. Taken together, these findings provide a significant medical rationale to study the signaling cascades regulated by PI3K α and PI3K β particularly in the context of their role in the development and maintenance of human cancer. Here, we summarize the current understanding of the upstream receptor regulation of the two PI3K isoforms and their roles in cancer as well as their functional requirements in downstream signaling cascades.

1 Introduction

The phosphatidylinositol 3-kinases (PI3Ks) constitute a family of intracellular lipid kinases, which phosphorylate the 3'-hydroxyl group (D-3) of phosphatidylinositol lipids in cellular membranes either acting constitutively or in response to extracellular stimuli such as growth factors and hormones. The D-3 phosphorylated phosphoinositides serve multiple functions in the cell, regulating cellular membrane trafficking and acting as second messengers, which serve to attract cytosolic signaling proteins containing pleckstrin-homology domains (PH-domains), unique binding domains for these lipids. Once recruited to the membrane, these cytosolic PH-domain containing signaling proteins activate diverse signal transduction pathways involved in the regulation of cellular growth, survival, metabolism, migration, and vesicular trafficking (Cantley 2002; Engelman et al. 2006).

The PI3Ks are divided into three classes (I-III) based on their substrate preferences and subunit composition. *In vivo* class I PI3Ks utilize phosphatidylinositol-4,5-bisphosphate (PI-4,5-P₂) to generate phosphatidylinositol-3,4,5-trisphosphate (PIP₃), while class II members generate both phosphatidylinositol-3,4-bisphosphate (PI-3,4-P₂) from phosphatidylinositol-4-phosphate (PI-4-P) and PI-3-P from phosphatidylinositol (PI) (Engelman et al. 2006; Katso et al. 2001). Class III enzymes generate only PI-3-P from PI (reviewed in Vanhaesebroeck et al. 2001; Fruman et al. 1998).

Notably, class I PI3Ks are activated by various cell surface receptors, leading to further subdivision of this class into subfamilies IA and IB based on the classical notion that the IA members are activated upon receptor tyrosine kinase (RTK) stimulation, whereas IB are activated by G-protein coupled receptors (GPCRs) (Engelman et al. 2006; Katso et al. 2001; Vanhaesebroeck et al. 2001). In addition, the two classes exhibit structural differences: class IA members are heterodimers consisting of a p110 catalytic subunit and a p85 regulatory subunit, whereas class IB has only one member consisting of a p110 γ catalytic subunit and a p101 regulatory subunit (Vanhaesebroeck et al. 2001). There are three class IA catalytic subunit isoforms in mammals, encoded by three genes PIK3CA (p110 α), PIK3CB (p110 β), and PIK3CD (p110 δ). In addition, three genes PIK3R1, PIK3R2, and PIK3R3 encode the class IA regulatory subunits p85 α , p85 β , and p55 γ (Vanhaesebroeck et al. 1997a).

2 Class IA PI3Ks

Due to their causal involvement in the genesis of human disease, class IA has attracted the most attention of all PI3Ks. Their p85/p55 regulatory subunits bind tyrosine phosphorylated residues of activated receptor proteins or specific adaptor molecules (Engelman et al. 2006; Vanhaesebroeck et al. 2001), and thereby recruit the catalytic p110 subunits to the plasma membrane, where p110s then phosphorylate available membrane lipid PI-4,5-P₂ substrates. Although p85/p55 binding is required for recruitment of p110s to the receptor, it is generally considered that its binding has an inhibitory effect on the catalytic activity of p110 in the cytoplasm, which is relieved upon membrane recruitment (Yu et al. 1998). Additionally, p110 can bind activated Ras and Ras family members, allowing its localization to the membrane and activation of its lipid kinase activity (Katso et al. 2001). As a signal attenuating mechanism, the reverse dephosphorylation reaction converting PIP₃ to PI-4,5-P₂ is catalyzed by the phosphatase PTEN (phosphatase and tensin homologue), a tumor suppressor protein.

Among the three class IA catalytic subunits, p110 α and p110 β are ubiquitously expressed among tissues, while p110 δ is found predominantly in leukocytes (Vanhaesebroeck et al. 1997a,b; Chantry et al. 1997). Even though the primary structures of p110 β and p110 δ are more homologous to each other than to that of p110 α , the overall domain structures of all three isoforms are quite similar (Vanhaesebroeck and Waterfield 1999). Specifically, they all contain an N-terminal adaptor binding domain (ABD), followed by a Ras-binding domain (RBD), a C2 domain, helical domain, and the catalytic kinase domain. In addition, all the regulatory subunits contain two Src-homology 2 (SH2) domains, which bind the tyrosine-phosphorylated receptors and adaptor molecules, and also an inter-SH2 domain, which is necessary for interaction with the catalytic p110 subunit. The longer adapter isoform, p85, also contains an N-terminal Src-homology 3 (SH3) domain and two proline-rich regions flanking a BCR homology domain, which is thought to interact with proline rich proteins and small GTPases, respectively (Engelman et al. 2006; Katso et al. 2001).

3 Mechanisms of Activation of Class IA p110 Isoforms

3.1 *Early Studies on In Vitro p110 α / β Activation*

Due to the structural and catalytic similarities among the class IA members, there has been considerable interest in elucidating any differences in their regulation and function, particularly with regard to the two ubiquitously expressed isoforms p110 α and p110 β . Although the primary structures of p110 α and p110 β are highly

homologous, the divergence among them is found in the Ras-binding domain, which has suggested altered specificities towards different small GTP-binding proteins (Deora et al. 1998). While it remains unclear whether the non-redundant functions of these isoforms can solely be explained by differential preferences towards various Ras-family members, there are several lines of evidence indicating that the two isoforms are differentially regulated by membrane receptors (Fig. 1). Classically, both p110 α and p110 β were thought to respond to RTK activation and a plethora of work has shown that p110 α and p110 β can indeed be recruited by various RTKs in response to numerous ligands including insulin (InsR), EGF (EGFR), and PDGF (PDGFR) (Roche et al. 1998; Hooshmand-Rad et al. 2000; Park et al. 2003). Notably, there have been indications of differential preference between the two isoforms by particular RTKs and to that end, *in vitro* experiments utilizing neutralizing anti-p110 α and -p110 β antibodies have suggested that p110 α is more important for PDGF-signaling than p110 β , whereas the opposite has been demonstrated for insulin-induced actin reorganization and signaling, suggesting a potential role for p110 β in RTK signaling (Roche et al. 1998; Hooshmand-Rad et al. 2000; Asano et al. 2000). On the other hand, additional *in vitro* work suggested that both p110 α and p110 β are required for insulin response (Roche et al. 1998).

Early studies suggested that, while p110 β (like p110 α) can be activated by a synthetic phosphotyrosyl peptide (containing the YMXM motif found in RTKs), it could also be activated by the $\beta\gamma$ subunit of G-proteins, thus indicating an additional mechanism of activation by GPCRs (Kurosu et al. 1997; Maier et al. 1999; Hazeki et al. 1998). Consistent with this notion, injection of p110 β -specific neutralizing antibodies into fibroblasts inhibited DNA synthesis induced by lysophosphatidic acid (LPA), a GPCR ligand, but did not significantly affect PDGFR signaling (Roche et al. 1998). On the other hand, p110 α -specific neutralizing antibodies did not significantly affect LPA-signaling, but did inhibit PDGFR signaling (Roche et al. 1994, 1998). Additional *in vitro* work also suggested that in contrast to p110 β , p110 α could not be activated by GPCR ligands (LPA and bombesin) (Roche et al. 1994, 1998; Murga et al. 2000) and a recent study using small-molecule inhibitors of p110 β showed that it is not a major effector downstream of RTKs, but rather couples to GPCRs (Guillemet-Guibert et al. 2008). Here, inhibition of p110 β did not block downstream signaling in response to RTK ligands PDGF, insulin, and IGF-1, but did affect signaling stimulated by GPCR-ligands stromal cell-derived factor (SDF-1 α), sphingosine-1-phosphate (S1P), and LPA (Table 1).

With the development of pharmacological inhibitors of PI3K, the use of isoform-specific inhibitors suggests, in contrast to earlier studies (Roche et al. 1998; Hooshmand-Rad et al. 2000), that p110 β only plays a minor role in insulin signaling (Foukas et al. 2006). Similarly, a pharmacological study utilizing a panel of isoform-specific inhibitors indicated that p110 α is the primary insulin-responsive isoform in culture (in adipocytes and myotubes), whereas p110 β remained dispensable under these conditions (Knight et al. 2006).

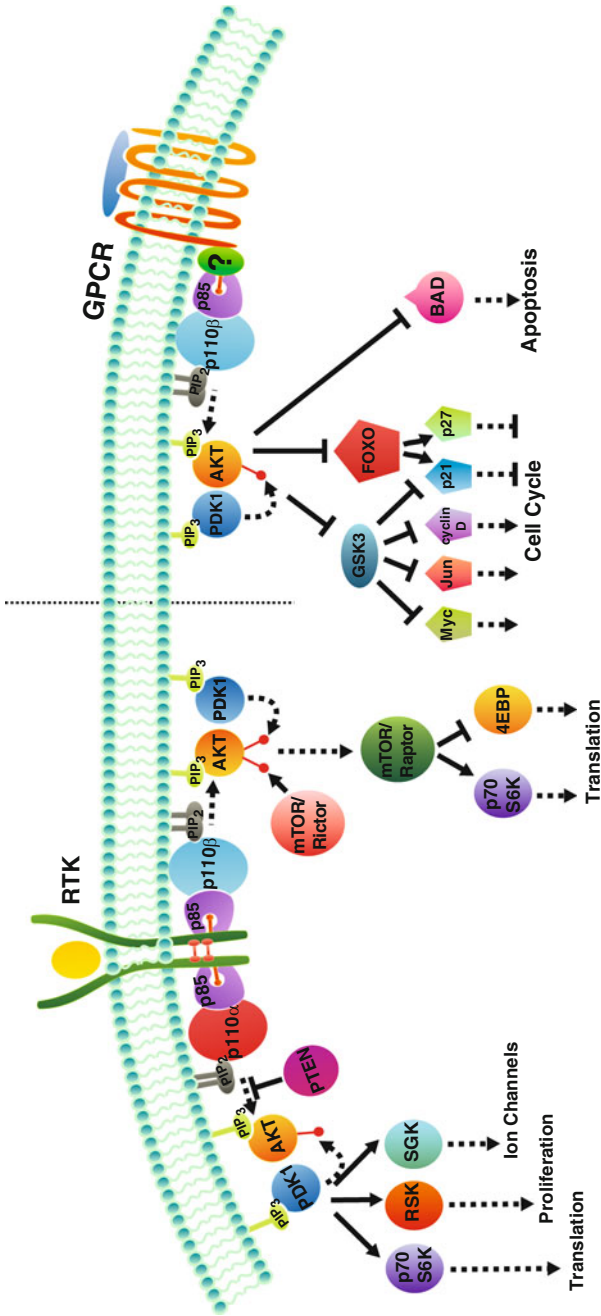


Fig. 1 Schematic view of the PI3K-pathway Upstream activation of p110 α and p110 β isoforms by receptor tyrosine kinase (RTK) or p110 β isoform by G-protein coupled receptor (GPCR) leading to downstream PI3K lipid kinase activity towards PIP₂. Membrane bound PIP₃ recruits AKT and PDK1, which leads to AKT phosphorylation and in turn suppression of GSK3 and FOXO-activities as well as mTOR pathway activation. In addition to activating AKT, PDK1 also directly phosphorylates and activates p70S6K, RSK, and SGK

Table 1 p110 isoform requirements for receptor signaling

p110 α		p110 β	
<i>In vitro</i>	<i>In vivo</i>	<i>In vitro</i>	<i>In vivo</i>
RTK activation by:			
Insulin	Insulin	Insulin	Insulin (+/)
PDGF (+/)	Leptin		
IGF 1			
EGF			
FGFR			
GPCR activation by:			
		LPA	
		S1P	
		SDF 1 α	
		Bombesin	

3.2 Studies on p110 Activation Using Engineered Mice

Gene-targeting experiments of the PI3K catalytic subunits in mice have shed additional light on upstream ligand and receptor activation requirements for p110 α and p110 β . Homozygous deletion of p110 α is embryonic lethal between E9.5 and E10.5 as a result of severe proliferative defects, and *Pik3ca*-nullizygous embryonic mouse fibroblasts fail to proliferate in culture even in the presence of growth factors (Bi et al. 1999; Foukas and Okkenhaug 2003). Similarly, mice carrying a kinase-dead knock-in mutation in p110 α (D933A) die early in embryonic development, but mice heterozygous for this allele are fertile and viable, although smaller than their wild type siblings (Foukas et al. 2006). In the heterozygous mice (p110 α ^{D933A/WT}) the relative insulin-stimulated PI3K activity (associated with the InsR and IRS-1/2 in skeletal muscle, liver, and fat tissue) is reduced by approximately 50% (as well as phosphorylation of Akt upon insulin stimulation), suggesting a critical role of p110 α in insulin signaling *in vivo*. Notably, these effects on insulin signaling are measurable despite the fact that the expression of insulin receptor (InsR) and its associated adaptor molecules IRS-1 and IRS-2, as well as the recruitment of p85 to the receptor complex remain unchanged. Similarly, in the central nervous system, specifically the hypothalamus, heterozygosity for the p110 α (D933A) allele resulted in markedly reduced IRS-1/2-associated PI3K activity in response to both insulin and leptin treatment. Importantly, insulin stimulation seemed to significantly enrich the IRS-1/2 complexes with p110 α compared to p110 β , thus resulting in the majority of the total PI3K activity at these complexes being contributed by p110 α . To bypass the issue of embryonic lethality of systemic p110 α loss-of-function, mice with a conditionally targeted *Pik3ca* allele have been generated (Zhao et al. 2006). Here, ablation of p110 α in MEFs severely reduced the response to insulin and insulin-like growth factor (IGF-1), as well as the epidermal growth factor (EGF). However, signaling response to PDGF was more moderately affected, and only at later time points than for the other RTK-ligands tested (Table 1).

Similarly, p110 β knock-out mice have also been generated, and the homozygous embryos were found to die early in embryonic development, like p110 α knockouts, while the heterozygous counterparts were viable (Bi et al. 2002; Brachmann et al. 2005). More recently, mice carrying a conditional *Pik3cb* allele were generated (Jia et al. 2008). Here, as in the p110 α conditional mouse knock-in study (Foukas et al. 2006) and the pharmacological studies (Knight et al. 2006), p110 β loss did not seem to have a major effect on signaling downstream of the InsR in the liver or in the isolated mouse embryonic fibroblasts (MEFs). Nevertheless, these animals displayed signs of impaired insulin metabolism demonstrated as increased levels of blood insulin, lower tolerance of glucose, and lower sensitivity to insulin as compared to control animals. In agreement with earlier *in vitro* work, deletion of p110 β had no negative effect on PDGF or EGF response, but did block the response to LPA stimulation (Roche et al. 1998). Knock-in mice expressing a catalytically inactive *Pik3cb*(K805R) allele (Ciraolo et al. 2008), also exhibited mild insulin resistance and partially impaired Akt activation, but showed no impairment of signaling downstream of RTK ligands (insulin, IGF-1, EGF, or PDGF) *in vitro*. In agreement with preceding work (Maier et al. 1999; Guillermet-Guibert et al. 2008; Knight et al. 2006; Jia et al. 2008), this study also demonstrated a requirement for p110 β for signaling downstream of GPCRs for LPA and S1P *in vitro* (Table 1).

While heterozygous loss of either p110 α or p110 β had no effect on insulin sensitivity, the double-heterozygous loss caused a significant impairment in the glucose tolerance test, which suggests that both p110 α and p110 β contribute to insulin response *in vivo* (Brachmann et al. 2005). However, the interpretation of these results is made difficult by the fact that the levels of the p85 regulatory subunit change dramatically in this setting, which may significantly affect insulin signaling (Ueki et al. 2003; Terauchi et al. 1999).

3.3 Unresolved Issues

Various studies to date have shown convincing evidence that p110 β mediates signaling through GPCRs (Roche et al. 1994, 1998; Kurosu et al. 1997; Maier et al. 1999; Hazeki et al. 1998; Murga et al. 2000; Guillermet-Guibert et al. 2008; Jia et al. 2008; Ciraolo et al. 2008), the precise mechanism of which still remains unclear. Since free p110 catalytic subunits have not been detected (Geering et al. 2007), it is likely that the p85 regulatory subunit participates in the heterodimer recruitment to the receptor complex. Analogous to class IB PI3K, which has previously been shown to mediate GPCR-signaling in leukocytes (Brock et al. 2003; al-Aoukaty et al. 1999; Naccache et al. 2000; Stoyanov et al. 1995; Stephens et al. 1997), it is conceivable that the regulatory subunit p85 mediates the interaction with the receptor complex by direct binding to the intracellular receptor domains. However, due to the poor homology between the class IA PI3K regulatory subunit (p85) and the class IB regulatory subunit (p101), this model seems less likely. The affinity of p85 for tyrosine-phosphorylated residues provides the

attractive alternative explanation that a cellular tyrosine-kinase mediates this interaction by providing tyrosine-phosphorylated residues that could serve as docking motifs for the regulatory subunit p85. Indeed, several members of the Src family of nonreceptor tyrosine kinases have been shown to localize to and become activated by GPCR-complexes, including receptors for LPA and bombesin (Luttrell et al. 1996; Rodriguez-Fernandez and Rozengurt 1996). Several mechanisms have been suggested as to how the Src family may be activated by GPCRs, including direct interaction of the intracellular SH3-domain binding motifs of the GPCRs with the SH3 domain of Src itself (Liu et al. 2004). Since p85 can directly interact with Src (Gentili et al. 2002; Burnham et al. 1999), this is a potential explanation of how p110 β is recruited to the GPCR complex. Additionally, Src has been shown to associate with GPCRs via its interaction with G α subunits of heteromeric G proteins (Ma et al. 2000), which Src can directly phosphorylate (Hausdorff et al. 1992), suggesting another possible mechanism of p85-p110 β recruitment to these receptors. It has also been shown that Src can directly bind β -arrestins, molecules associated with GPCRs that are involved in attenuation of the signal from the receptor and targeting it to clathrin-coated pits (Krupnick and Benovic 1998; Luttrell et al. 1999), providing yet another explanation of how p110 β may be recruited to the GPCR complex by p85-Src interaction.

Meanwhile, it remains puzzling why p110 β is found in RTK-complexes, when its ablation does not seem to significantly affect the downstream signaling (Guillemet-Guibert et al. 2008; Foukas et al. 2006; Knight et al. 2006; Jia et al. 2008; Ciraolo et al. 2008). One potential explanation is that in RTK-complexes this isoform only plays a scaffolding function. In this regard, it is worth noting, however, that three independent studies have reported that p110 β loss does impair insulin metabolism on the organismal level, even though Akt phosphorylation downstream of InsR does not seem to be affected (Brachmann et al. 2005; Jia et al. 2008; Ciraolo et al. 2008).

In addition, assuming that p110 α is the predominant isoform signaling downstream of RTKs, it is unclear why its ablation does not seem to significantly affect signaling downstream of particular RTKs, such as PDGFR (Zhao et al. 2006) or colony-stimulating growth factor receptor (CSF-1R, closely related to PDGFR) (Roche et al. 1994). Interestingly, it has been reported that p110 β can also be recruited to PDGFR (Roche et al. 1998; Hooshmand-Rad et al. 2000), where (unlike p110 α) it does not seem to affect actin reorganization upon PDGF stimulation (Roche et al. 1998; Hooshmand-Rad et al. 2000; Park et al. 2003). Several possible explanations present themselves for this apparent paradox, perhaps the simplest being that in the absence of p110 α , p110 β plays a compensatory signaling role on the level of PDGFR by taking over the signaling. This scenario could occur if approximately equal amounts of p110 α and p110 β bound to a given RTK. In this case, most of the PIP $_3$ would be produced by p110 α , which has a roughly tenfold higher specific activity than p110 β , and deletion of p110 β would have little effect on receptor signaling. Consequently, in the absence of p110 α , signaling through p110 β would be relatively weak for non-abundant receptors such as EGFR present in MEFs, while for abundant receptors such as PDGFR, the amount of p110 β bound might still be adequate to saturate downstream signaling.

4 Downstream Signaling: Acting Out Through AKT and PDK1

Signaling downstream of p110 α and p110 β involves recruitment of proteins that bind PIP₃, the shared second messenger of both isoforms. The current consensus is that proteins containing pleckstrin-homology (PH) domains directly bind PIP₃ generated in the membrane at the sites of PI3K activation. Here, the most well known of the PH-domain proteins are the serine-threonine kinases AKT (also known as protein kinase B (PKB)) and phosphoinositide-dependent protein kinase (PDK1) (Bellacosa et al. 1993; Coffey and Woodgett 1991; Jones et al. 1991) (Fig. 1). It is thought that binding of AKT and PDK1 to PIP₃ brings the two in close proximity to each other and allows for activating phosphorylation of AKT by PDK1 (Vanhaesebroeck and Alessi 2000; Alessi et al. 1997; Toker and Newton 2000). Subsequently AKT is phosphorylated by a second activating kinase termed PDK2, now believed to be a complex of mTOR (mammalian target of rapamycin) and RICTOR (TORC2 complex) (Sarbasov et al. 2005a). Activated AKT as well as PDK1 can phosphorylate a wide range of proteins, which impact cellular growth, proliferation, motility, and survival. PDK1 serves as the master regulator of the AGC-family of kinases, most of which require an additional phosphorylation event for complete activation through a parallel-acting pathway (reviewed in Mora et al. 2004; Bayascas 2008).

4.1 AKT Signaling

PI3K signaling via AKT controls the initiation of protein translation by regulating the mTOR signaling pathway. This protein signaling cascade proceeds through the tuberous sclerosis complex (TSC), which AKT inhibits via phosphorylation, followed by the continuation of signal transduction through the small G protein RheB (Ras homolog enriched in brain), and mTOR/RAPTOR (Regulatory associated protein of mTOR) complex (TORC1). The two critical downstream effectors of the mTOR pathway are S6K (p70 S6 kinase) and 4EBP (eukaryotic initiation factor 4E-binding protein), both of which when phosphorylated can promote protein synthesis and increase in cell volume (Zhao and Vogt 2008; Inoki et al. 2002, 2003; Garami et al. 2003; Tee et al. 2003; Zhang et al. 2003; Sarbasov et al. 2005b; Hanrahan and Blenis 2006; Fingar and Blenis 2004). There is also a negative feedback loop via which mTOR can attenuate the PI3K activity by phosphorylation of IRS1 (insulin-receptor substrate) by S6K (Manning 2004; Harrington et al. 2005). On the other hand, in a positive feedback loop, mTOR in a complex with RICTOR (rapamycin-insensitive companion of TOR) protein additionally activates AKT (Sarbasov et al. 2005a).

Another important target of AKT is glycogen synthase kinase 3 (GSK3) through which AKT regulates a range of different transcription factors and cell cycle entry. The phosphorylation of a constitutively active kinase GSK3 by AKT has also an

inhibitory effect. When active, GSK3 phosphorylates many transcription factors, including Myc and Jun, as well as cell cycle regulators, among which are cyclin D and p21, thereby keeping them in inactive states or promoting their proteasomal degradation (Rossig et al. 2002; Nikolakaki et al. 1993; de Groot et al. 1993; Sears et al. 2000; Gregory et al. 2003; Wei et al. 2005). However, when GSK3 activity is suppressed by AKT function, these downstream pathways are activated, underscored by their causal involvement as oncogenes.

Additionally, AKT can suppress apoptosis, working via inhibitory phosphorylation of its target, the FOXO (forkhead box O transcription factor) family of transcription factors, thereby mediating their retention in the cytosol by a formation of a complex with the 14-3-3 family of proteins and inhibiting transcription of anti-apoptotic genes normally stimulated by FOXOs, such as p27 and p21 (Brunet et al. 1999, 2001; Biggs et al. 1999; Kops et al. 1999; Takaishi et al. 1999; Tang et al. 1999; Medema et al. 2000; Seoane et al. 2004). Similarly, AKT negatively regulates the pro-apoptotic protein BAD (BCL2-antagonist of death) by generating a binding site for 14-3-3 proteins, thus barring BAD from an inhibitory interaction with BCL2 family members and allowing them to proceed with a cell survival response (Brunet et al. 2001; Zha et al. 1996; Franke and Cantley 1997).

Finally, signaling by PI3K is attenuated by dephosphorylation of PIP₃ by at least two types of phosphatases – the Src-homology 2 (SH2)-containing phosphatases (SHIP) and PTEN. Notably, these two classes of phosphatases differ by the position of the phosphate on the inositol ring that they can remove. SHIP1 and SHIP2 dephosphorylate the 5 position of PIP₃, thus generating PI-3,4-P₂; in contrast, PTEN dephosphorylates the 3 position to generate PI-4,5-P₂ (Clement et al. 2001; Lee et al. 1999; Maehama and Dixon 1999), which is the substrate for PI3K.

Since different isoforms of PI3K mediate signaling downstream of RTKs or GPCRs (or both, in case of p110β), it is intriguing to speculate that PI3K signaling effectors downstream of different receptor types might also be differentially regulated. Currently, there is almost no evidence available to suggest divergent signaling; however, the majority of studies to date rely on phosphorylation of total AKT as the major downstream read-out. Utilizing PIP₃ as a shared second messenger for all PI3K isoforms, it is possible that many of the effectors are shared as well. On the other hand, there are many PH-domain containing proteins including multiple AKT isoforms which could potentially mediate differential signaling by colocalizing to particular cellular microdomains with particular membrane receptors.

5 PI3K Isoforms in Cancer

Deregulation of the PI3K pathway is frequently found in human cancer where components of this pathway are mutated, amplified, or deleted in various tumors types. Importantly, PI3K itself frequently bears oncogenic mutations or amplifications. Specifically, the *PIK3CA*-gene, encoding p110α, undergoes activating mutations in breast (26%), endometrial (23%), ovarian (7%), urinary tract (17%),

colorectal (14%), pancreatic (8%), stomach (8%), liver (6%), and lung (3%) cancers (<http://www.sanger.ac.uk/genetics/CGP/cosmic/>). Here, the major missense mutations in *PIK3CA* are single point-mutations clustered in two regions of the gene, corresponding to the helical (E542K and E545K) and the kinase (H1047R) domain of the protein, all of which are thought to contribute to constitutive lipid kinase activity of p110 α (Samuels et al. 2004, 2005; Samuels and Ericson 2006; Ikenoue et al. 2005; Kang et al. 2005). In addition to point mutations, amplification of the *PIK3CA* gene is found in a subset of head and neck, squamous cell lung carcinoma, cervical and gastric cancers (Engelman et al. 2006).

Mutant p110 α expressed in cultured mammalian cells displays the oncogenic potential inferred from patient tumor material, demonstrated as its ability to transform primary cells as measured by anchorage-independent growth and tumor formation in xenograft experiments (Ikenoue et al. 2005; Kang et al. 2005; Zhao et al. 2005; Isakoff et al. 2005; Bader et al. 2006). Consistently, expression of oncogenic p110 α in cells and tissues drives upregulation of signaling to many downstream PI3K effectors, such as AKT, S6K, GSK, FOXOs, etc.

Notably p110 β has not been found mutant in human cancer as yet, even though it shares significant sequence homology and ubiquitous expression with p110 α . Nonetheless, its potential as an oncogene has been studied in cell culture (Zhao et al. 2005; Kang et al. 2006). In immortalized human mammary epithelial cells (HMECs), p110 β with an amino-terminal myristylation signal (for constitutive membrane localization) drives anchorage independent growth, xenograft tumor growth, and induces potent activation of downstream AKT signaling (Zhao et al. 2005). Contrary to synthetic activation by myristylation, an engineered p110 β -allele mutant at E522K, the position analogous to E545K in p110 α , fails to induce strong AKT phosphorylation or to promote anchorage independent growth in the same HMEC system (Zhao et al. 2005). In addition, when expressed in chicken embryonic fibroblasts (CEFs), wild-type p110 β induces transformation which becomes more pronounced when a myristylation sequence is added; however, downstream AKT phosphorylation was only induced by myristylation but not by overexpression alone (Kang et al. 2006; Denley et al. 2008). Interestingly, the transforming potential of wild-type p110 β is inhibited by a Ras-binding domain mutation (K230E) or by pharmacological inhibition of the MAPK pathway, suggesting the necessity of Ras binding and MAPK-activation for p110 β -induced transformation. Notably, addition of a myristylation sequence to p110 β was able to transform the cells even in the presence of the Ras-binding domain mutation (Kang et al. 2006; Denley et al. 2008), suggesting that interaction with Ras or a Ras-family member enables membrane recruitment.

5.1 *Deregulated PI3K Pathway Components*

In addition to p110 α mutations, many other pathway components, both upstream and downstream of PI3K, are altered in cancer. Among these are various upstream

RTKs necessary for PI3K membrane recruitment and activation. Some of the most frequently deregulated RTKs are HER2, overexpressed or amplified in a large fraction of breast and ovarian cancers; epidermal growth factor receptor (EGFR), amplified or constitutively activated by mutation in gliomas and lung cancers; KIT and PDGFR α , bearing activating mutations in GISTs (gastrointestinal stromal tumors) (Yuan and Cantley 2008; Moasser 2007; Sauter et al. 1996; Narita et al. 2002; Arteaga 2006; Tornillo and Terracciano 2006). Notably, the most frequently deregulated component of the pathway downstream of PI3K is the tumor suppressor PTEN, which can be found inactivated either via point mutation or deleted in human cancers (reviewed in Keniry and Parsons 2008). Loss of PTEN results in constitutive activation of the PI3K axis due to the loss of one of its major suppressors, an effect which is exacerbated by a gain of an activating mutation in an RTK or p110 α with concomitant loss of PTEN.

5.2 Targeting PI3K in Cancer

Considering the frequent oncogenic alterations in various PI3K-pathway components, this pathway has attracted substantial interest from pharmaceutical companies for therapeutic intervention with targeted therapy solutions. The rationale here is that PI3K-signaling attenuation would result in a cytotoxic or at least cytostatic effect on tumors that have acquired dependence on this pathway. Towards this goal, several class I PI3K inhibitors have entered phase I clinical trials and, in addition, compounds that exhibit isoform-specificity are emerging (reviewed in Garcia-Echeverria and Sellers 2008). However, it is not clear which PI3K-pathway component inhibition would give the most profound results when treating different types of tumors. It is likely that tumors bearing oncogenic *PIK3CA* mutations would indeed be sensitive to direct p110 α inhibition. Nonetheless, it is not yet fully substantiated if p110 α -specific inhibition would suppress tumors carrying other genetic alterations in the pathway, such as PTEN tumor-suppressor loss or conversely upstream RTK oncogene activation. To address these issues, several recent genetic studies have shed some light onto the question of which PI3K isoforms are responsible for driving several different tumor types.

5.3 p110 α as a Viable Tumor Target

Generation of conditional *PIK3CA* knock-out (Zhao et al. 2006) and kinase-dead PIK3CA (D933A) knock-in mice (Foukas et al. 2006) has provided valuable genetic tools for studying the role of p110 α in many different genetic tumor contexts. *In vitro* studies of immortalized MEFs from conditional *PIK3CA* knock-out mice suggest that p110 α is necessary for transformation of cells driven by RTKs such as insulin-like growth factor 1 receptor (IGF-1R), EGFR, and HER2, but

Table 2 p110 isoform ablation in cancer

Lesion\isoform	p110 α	p110 β
IGF 1R	✓ (<i>in vitro</i>)	Not tested
EGFR	✓ (<i>in vitro</i>)	✓ (<i>in vitro</i> , level dependent)
HER 2	✓ (<i>in vitro</i>)	✓ (<i>in vivo</i> , kinase dead)
vSRC	X (<i>in vitro</i>)	Not tested
HRAS	Not tested	✓ (<i>in vitro</i> , kinase independent)
PTEN /	X (<i>in vivo</i>)	✓ (<i>in vivo</i>)
KRAS	✓ (<i>in vivo</i> , RBS ^a mutant)	Not tested

^aRBS RAS binding site

interestingly v-Src induced transformation was not affected (Zhao et al. 2006) (Table 2). Although not yet corroborated in a genetic mouse model, this data suggests that specific targeting of p110 α with small molecules might be a therapeutic choice for tumors with RTK alterations upstream of p110 α . However, when the p110 α knock-out mice were crossed to a prostate cancer-prone mouse model driven by *Pten* loss (Jia et al. 2008), surprisingly tumor formation was found to be independent of p110 α function.

In certain tumor types, *PIK3CA* mutations are coexistent with RAS (KRAS in particular) mutations, whereas in other types they have been suggested to be mutually exclusive (reviewed in Yuan and Cantley 2008). As discussed earlier, the p110 subunits of PI3K contain a RAS-binding domain and therefore, it is important to determine if PI3K is necessary for RAS-induced tumorigenesis. To this end, it would be beneficial to determine if PI3K-inhibition would be a suitable therapy for RAS-driven tumors. To address the importance of RAS-PI3K molecular interaction, knock-in mice were generated bearing two *Pik3ca* point mutations (T280D and K227A) which prevent its interaction with endogenous RAS (Gupta et al. 2007). The homozygous MEFs isolated from these mice had significantly attenuated growth factor signaling towards the PI3K axis, particularly evident in response to EGF and fibroblast growth factor 2 (FGF2) stimulation. Furthermore, the mutant MEFs had a markedly decreased number of cells in S-phase, implying a proliferative defect induced by the loss of PI3K-RAS interaction. *In vitro*, the MEFs could not be efficiently transformed by oncogenic HRAS or EGFR, potentially highlighting the necessity for p110 α inhibition in tumors driven by these oncogenes. In addition, when these mice were crossed to mice expressing an oncogenic *Kras* allele (which normally develop lung adenocarcinoma), tumor formation was virtually completely abrogated, suggesting importance of this interaction for the initiation of KRAS-induced tumorigenesis. As a complementary approach, it would be interesting to investigate tumor formation in mice that co-express an oncogenic allele of *Pik3ca* (E545K or H1047R) and the RAS-effector mutant that does not interact with p110. Recently, the question of RAS-PI3K interaction in tumorigenesis was addressed by the use of a small molecule inhibitor NVP-BEZ235, a dual pan-PI3K and mTOR inhibitor in clinical trials (Engelman et al. 2008). In this study, a mouse model for lung adenocarcinoma was generated by expression of *Pik3ca*(H1047R) in the lung. As expected, the mice that developed tumors responded well to PI3K/mTOR inhibition, measured as very potent reduction in

lung tumor burden. Strikingly, the lung-targeted oncogenic Kras mutant mice did not show substantial tumor effects when treated with the PI3K/mTOR inhibitor. However, significant tumor shrinkage could be achieved by the combination therapy including the PI3K/mTOR inhibitor and a MEK inhibitor. Notably, the results of this pharmacological study (in contrast to the previously discussed genetic study) suggest that PI3K inhibition may not be sufficient for the treatment of *KRAS* tumors once established, which in addition may require the inhibition of the RAS-RAF-MAPK signaling pathway.

5.4 *p110 β as a Drug Target*

As most PI3K inhibitors which have therapeutic potential inhibit all class I PI3K isoforms (and not p110 α specifically) (Garcia-Echeverria and Sellers 2008), it has become exceedingly important to investigate if inhibition of isoforms other than p110 α would be beneficial in cancer therapy (as these have not been found mutant in human tumors). Since p110 β is the only other ubiquitously expressed class I isoform, p110 β mouse knock-out studies have provided useful clues towards answering this question. Notably, *in vitro* results suggest that targeted knock-out of p110 β in MEFs prevents transformation driven by mutant HRAS and mutant EGFR, suggesting necessity for p110 β in this setting (Jia et al. 2008) (Table 2). Surprisingly, molecular replacement with a kinase-dead allele of p110 β rescued most or all of the observed phenotypes by allowing for MEF transformation, suggesting a potential scaffolding role for p110 β . A possible explanation for this data is that lack of p110 β makes cells sensitive to oncogene induced stress, a condition that can largely be rescued by kinase dead allele of p110 β . In the case of the breast-targeted Her2 mouse model for breast cancer, mice with a knock-in of a kinase dead allele of p110 β showed slower tumor development than did controls (Ciraolo et al. 2008). In addition, p110 β -loss in the *Pten*-null tumor setting, in contrast to p110 α -loss, seems to prevent tumor formation in the anterior prostate, suggesting that specific inhibition of p110 β would be a reasonable therapeutic approach in PTEN-null prostate cancers (Jia et al. 2008) and that tumor formation driven by PTEN in the prostate might depend on p110 β and not p110 α . Consistent with this notion, it has been shown using shRNA approaches that knock-down of p110 α does not affect growth and colony formation of three PTEN-null human cancer cell lines (originating from prostate, brain, and breast tumors), whereas knock-down of p110 β profoundly affected their growth and signaling to the downstream AKT/mTOR pathway (Wee et al. 2008). Here, the growth retardation and signaling could not be rescued by the expression of the kinase-inactive p110 β allele. Moreover, p110 β also seemed to be required for tumor formation by these cells in nude mice, thus highlighting the role of p110 β in PTEN-deficient tumors. Nonetheless, due to the limited number of cancer cell-lines used in this study and our lack of understanding of all genetic lesions that these cancer cell lines bear, it will be important to determine if p110 β is important for tumorigenic state in all

PTEN-null tumors, or only within a subset of them where PTEN-loss co-occurs with particular other lesions. Collectively, this suggests that additional genetic mouse models need to be generated in order to obtain more definitive data on the role of p110 β in tumor formation and maintenance.

5.5 What Are the Take-Home Messages from p110-Isoform Knock-Out Studies In Vivo?

As targeted therapies for various tumor lesions are developed, genetic knock-out mouse models have become important tools in determining the use of these therapies in particular cancer settings. Thus, as discussed above, genetic ablation of p110-isoforms serves as a tool in helping to elucidate whether PI3K (and which particular PI3K isoform) should be targeted in various tumor types. However, one issue with these genetic approaches is that the ablation of p110-isoforms (i.e., conditional tissue-specific knock-out) usually takes place before a tumor driven by gain of a particular oncogene or loss of a tumor suppressor (e.g., gain of oncogenic Kras, loss of Pten, etc.) is formed (Jia et al. 2008; Cirao et al. 2008; Gupta et al. 2007). Thus, the question that can readily be answered from these types of studies is whether a particular isoform is involved in the process of tumor nucleation or initiation. Based on the *in vivo* studies available to date, it seems likely that one or both of the p110-isoforms are required for tumor initiation. However, it remains unclear if ablation of these isoforms in an already formed tumor would have an effect, or in other words, if p110-isoforms are necessary for tumor maintenance. Currently, the way to answer this question *in vivo* is either by using targeted therapies to treat the already formed tumors (provided that these therapies are available, as described in Engelman et al. (2008)) or by designing animal models where the expression of the Cre recombinase used for p110 ablation can be regulated by use of externally administered chemicals (such as doxycycline, Chin et al. 1999) independently from the mutation driving tumor formation. In addition, *in vitro* studies utilizing cancer cell lines with isoform-specific shRNA knock-down (such as Wei et al. 2005) may provide significant information as to which isoform should be inhibited in a particular setting of tumor lesions. However, in order to obtain firm conclusions from these studies, it is likely that large numbers of human cancer cell lines need to be used as this may bypass the recurring issue of poor annotation of genetic lesions present in them and provide significant statistical cohort analysis.

5.6 Kinase-Independent Roles of p110-Isoforms

Due to the suggested scaffolding (kinase-independent) role of p110 β for transformation *in vitro*, it was of considerable interest to investigate the functions of kinase-dead

alleles *in vivo*. Mice carrying a germline knock-in mutation of kinase-dead allele of p110 α (D933A) die during embryonic development (Foukas et al. 2006), thus no *in vivo* studies using these animals have been possible. In contrast, mice bearing kinase-inactive *Pik3cb*(K805R) allele survive to adulthood, however with growth retardation features (Ciraolo et al. 2008). Since classical knockout of *Pik3cb* is early embryonic lethal this data argues strongly for an important kinase-independent function for p110 β during development. When crossed to breast cancer prone Her2 knock-in mice, the compound homozygous mice develop breast tumors much more slowly than control mice, implying that the kinase activity of p110 β could be functionally necessary downstream of HER2 in human breast cancer. However, due to the lack of the corresponding p110 α kinase-dead knock-in parallel experiments (such as the model described in Foukas et al. 2006), it is yet uncertain if p110 α is necessary in Her2 tumorigenesis.

Correspondingly, it remains to be experimentally addressed if kinase activity of p110 β contributes to Pten-induced transformation *in vivo* (Jia et al. 2008) or if prostate neoplasia can be attributed to its scaffolding role alone. Since ablation of p110 β blocked the appearance of phosphorylated Akt in the prostate, it is possible that p110 β kinase activity is required for tumorigenesis. In any event, it is currently unclear how the inferred scaffolding function of p110 β may be carried out at a molecular level. One possible explanation is that p110 β functions as an adaptor protein, serving to recruit a subset of signaling molecules to RTKs. Notably, p110 β found in RTK-associated complexes exhibits low lipid kinase activity, measured as its contribution relative to co-precipitating p110 α (Guillemet-Guibert et al. 2008; Foukas et al. 2006) and hence, it may be possible that its scaffolding function is more significant than its inherent kinase function at these receptors. Results showing that transformation of p110 β -null MEFs by activated EGFR was rescued by the exogenous expression of the kinase-dead allele of p110 β despite its negligible contribution to Akt signaling downstream of EGFR are in line with this notion (Jia et al. 2008). An alternative mechanism could be that the scaffolding role of p110 β is exhibited at the level of cellular endocytosis. Specifically, p85 α -p110 β heterodimers have been found to directly bind the small GTPase Rab5 which regulates docking and fusion of early clathrin-coated endosomes during endocytosis (Christoforidis et al. 1999; Shin et al. 2005). In agreement, p110 β -null MEFs exhibit a defect in the uptake of transferrin (marker for receptor-mediated endocytosis) which could be functionally rescued by overexpression of the kinase-dead p110 β allele (Jia et al. 2008). Similarly, a defect in EGFR internalization and formation of clathrin-coated vesicles beneath the plasma membrane observed in knock-in MEFs expressing low levels of kinase-dead p110 β allele was rescued by high levels of expression of the same allele (Ciraolo et al. 2008), also suggesting a scaffolding role during endocytosis. Although not yet studied in detail, it is possible that p110 β can exhibit its scaffolding function at the level of GPCRs as well. Most studies to date have used phosphorylation of downstream AKT as a read-out of GPCR stimulation by various ligands (Guillemet-Guibert et al. 2008; Jia et al. 2008; Ciraolo et al. 2008). Notably, this particular process seems to depend on the lipid kinase activity of p110 β . Nonetheless, it would be interesting to identify an

alternative experimental read-out that could enable the assessment of p110 β kinase-independent role in GPCR-associated complexes.

6 Conclusions

Although PI3Ks are well studied enzymes, still many important questions remain to be answered as we have tried to illustrate herein. The recent animal models (Foukas et al. 2006; Jia et al. 2008; Ciraolo et al. 2008) and an ever-increasing number of sophisticated and highly isoform-specific PI3K inhibitors will serve as invaluable tools in the elucidation of the roles of PI3K isoforms during development and in disease. Of central mechanistic importance, it remains unanswered whether different PI3K isoforms (catalyzing the same chemical reaction) mediate differential downstream signaling, to what extent this may be exhibited in the context of different upstream receptor types (RTKs and GPCRs) and how signaling differs between cell-types. Pertaining to human cancer, we await conclusive determination of which PI3K isoforms are most valuable for therapeutic inhibition in different stages and types of tumors. Moreover, particular genetic lesions (such as KRAS-mutation or HER2-amplification) in combination with PI3K-mutation could lead to oncogene-dependence (tumor cell-addiction) whereby pharmacological inhibition would result in induction of apoptosis in the targeted tumor cells. Notably, several recent studies have already begun to answer this question (Jia et al. 2008; Ciraolo et al. 2008; Engelman et al. 2008; Wee et al. 2008), but still need to be complemented by reciprocal studies with remaining isoforms and performed in additional tumor genetic backgrounds relevant to human cancer.

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References

- al Aoukaty A, Rolstad B, Maghazachi AA (1999) Recruitment of pleckstrin and phosphoinositide 3 kinase gamma into the cell membranes, and their association with G beta gamma after activation of NK cells with chemokines. *J Immunol* 162:3249–3255
- Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PR, Reese CB, Cohen P (1997) Characterization of a 3 phosphoinositide dependent protein kinase which phosphorylates and activates protein kinase Balpha. *Curr Biol* 7:261–269
- Arteaga CL (2006) EGF receptor mutations in lung cancer: from humans to mice and maybe back to humans. *Cancer Cell* 9:421–423
- Asano T, Kanda A, Katagiri H, Nawano M, Ogihara T, Inukai K, Anai M, Fukushima Y, Yazaki Y, Kikuchi M, Hooshmand Rad R, Heldin CH, Oka Y, Funaki M (2000) p110beta is up regulated

- during differentiation of 3T3 L1 cells and contributes to the highly insulin responsive glucose transport activity. *J Biol Chem* 275:17671-17676
- Bader AG, Kang S, Vogt PK (2006) Cancer specific mutations in PIK3CA are oncogenic in vivo. *Proc Natl Acad Sci USA* 103:1475-1479
- Bayascas JR (2008) Dissecting the role of the 3 phosphoinositide dependent protein kinase 1 (PDK1) signalling pathways. *Cell Cycle* 7:2978-2982
- Bellacosa A, Franke TF, Gonzalez Portal ME, Datta K, Taguchi T, Gardner J, Cheng JQ, Testa JR, Tsichlis PN (1993) Structure, expression and chromosomal mapping of c-akt: relationship to v-akt and its implications. *Oncogene* 8:745-754
- Bi L, Okabe I, Bernard DJ, Wynshaw-Boris A, Nussbaum RL (1999) Proliferative defect and embryonic lethality in mice homozygous for a deletion in the p110alpha subunit of phosphoinositide 3 kinase. *J Biol Chem* 274:10963-10968
- Bi L, Okabe I, Bernard DJ, Nussbaum RL (2002) Early embryonic lethality in mice deficient in the p110beta catalytic subunit of PI 3 kinase. *Mamm Genome* 13:169-172
- Biggs WH III, Meisenhelder J, Hunter T, Cavenee WK, Arden KC (1999) Protein kinase B/Akt mediated phosphorylation promotes nuclear exclusion of the winged helix transcription factor FKHR1. *Proc Natl Acad Sci USA* 96:7421-7426
- Brachmann SM, Ueki K, Engelman JA, Kahn RC, Cantley LC (2005) Phosphoinositide 3 kinase catalytic subunit deletion and regulatory subunit deletion have opposite effects on insulin sensitivity in mice. *Mol Cell Biol* 25:1596-1607
- Brock C, Schaefer M, Reusch HP, Czupalla C, Michalke M, Spicher K, Schultz G, Nurnberg B (2003) Roles of G beta gamma in membrane recruitment and activation of p110 gamma/p101 phosphoinositide 3 kinase gamma. *J Cell Biol* 160:89-99
- Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis J, Greenberg ME (1999) Akt promotes cell survival by phosphorylating and inhibiting a Fork head transcription factor. *Cell* 96:857-868
- Brunet A, Datta SR, Greenberg ME (2001) Transcription dependent and independent control of neuronal survival by the PI3K Akt signaling pathway. *Curr Opin Neurobiol* 11:297-305
- Burnham MR, Harte MT, Bouton AH (1999) The role of SRC CAS interactions in cellular transformation: ectopic expression of the carboxy terminus of CAS inhibits SRC CAS interaction but has no effect on cellular transformation. *Mol Carcinog* 26:20-31
- Cantley LC (2002) The phosphoinositide 3 kinase pathway. *Science* 296:1655-1657
- Chantry D, Vojtek A, Kashishian A, Holtzman DA, Wood C, Gray PW, Cooper JA, Hoekstra MF (1997) p110delta, a novel phosphatidylinositol 3 kinase catalytic subunit that associates with p85 and is expressed predominantly in leukocytes. *J Biol Chem* 272:19236-19241
- Chin L, Tam A, Pomerantz J, Wong M, Holash J, Bardeesy N, Shen Q, O'Hagan R, Pantginis J, Zhou H, Horner JW II, Cordon-Cardo C, Yancopoulos GD, DePinho RA (1999) Essential role for oncogenic Ras in tumour maintenance. *Nature* 400:468-472
- Christoforidis S, Miaczynska M, Ashman K, Wilm M, Zhao L, Yip SC, Waterfield MD, Backer JM, Zerial M (1999) Phosphatidylinositol 3 OH kinases are Rab5 effectors. *Nat Cell Biol* 1:249-252
- Ciraolo E, Iezzi M, Marone R, Marengo S, Curcio C, Costa C, Azzolino O, Gonella C, Rubinetto C, Wu H, Dastru W, Martin EL, Silengo L, Altruda F, Turco E, Lanzetti L, Musiani P, Ruckle T, Rommel C, Backer JM, Forni G, Wymann MP, Hirsch E (2008) Phosphoinositide 3 kinase p110beta activity: key role in metabolism and mammary gland cancer but not development. *Sci Signal* 1:ra3
- Clement S, Krause U, Desmedt F, Tanti JF, Behrends J, Pesesse X, Sasaki T, Penninger J, Doherty M, Malaisse W, Dumont JE, Le Marchand-Brustel Y, Erneux C, Hue L, Schurmans S (2001) The lipid phosphatase SHIP2 controls insulin sensitivity. *Nature* 409:92-97
- Coffer PJ, Woodgett JR (1991) Molecular cloning and characterisation of a novel putative protein serine kinase related to the cAMP dependent and protein kinase C families. *Eur J Biochem* 201:475-481

- de Groot RP, Auwerx J, Bourouis M, Sassone Corsi P (1993) Negative regulation of Jun/AP 1: conserved function of glycogen synthase kinase 3 and the Drosophila kinase shaggy. *Oncogene* 8:841 847
- Denley A, Kang S, Karst U, Vogt PK (2008) Oncogenic signaling of class I PI3K isoforms. *Oncogene* 27:2561 2574
- Deora AA, Win T, Vanhaesebroeck B, Lander HM (1998) A redox triggered ras effector interaction. Recruitment of phosphatidylinositol 3' kinase to Ras by redox stress. *J Biol Chem* 273:29923 29928
- Engelman JA, Luo J, Cantley LC (2006) The evolution of phosphatidylinositol 3 kinases as regulators of growth and metabolism. *Nat Rev Genet* 7:606 619
- Engelman JA, Chen L, Tan X, Crosby K, Guimaraes AR, Upadhyay R, Maira M, McNamara K, Perera SA, Song Y, Chirieac LR, Kaur R, Lightbown A, Simendinger J, Li T, Padera RF, Garcia Echeverria C, Weissleder R, Mahmood U, Cantley LC, Wong KK (2008) Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. *Nat Med* 14:1351 1356
- Fingar DC, Blenis J (2004) Target of rapamycin (TOR): an integrator of nutrient and growth factor signals and coordinator of cell growth and cell cycle progression. *Oncogene* 23:3151 3171
- Foukas LC, Okkenhaug K (2003) Gene targeting reveals physiological roles and complex regulation of the phosphoinositide 3 kinases. *Arch Biochem Biophys* 414:13 18
- Foukas LC, Claret M, Pearce W, Okkenhaug K, Meek S, Peskett E, Sancho S, Smith AJ, Withers DJ, Vanhaesebroeck B (2006) Critical role for the p110 α phosphoinositide 3 OH kinase in growth and metabolic regulation. *Nature* 441:366 370
- Franke TF, Cantley LC (1997) Apoptosis. A Bad kinase makes good. *Nature* 390:116 117
- Fruman DA, Meyers RE, Cantley LC (1998) Phosphoinositide kinases. *Annu Rev Biochem* 67:481 507
- Garami A, Zwartkruis FJ, Nobukuni T, Joaquin M, Rocco M, Stocker H, Kozma SC, Hafen E, Bos JL, Thomas G (2003) Insulin activation of Rheb, a mediator of mTOR/S6K/4E BP signaling, is inhibited by TSC1 and 2. *Mol Cell* 11:1457 1466
- Garcia Echeverria C, Sellers WR (2008) Drug discovery approaches targeting the PI3K/Akt pathway in cancer. *Oncogene* 27:5511 5526
- Geering B, Cutillas PR, Nock G, Gharbi SI, Vanhaesebroeck B (2007) Class IA phosphoinositide 3 kinases are obligate p85 p110 heterodimers. *Proc Natl Acad Sci USA* 104:7809 7814
- Gentili C, Morelli S, Russo De Boland A (2002) Involvement of PI3 kinase and its association with c Src in PTH stimulated rat enterocytes. *J Cell Biochem* 86:773 783
- Gregory MA, Qi Y, Hann SR (2003) Phosphorylation by glycogen synthase kinase 3 controls c myc proteolysis and subnuclear localization. *J Biol Chem* 278:51606 51612
- Guillemet Guibert J, Bjorklof K, Salpekar A, Gonella C, Ramadani F, Bilancio A, Meek S, Smith AJ, Okkenhaug K, Vanhaesebroeck B (2008) The p110 β isoform of phosphoinositide 3 kinase signals downstream of G protein coupled receptors and is functionally redundant with p110 γ . *Proc Natl Acad Sci USA* 105:8292 8297
- Gupta S, Ramjaun AR, Haiko P, Wang Y, Warne PH, Nicke B, Nye E, Stamp G, Alitalo K, Downward J (2007) Binding of ras to phosphoinositide 3 kinase p110 α is required for ras driven tumorigenesis in mice. *Cell* 129:957 968
- Hanrahan J, Blenis J (2006) Rheb activation of mTOR and S6K1 signaling. *Methods Enzymol* 407:542 555
- Harrington LS, Findlay GM, Lamb RF (2005) Restraining PI3K: mTOR signalling goes back to the membrane. *Trends Biochem Sci* 30:35 42
- Hausdorff WP, Pitcher JA, Luttrell DK, Linder ME, Kurose H, Parsons SJ, Caron MG, Lefkowitz RJ (1992) Tyrosine phosphorylation of G protein alpha subunits by pp 60c src. *Proc Natl Acad Sci USA* 89:5720 5724
- Hazeki O, Okada T, Kurosu H, Takasuga S, Suzuki T, Katada T (1998) Activation of PI 3 kinase by G protein betagamma subunits. *Life Sci* 62:1555 1559

- Hooshmand Rad R, Hajkova L, Klint P, Karlsson R, Vanhaesebroeck B, Claesson Welsh L, Heldin CH (2000) The PI 3 kinase isoforms p110(alpha) and p110(beta) have differential roles in PDGF and insulin mediated signaling. *J Cell Sci* 113(Pt 2):207-214
- Ikenoue T, Kanai F, Hikiba Y, Obata T, Tanaka Y, Imamura J, Ohta M, Jazag A, Guleng B, Tateishi K, Asaoka Y, Matsumura M, Kawabe T, Omata M (2005) Functional analysis of PIK3CA gene mutations in human colorectal cancer. *Cancer Res* 65:4562-4567
- Inoki K, Li Y, Zhu T, Wu J, Guan KL (2002) TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nat Cell Biol* 4:648-657
- Inoki K, Li Y, Xu T, Guan KL (2003) Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes Dev* 17:1829-1834
- Isakoff SJ, Engelman JA, Irie HY, Luo J, Brachmann SM, Pearline RV, Cantley LC, Brugge JS (2005) Breast cancer associated PIK3CA mutations are oncogenic in mammary epithelial cells. *Cancer Res* 65:10992-11000
- Jia S, Liu Z, Zhang S, Liu P, Zhang L, Lee SH, Zhang J, Signoretti S, Loda M, Roberts TM, Zhao JJ (2008) Essential roles of PI(3)K p110beta in cell growth, metabolism and tumorigenesis. *Nature* 454:776-779
- Jones PF, Jakubowicz T, Pitossi FJ, Maurer F, Hemmings BA (1991) Molecular cloning and identification of a serine/threonine protein kinase of the second messenger subfamily. *Proc Natl Acad Sci USA* 88:4171-4175
- Kang S, Bader AG, Vogt PK (2005) Phosphatidylinositol 3 kinase mutations identified in human cancer are oncogenic. *Proc Natl Acad Sci USA* 102:802-807
- Kang S, Denley A, Vanhaesebroeck B, Vogt PK (2006) Oncogenic transformation induced by the p110beta, gamma, and delta isoforms of class I phosphoinositide 3 kinase. *Proc Natl Acad Sci USA* 103:1289-1294
- Katso R, Okkenhaug K, Ahmadi K, White S, Timms J, Waterfield MD (2001) Cellular function of phosphoinositide 3 kinases: implications for development, homeostasis, and cancer. *Annu Rev Cell Dev Biol* 17:615-675
- Keniry M, Parsons R (2008) The role of PTEN signaling perturbations in cancer and in targeted therapy. *Oncogene* 27:5477-5485
- Knight ZA, Gonzalez B, Feldman ME, Zunder ER, Goldenberg DD, Williams O, Loewith R, Stokoe D, Balla A, Toth B, Balla T, Weiss WA, Williams RL, Shokat KM (2006) A pharmacological map of the PI3 K family defines a role for p110alpha in insulin signaling. *Cell* 125:733-747
- Kops GJ, de Ruyter ND, De Vries Smits AM, Powell DR, Bos JL, Burgering BM (1999) Direct control of the Forkhead transcription factor AFX by protein kinase B. *Nature* 398:630-634
- Krupnick JG, Benovic JL (1998) The role of receptor kinases and arrestins in G protein coupled receptor regulation. *Annu Rev Pharmacol Toxicol* 38:289-319
- Kurosu H, Maehama T, Okada T, Yamamoto T, Hoshino S, Fukui Y, Ui M, Hazeki O, Katada T (1997) Heterodimeric phosphoinositide 3 kinase consisting of p85 and p110beta is synergistically activated by the betagamma subunits of G proteins and phosphotyrosyl peptide. *J Biol Chem* 272:24252-24256
- Lee JO, Yang H, Georgescu MM, Di Cristofano A, Maehama T, Shi Y, Dixon JE, Pandolfi P, Pavletich NP (1999) Crystal structure of the PTEN tumor suppressor: implications for its phosphoinositide phosphatase activity and membrane association. *Cell* 99:323-334
- Liu J, Liao Z, Camden J, Griffin KD, Garrad RC, Santiago Perez LI, Gonzalez FA, Seye CI, Weisman GA, Erb L (2004) Src homology 3 binding sites in the P2Y2 nucleotide receptor interact with Src and regulate activities of Src, proline rich tyrosine kinase 2, and growth factor receptors. *J Biol Chem* 279:8212-8218
- Luttrell LM, Hawes BE, van Biesen T, Luttrell DK, Lansing TJ, Lefkowitz RJ (1996) Role of c Src tyrosine kinase in G protein coupled receptor and Gbetagamma subunit mediated activation of mitogen activated protein kinases. *J Biol Chem* 271:19443-19450
- Luttrell LM, Ferguson SS, Daaka Y, Miller WE, Maudsley S, Della Rocca GJ, Lin F, Kawakatsu H, Owada K, Luttrell DK, Caron MG, Lefkowitz RJ (1999) Beta arrestin dependent formation of beta2 adrenergic receptor Src protein kinase complexes. *Science* 283:655-661

- Ma YC, Huang J, Ali S, Lowry W, Huang XY (2000) Src tyrosine kinase is a novel direct effector of G proteins. *Cell* 102:635-646
- Maehama T, Dixon JE (1999) PTEN: a tumour suppressor that functions as a phospholipid phosphatase. *Trends Cell Biol* 9:125-128
- Maier U, Babich A, Nurnberg B (1999) Roles of non catalytic subunits in gbetagamma induced activation of class I phosphoinositide 3 kinase isoforms beta and gamma. *J Biol Chem* 274:29311-29317
- Manning BD (2004) Balancing Akt with S6K: implications for both metabolic diseases and tumorigenesis. *J Cell Biol* 167:399-403
- Medema RH, Kops GJ, Bos JL, Burgering BM (2000) AFX like Forkhead transcription factors mediate cell cycle regulation by Ras and PKB through p27kip1. *Nature* 404:782-787
- Moasser MM (2007) The oncogene HER2: its signaling and transforming functions and its role in human cancer pathogenesis. *Oncogene* 26:6469-6487
- Mora A, Komander D, van Aalten DM, Alessi DR (2004) PDK1, the master regulator of AGC kinase signal transduction. *Semin Cell Dev Biol* 15:161-170
- Murga C, Fukuhara S, Gutkind JS (2000) A novel role for phosphatidylinositol 3 kinase beta in signaling from G protein coupled receptors to Akt. *J Biol Chem* 275:12069-12073
- Naccache PH, Levasseur S, Lachance G, Chakravarti S, Bourgoin SG, McColl SR (2000) Stimulation of human neutrophils by chemotactic factors is associated with the activation of phosphatidylinositol 3 kinase gamma. *J Biol Chem* 275:23636-23641
- Narita Y, Nagane M, Mishima K, Huang HJ, Furnari FB, Cavenee WK (2002) Mutant epidermal growth factor receptor signaling down regulates p27 through activation of the phosphatidylinositol 3 kinase/Akt pathway in glioblastomas. *Cancer Res* 62:6764-6769
- Nikolakaki E, Coffey PJ, Hemelsoet R, Woodgett JR, Defize LH (1993) Glycogen synthase kinase 3 phosphorylates Jun family members in vitro and negatively regulates their transactivating potential in intact cells. *Oncogene* 8:833-840
- Park CS, Schneider IC, Haugh JM (2003) Kinetic analysis of platelet derived growth factor receptor/phosphoinositide 3 kinase/Akt signaling in fibroblasts. *J Biol Chem* 278:37064-37072
- Roche S, Koegl M, Courtneidge SA (1994) The phosphatidylinositol 3 kinase alpha is required for DNA synthesis induced by some, but not all, growth factors. *Proc Natl Acad Sci USA* 91:9185-9189
- Roche S, Downward J, Raynal P, Courtneidge SA (1998) A function for phosphatidylinositol 3 kinase beta (p85alpha p110beta) in fibroblasts during mitogenesis: requirement for insulin and lysophosphatidic acid mediated signal transduction. *Mol Cell Biol* 18:7119-7129
- Rodriguez Fernandez JL, Rozengurt E (1996) Bombesin, bradykinin, vasopressin, and phorbol esters rapidly and transiently activate Src family tyrosine kinases in Swiss 3T3 cells. Dissociation from tyrosine phosphorylation of p125 focal adhesion kinase. *J Biol Chem* 271:27895-27901
- Rossig L, Badorff C, Holzmann Y, Zeiher AM, Dimmeler S (2002) Glycogen synthase kinase 3 couples AKT dependent signaling to the regulation of p21Cip1 degradation. *J Biol Chem* 277:9684-9689
- Samuels Y, Ericson K (2006) Oncogenic PI3K and its role in cancer. *Curr Opin Oncol* 18:77-82
- Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell SM, Riggins GJ, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Velculescu VE (2004) High frequency of mutations of the PIK3CA gene in human cancers. *Science* 304:554
- Samuels Y, Diaz LA Jr, Schmidt Kittler O, Cummins JM, DeLong L, Cheong I, Rago C, Huso DL, Lengauer C, Kinzler KW, Vogelstein B, Velculescu VE (2005) Mutant PIK3CA promotes cell growth and invasion of human cancer cells. *Cancer Cell* 7:561-573
- Sarbassov DD, Guertin DA, Ali SM, Sabatini DM (2005a) Phosphorylation and regulation of Akt/PKB by the rictor mTOR complex. *Science* 307:1098-1101
- Sarbassov DD, Ali SM, Sabatini DM (2005b) Growing roles for the mTOR pathway. *Curr Opin Cell Biol* 17:596-603

- Sauter G, Maeda T, Waldman FM, Davis RL, Feuerstein BG (1996) Patterns of epidermal growth factor receptor amplification in malignant gliomas. *Am J Pathol* 148:1047-1053
- Sears R, Nuckolls F, Haura E, Taya Y, Tamai K, Nevins JR (2000) Multiple Ras dependent phosphorylation pathways regulate Myc protein stability. *Genes Dev* 14:2501-2514
- Seoane J, Le HV, Shen L, Anderson SA, Massague J (2004) Integration of Smad and forkhead pathways in the control of neuroepithelial and glioblastoma cell proliferation. *Cell* 117:211-223
- Shin HW, Hayashi M, Christoforidis S, Lacas Gervais S, Hoepfner S, Wenk MR, Modregger J, Uttenweiler Joseph S, Wilm M, Nystuen A, Frankel WN, Solimena M, De Camilli P, Zerial M (2005) An enzymatic cascade of Rab5 effectors regulates phosphoinositide turnover in the endocytic pathway. *J Cell Biol* 170:607-618
- Stephens LR, Eguinoa A, Erdjument Bromage H, Lui M, Cooke F, Coadwell J, Smrcka AS, Thelen M, Cadwallader K, Tempst P, Hawkins PT (1997) The G beta gamma sensitivity of a PI3K is dependent upon a tightly associated adaptor, p101. *Cell* 89:105-114
- Stoyanov B, Volinia S, Hanck T, Rubio I, Loubtchenkov M, Malek D, Stoyanova S, Vanhaesebroeck B, Dhand R, Nurnberg B et al (1995) Cloning and characterization of a G protein activated human phosphoinositide 3 kinase. *Science* 269:690-693
- Takaishi H, Konishi H, Matsuzaki H, Ono Y, Shirai Y, Saito N, Kitamura T, Ogawa W, Kasuga M, Kikkawa U, Nishizuka Y (1999) Regulation of nuclear translocation of forkhead transcription factor AFX by protein kinase B. *Proc Natl Acad Sci USA* 96:11836-11841
- Tang ED, Nunez G, Barr FG, Guan KL (1999) Negative regulation of the forkhead transcription factor FKHR by Akt. *J Biol Chem* 274:16741-16746
- Tee AR, Manning BD, Roux PP, Cantley LC, Blenis J (2003) Tuberous sclerosis complex gene products, Tuberin and Hamartin, control mTOR signaling by acting as a GTPase activating protein complex toward Rheb. *Curr Biol* 13:1259-1268
- Terauchi Y, Tsuji Y, Satoh S, Minoura H, Murakami K, Okuno A, Inukai K, Asano T, Kaburagi Y, Ueki K, Nakajima H, Hanafusa T, Matsuzawa Y, Sekihara H, Yin Y, Barrett JC, Oda H, Ishikawa T, Akanuma Y, Komuro I, Suzuki M, Yamamura K, Kodama T, Suzuki H, Yamamura K, Kodama T, Suzuki H, Koyasu S, Aizawa S, Tobe K, Fukui Y, Yazaki Y, Kadowaki T (1999) Increased insulin sensitivity and hypoglycaemia in mice lacking the p85 alpha subunit of phosphoinositide 3 kinase. *Nat Genet* 21:230-235
- Toker A, Newton AC (2000) Cellular signaling: pivoting around PDK 1. *Cell* 103:185-188
- Tornillo L, Terracciano LM (2006) An update on molecular genetics of gastrointestinal stromal tumours. *J Clin Pathol* 59:557-563
- Ueki K, Fruman DA, Yballe CM, Fasshauer M, Klein J, Asano T, Cantley LC, Kahn CR (2003) Positive and negative roles of p85 alpha and p85 beta regulatory subunits of phosphoinositide 3 kinase in insulin signaling. *J Biol Chem* 278:48453-48466
- Vanhaesebroeck B, Alessi DR (2000) The PI3K PDK1 connection: more than just a road to PKB. *Biochem J* 346(Pt 3):561-576
- Vanhaesebroeck B, Waterfield MD (1999) Signaling by distinct classes of phosphoinositide 3 kinases. *Exp Cell Res* 253:239-254
- Vanhaesebroeck B, Leever SJ, Panayotou G, Waterfield MD (1997a) Phosphoinositide 3 kinases: a conserved family of signal transducers. *Trends Biochem Sci* 22:267-272
- Vanhaesebroeck B, Welham MJ, Kotani K, Stein R, Wame PH, Zvelebil MJ, Higashi K, Volinia S, Downward J, Waterfield MD (1997b) PI10delta, a novel phosphoinositide 3 kinase in leukocytes. *Proc Natl Acad Sci USA* 94:4330-4335
- Vanhaesebroeck B, Leever SJ, Ahmadi K, Timms J, Katso R, Driscoll PC, Woscholski R, Parker PJ, Waterfield MD (2001) Synthesis and function of 3 phosphorylated inositol lipids. *Annu Rev Biochem* 70:535-602
- Wee S, Wiederschain D, Maira SM, Loo A, Miller C, deBeaumont R, Stegmeier F, Yao YM, Lengauer C (2008) PTEN deficient cancers depend on PIK3CB. *Proc Natl Acad Sci USA* 105:13057-13062

- Wei W, Jin J, Schlisio S, Harper JW, Kaelin WG Jr (2005) The v Jun point mutation allows c Jun to escape GSK3 dependent recognition and destruction by the Fbw7 ubiquitin ligase. *Cancer Cell* 8:25-33
- Yu J, Zhang Y, McIlroy J, Rordorf Nikolic T, Orr GA, Backer JM (1998) Regulation of the p85/p110 phosphatidylinositol 3' kinase: stabilization and inhibition of the p110 α catalytic subunit by the p85 regulatory subunit. *Mol Cell Biol* 18:1379-1387
- Yuan TL, Cantley LC (2008) PI3K pathway alterations in cancer: variations on a theme. *Oncogene* 27:5497-5510
- Zha J, Harada H, Yang E, Jockel J, Korsmeyer SJ (1996) Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3 not BCL-X(L). *Cell* 87:619-628
- Zhang Y, Gao X, Saucedo LJ, Ru B, Edgar BA, Pan D (2003) Rheb is a direct target of the tuberous sclerosis tumour suppressor proteins. *Nat Cell Biol* 5:578-581
- Zhao L, Vogt PK (2008) Class I PI3K in oncogenic cellular transformation. *Oncogene* 27:5486-5496
- Zhao JJ, Liu Z, Wang L, Shin E, Loda MF, Roberts TM (2005) The oncogenic properties of mutant p110 α and p110 β phosphatidylinositol 3 kinases in human mammary epithelial cells. *Proc Natl Acad Sci USA* 102:18443-18448
- Zhao JJ, Cheng H, Jia S, Wang L, Gjoerup OV, Mikami A, Roberts TM (2006) The p110 α isoform of PI3K is essential for proper growth factor signaling and oncogenic transformation. *Proc Natl Acad Sci USA* 103:16296-16300

Phosphatidylinositol 3-Kinase: The Oncoprotein

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Abstract The catalytic and regulatory subunits of class I phosphoinositide 3-kinase (PI3K) have oncogenic potential. The catalytic subunit p110 α and the regulatory subunit p85 undergo cancer-specific gain-of-function mutations that lead to enhanced enzymatic activity, ability to signal constitutively, and oncogenicity. The β , γ , and δ isoforms of p110 are cell-transforming as overexpressed wild-type proteins. Class I PI3Ks have the unique ability to generate phosphoinositide 3,4,5 trisphosphate (PIP₃). Class II and class III PI3Ks lack this ability. Genetic and cell biological evidence suggests that PIP₃ is essential for PI3K-mediated oncogenicity, explaining why class II and class III enzymes have not been linked to cancer. Mutational analysis reveals the existence of at least two distinct molecular mechanisms for the gain of function seen with cancer-specific mutations in p110 α ; one causing independence from upstream receptor tyrosine kinases, the other inducing

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independence from Ras. An essential component of the oncogenic signal that is initiated by PI3K is the TOR (target of rapamycin) kinase. TOR is an integrator of growth and of metabolic inputs. In complex with the raptor protein (TORC1), it controls cap-dependent translation, and this function is essential for PI3K-initiated oncogenesis.

1 Phosphatidylinositol 3-Kinases and Cancer

The phosphatidylinositol 3-kinases (PI3Ks) are grouped into three classes (I–III) which differ in structure and function (Fruman et al. 1998; Vanhaesebroeck et al. 1997; Vanhaesebroeck and Waterfield 1999). Class I enzymes have been intensely studied and have emerged as promising drug targets in cancer and in immune disorders (Brachmann et al. 2009; Ghigo and Hirsch 2008). They are heterodimeric enzymes consisting of a catalytic subunit p110 that associates with a regulatory subunit. The vertebrate genome codes for four isoforms of p110 (α , β , γ , δ). Several regulatory subunits have been identified. For p110 α , β , and δ , the regulatory subunit p85 is the most prevalent. p110 γ associates with separate, specific regulatory subunits of which p101 is the most common (Stephens et al. 1997). Class I PI3Ks occur as obligatory dimers in the cell (Geering et al. 2007). Regulatory and catalytic subunits show distinct structure-function domains that are illustrated in Fig. 1 (Amzel et al. 2008; Huang et al. 2007; Walker et al. 1999). Class I PI3Ks act on three substrates, the nonphosphorylated phosphatidylinositol, PI, the inositol monophosphate (PI(4)P) and the bisphosphate (PI(4,5)P₂), to add a phosphate group in the D-3 position of the inositol ring and generate PI(3)P, PI(3,4)P₂ and PI(3,4,5)P₃, respectively (Carpenter et al. 1990). The latter, also referred to as PIP₃, functions as an important second messenger in the cell and is the predominant mediator of PI3K

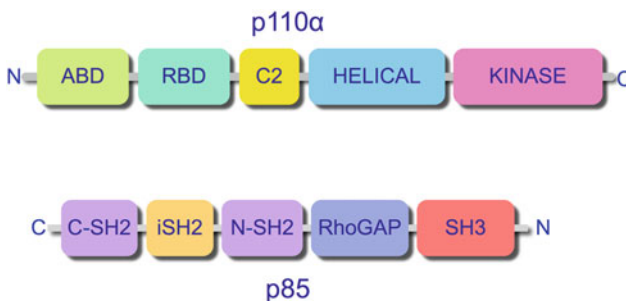


Fig. 1 Domain structure of catalytic and regulatory subunits of PI3K. *ABD* adaptor binding domain; *RBD* RAS binding domain; *C2* C2 domain; *HELICAL* helical domain; *KINASE* kinase domain; *C-SH2* C terminal SH2 domain; *iSH2* inter SH2 domain; *N-SH2* N terminal SH2 domain; *RhoGAP* Rho GTPase activating protein homology domain; *SH3* SH3 domain

activity. The phosphatase PTEN (phosphatase and tensin homolog deleted on chromosome 10) removes the phosphate group from the D-3 position of phosphatidylinositol, acting as the direct catalytic antagonist of PI3K (Li et al. 1997; Maehama and Dixon 1998).

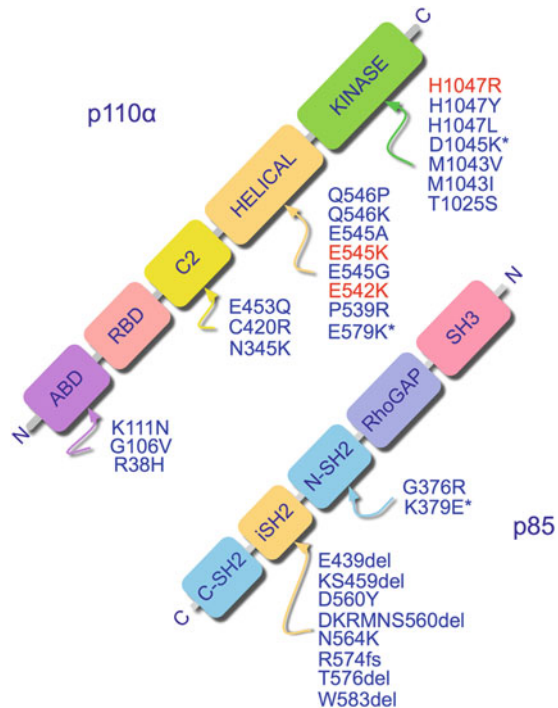
Class I PI3Ks have a long history of association with cancer (Yuan and Cantley 2008; Zhao and Roberts 2006; Zhao and Vogt 2008a). Extensive studies in the 1980s have documented a tight link of PI3K activity with tyrosine kinase oncoproteins and with the polyoma virus middle T oncoprotein (Sugimoto et al. 1984; Whitman et al. 1985). A representative interaction of this type is the binding of middle T to the Src oncoprotein, leading to an activation of the Src kinase which results in the phosphorylation of several tyrosine residues on middle T and the subsequent recruitment and activation of the p85-p110 α dimer (Courtneidge and Smith 1984; Utermark et al. 2007). The cell-transforming activity of tyrosine kinase oncoproteins is correlated with their ability to associate with PI3K (Engelman et al. 2006; Kaplan et al. 1989; Schaffhausen and Roberts 2009). In 1997, the gene encoding the p110 α catalytic subunit of PI3K was identified as the cell-derived oncogene in an avian retrovirus and shown to be constitutively activated by N-terminal fusion to viral sequences (Chang et al. 1997; Aoki et al. 2000). The isolation of this avian retrovirus documented the direct oncogenic potential of p110 α .

2 Cancer-Specific Mutations in PI3K

In 2004, the discovery of cancer-specific mutations in *PIK3CA*, the gene encoding the catalytic subunit p110 of PI3K, put PI3Ks in the limelight as clinically relevant oncoproteins and as drug targets (Liu and Roberts 2006; Samuels and Velculescu 2004; Samuels et al. 2004; Stephens et al. 2005). Mutations have now been identified in the genes coding for both subunits of PI3K, in *PIK3CA* and in *PIK3RI*, the gene encoding p85 (Fig. 2) (Cancer Genome Atlas Research Network 2008; Samuels et al. 2004). These mutations occur at frequencies extending from 5 to 25% in several common cancers, including cancers of the breast, endometrium, and the large intestine (<http://www.sanger.ac.uk>). The PI3K antagonist PTEN functions as an important tumor suppressor and is frequently inactivated by mutation or deletion in cancer (Di Cristofano and Pandolfi 2000).

The *PIK3CA* mutations are concentrated in three hot spots in the coding sequence (Samuels et al. 2004). Two of these hot spots are located in the helical domain of p110 α , and one is situated in the catalytic domain. These hot spot mutations are single nucleotide substitutions that lead to the amino acid substitutions: E542K, E545K and H1047R (Samuels et al. 2004). The preferential mapping of cancer-specific mutations to hot spots suggested immediately a strong positive selection for such mutations, possibly reflecting a powerful replicative advantage of mutant-carrying cells. Studies of the mutant proteins rapidly revealed a mutation-induced gain of function as compared to the wild-type enzyme. The PIP₃-generating

Fig. 2 Cancer derived and engineered gain of function mutations in p110 α and in p85. p110 α : The hot spot mutations are in *red*, rare mutations in *blue*; engineered gain of function mutations are marked by an *asterisk*. p85: The engineered gain of function mutation is marked by an *asterisk*



lipid kinase activity of the mutants is increased several fold (Carson et al. 2008; Chaussade et al. 2009; Ikenoue et al. 2005; Kang et al. 2005; Sugita et al. 2008; Zhao et al. 2005). Downstream signaling is no longer dependent on upstream stimulation by growth factors. It is constitutive and operates in serum-starved cells. This downstream signaling manifests itself in the phosphorylation of AKT (murine thymoma viral oncoprotein homolog) at T308 and S473, of the eukaryotic initiation factor 4E-binding protein (4E-BP) at T37 and T46 and of p70 S6 kinase (S6K) at T389. The three hot spot mutations activate the oncogenic potential of p110 α . Expression of wild-type p110 α does not detectably affect the growth behavior and the morphology of the cell. In contrast, expression of the hot spot mutants induces oncogenic transformation in avian and in mammalian cell culture (Ikenoue et al. 2005; Isakoff et al. 2005; Kang et al. 2005; Zhang et al. 2008; Zhao et al. 2005). The transformed cells are also tumorigenic in animal model systems (Bader et al. 2006; Zhao et al. 2005). In a mouse model, transgenic expression of the H1047R mutant p110 α in the lung induces adenocarcinomas (Engelman et al. 2008). These data on enhanced enzymatic activity, constitutive downstream signaling and oncogenic potency strongly suggest that the hot spot mutations function as “drivers” in human cancer, responsible for at least part of the oncogenic phenotype of the cancer cell.

The hot spot mutations account for about 80% of the mutated *PIK3CA* genes in cancer. But there are also numerous cancer-specific mutations that occur at lower frequencies. Most of these are again single nucleotide substitutions, but recently two in-frame deletions have also been identified (Cancer Genome Atlas Research Network 2008). An investigation of seventeen rare point mutations led to the surprising finding that most of these (16 out of 17) also show a gain of function (Gymnopoulos et al. 2007). However, compared with the hot spot mutants, the rare mutations induce smaller gains of function. The mutant proteins show lower enzymatic activity, mediate lower levels of downstream phosphorylation and induce decreased oncogenic transformation in cell culture as measured by the number of transformed cell foci per ng of DNA. These lesser gains of function may explain the low frequencies at which such mutants are found in cancer. The broad distribution of rare, cancer-specific mutations over almost the entire coding sequence of p110 α , with the notable and so far unexplained exception of the RAS-binding domain, raised the possibility that any random mutation may induce a gain of function, perhaps by triggering a conformational change. However, several random mutations introduced into *PIK3CA* had no phenotype, suggesting that cancer-specific mutations, no matter how rare, are still the result of positive selection (Gymnopoulos et al. 2007).

The mutations in *PIK3R1* are also clustered (Cancer Genome Atlas Research Network 2008). Most occur within a stretch of six residues (560–565) located in the inter-SH2 domain of p85 (Cancer Genome Atlas Research Network 2008). This portion of p85 includes the contact points with residues in the C2 domain of p110 α . The mutations in *PIK3R1* interfere with the proper binding to p110 α , relieving an inhibitory interaction. Several of these mutations in the inter-SH2 domain of p85 have recently been shown to induce a gain of function in PI3K including enhanced signaling to Akt, stimulation of cell replication and oncogenic transformation (Jaiswal et al. 2009; Wu et al. 2009; Sun et al. 2010, submitted). A cancer-derived *PIK3R1* mutation in the N-terminal SH2 domain of p85 (G376R) may reduce the inhibitory interaction with the helical domain of p110 α as is the case with the engineered p85 mutation K379E (Sun et al. 2010, submitted). Thus, the mutations in the inter-SH2 domain of p85 may be functionally equivalent to the mutations in the C2 domain of p110 α , and the p85 mutations in the N-terminal SH2 domain may have the same effect as the helical domain mutations in p110 α .

The map of the gain-of-function mutations on the structure of p110 α (Amzel et al. 2008; Huang et al. 2007, 2008; Miled et al. 2007) reveals two properties that are shared by several mutants: location on the surface of the protein and a change from an acidic to a basic amino acid. This observation suggests that many of the gain-of-function mutations change the surface properties of the enzyme, probably affecting the interaction with other proteins or with membranes. In fact, of three engineered mutants inducing an acidic to basic change on the protein surface, two showed oncogenic activity (Gymnopoulos et al. 2007).

3 Several Molecular Mechanisms Can Induce a Gain of Function in p110

The occurrence of gain-of-function mutations distributed over several domains of p110 α raises the question of the molecular mechanism responsible for increased activity. Do all these mutants operate by the same mechanism, or are there several distinct ways of enhancing p110 α function? The available evidence strongly favors the existence of several molecular mechanisms leading to a gain of function. The combination of two hot spot mutations, one from the helical and the other from the kinase domain, in one protein has a strong synergistic effect on p110 α activity. In contrast, introducing both helical domain mutations into the same molecule results in an only moderately additive effect (Zhao and Vogt 2008b). These results suggest that helical and kinase domain mutations work by different mechanisms that can cooperate. Several additional mutant combinations have been studied. Combinations of mutations located in different domains of p110 α often show a synergistic effect, but combinations of mutations in the same domain are merely additive. There is also one pair of mutations, E545K/Y1021C, that shows loss of function when introduced in the same molecule, indicating incompatibility of the combined mutation-induced changes with p110 α function (Gymnopoulos and Vogt 2009, to be submitted).

The distinction between helical and kinase domain mutations is further illuminated by investigations that explore the interactions of the mutant proteins with the p85 regulatory subunit and with RAS (Zhao and Vogt 2008b). An N-terminal deletion of p110 α that still permits expression of the protein but eliminates binding to p85, has contrasting effects on helical and kinase domain mutants. The oncogenic activity of the kinase domain mutant that lacks p85 binding is completely inactivated, and its downstream signaling is greatly reduced. The two helical domain mutants are much less affected by a lack of p85 binding. Their oncogenic activity in cell culture is only moderately reduced, and the effect on signaling is also minor. For wild-type p110 α , deletion of the p85-binding domain has an activating effect, resulting in constitutive signaling and oncogenicity (Zhao et al. 2005). This somewhat surprising observation is explained by the fact that in cells devoid of upstream signaling, the p85-p110 α interaction is both inhibitory and stabilizing for p110 α . Upon growth factor stimulation, the SH2 domains of p85 interact with phosphorylated tyrosine on upstream signaling molecules, relieving the inhibition on p110 α . Deletion of the p85-binding domain has a similar disinhibitory effect on p110 α and reveals its latent oncogenic activity and signaling potential.

A mutational inactivation of the ability of p110 α to interact with RAS has the opposite effect on the hot spot mutants. Interference with RAS binding decreases the oncogenicity of the helical domain mutants and drastically diminishes their downstream signaling. The kinase domain mutant is largely independent of RAS binding. Its oncogenicity is preserved in the absence of RAS binding, and signaling to AKT is only mildly affected. Complementary data have emerged from a study of mutant enzyme kinetics (Chaussade et al. 2009). The V_{\max} values of the hot spot

p110 α mutants are significantly above that of the wild-type. Wild-type p110 α can be activated by a PDGFR (platelet-derived growth factor receptor)-derived diphosphoryl peptide (Cuevas et al. 2001; Shekar et al. 2005). The phosphorylated tyrosines of this peptide interact with the N-terminal SH2 domain of p85 and thereby alleviate p85-mediated inhibition of p110 α . Significantly, this PDGFR-derived peptide has no effect on the activity of the helical domain mutants, but it inhibits the kinase domain mutant (Chaussade et al. 2009). These data on the functional differences between helical and kinase domain mutations suggest that the kinase domain mutant is independent of activation by RAS; the mutation appears to have induced the same or a similar activating change that in the wild-type enzyme and in the helical domain mutants is achieved by the interaction with RAS. However, the kinase domain mutant still remains critically dependent on an interaction with p85, and this dependence requires further clarification. One possibility is suggested the crystal structure of the p110 α -p85 complex which reveals an unexpected interaction between the p85-binding domain and the kinase domain (Huang et al. 2007, 2008). This interaction might be important for the active conformation induced by H1047R and could explain the sensitivity of H1047R to a loss of p85 binding. Interactions between p85 and p110 α that are relevant to the properties of the mutant proteins are summarized in Fig. 3.

The kinase domain mutation H1047R maps to the hinge region of the activation loop. It could affect the position and the mobility of the activation loop. RAS also interacts with the kinase domain and could induce a change that is similar to the one caused by the H1047R mutation. The helical domain mutants remain dependent

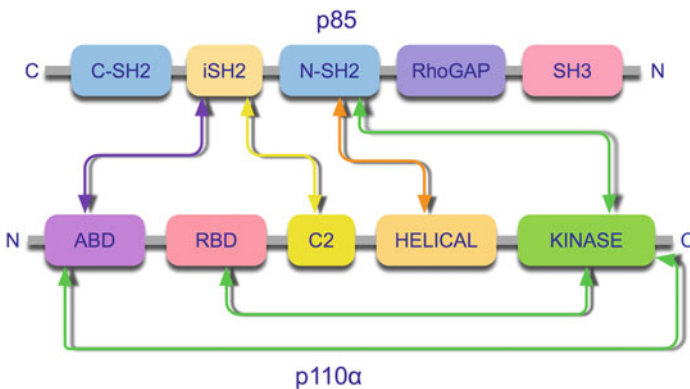


Fig. 3 p110 α p85 domain interactions of importance for gain of function mutations. *Green arrows* mark interactions with the kinase domain that could explain why the H1047R mutant is RAS independent, but p85 dependent. The *orange arrow* signifies the inhibitory interaction between the N terminal SH2 domain of p85 and the helical domain of p110 α that is released by the helical domain mutation in p110 α and the N terminal SH2 domain mutation in p85. The *orange and yellow arrows* mark similar, probably inhibitory interactions between the inter SH2 domain of p85 and the C2 or adaptor binding domain of p110 α , respectively. These interactions may be modulated by mutations in the respective domains of p110 α .

on binding to RAS, but are largely independent of p85-binding, probably because they mimic the activating event that follows the growth factor-induced relief of the helical domain-p85 interaction. The same effect of disrupting the helical domain-p85 binding probably also results from the cancer-specific mutation in the N-terminal SH2 domain of p85; it can also be achieved experimentally by mutating critical helical domain-interacting residues R340E and K379E in the p85 which are postulated to interact with E542 and E545 residues of p110 α (Miled et al. 2007). Overexpression of such experimentally mutated p85 induces oncogenic transformation in cell culture (Sun et al. 2010, submitted).

The molecular mechanisms for the mutation-induced gain of function in helical and in kinase domain mutations are complementary and reciprocal. The helical domain mutations have gained activation through p85-independence, but still need the interaction with RAS. The kinase domain mutation is in a state of constitutive RAS activation, but still requires the interaction with p85. The helical-kinase domain double mutant is both independent of RAS and of p85.

Since the hot spot mutations account for about 80% of all cancer-specific mutations in p110 α , inhibitors specific for these mutants could benefit the majority of the affected patients. Mutant-specific inhibitors would not induce side effects that can result from interfering with the life-sustaining functions of PI3K. The identification of small molecules that discriminate between mutant and wild-type is a challenging task for drug discovery. It would be greatly facilitated by structural information that is specific for the mutants.

4 Non-alpha Isoforms of Class I PI3K in Cancer

There are four isoforms of the catalytic subunit of class I PI3Ks: p110 α , p110 β , p110 γ , and p110 δ (Deane and Fruman 2004; Engelman et al. 2006; Fruman and Bismuth 2009; Hawkins et al. 2006; Stephens et al. 1996; Vanhaesebroeck et al. 2001). They are encoded by different genes, but share a basic domain structure. The α , β , and δ isoforms use the same regulatory subunits. The α and δ isoforms are linked primarily to upstream receptor tyrosine kinases, the upstream activation of p110 β appears to be context-dependent, involving receptor tyrosine kinases and G-protein-coupled receptors (Ciraolo et al. 2008; Guillermet-Guibert et al. 2008). The p110 γ isoform interacts with separate distinct regulatory subunits and is linked to G-protein-coupled receptors (Stoyanov et al. 1995; Yart et al. 2002). The α and β isoforms are expressed ubiquitously. Expression of γ and δ isoforms is restricted mainly to lymphocytes. Genetic inactivation of the α and β isoforms causes early embryonic lethality (Bi et al. 1999, 2002), whereas γ and δ knockouts are viable but suffer from immune deficiencies (Ali et al. 2004; Cantley 2002; Clayton et al. 2002; Hirsch et al. 2000; Ji et al. 2007; Jou et al. 2002; Laffargue et al. 2002; Okkenhaug et al. 2002; Rodriguez-Borlado et al. 2003; Sasaki et al. 2000). The functions of the p110 isoforms are overlapping, but they are clearly not redundant. Conditional and

tissue-specific mutations have defined isoform-specific roles in cellular signaling. The principal roles of p110 γ and p110 δ are in the immune system (Alcazar et al. 2007; Ali et al. 2008; Ji et al. 2007; Okkenhaug et al. 2004; Patton et al. 2007), and p110 α and p110 β have distinct, complementary, context-dependent and cell type-dependent roles in the control of cell growth and metabolism (Graupera et al. 2008; Marone et al. 2008; Vanhaesebroeck et al. 2005). The functions of specific p110 isoforms are explored in greater detail in other chapters of this volume.

The oncogenic potential of p110 α is well documented in experimental systems; the gain-of-function mutations in human cancer add significance to this activity (Samuels et al. 2004). For the non-alpha isoforms of class I PI3K, the connection to cancer is more tenuous. There are no cancer-specific mutations in these isoforms, but differential expression is observed in several cancers. The p110 δ isoform is consistently overexpressed in acute myeloblastic leukemia, and inhibitors of p110 δ specifically interfere with the growth of these leukemic cells, suggesting a role of p110 δ in leukemogenesis (Samuels et al. 2004; Sujobert et al. 2005). Specific inhibitors of p110 δ are in clinical trials for hematopoietic malignancies (<http://clinicaltrials.gov/ct2/show/NCT00710528>). Increased expression of p110 γ is seen in chronic myeloid leukemia (Hickey and Cotter 2006; Knobbe et al. 2005). There are also data that suggest involvement of non-alpha isoforms of class I PI3K in solid tumors (Bénistant et al. 2000; Knobbe et al. 2005; Mizoguchi et al. 2004). The wild-type non-alpha isoforms have the ability to induce oncogenic transformation when overexpressed in cell culture, whereas wild-type p110 α lacks this ability (Kang et al. 2006). The elevated expression of non-alpha isoforms in some cancers may therefore be a determinant of the oncogenic cellular phenotype. The oncogenic activity of wild-type non-alpha isoforms has so far been shown only in chicken embryo fibroblasts which are exquisitely sensitive to transformation by single oncoproteins. In this cell culture system, the various isoforms reveal distinctly different characteristics in their signaling through AKT, their interactions with RAS and sensitivity to inhibitors of the MAP kinase pathway (Denley et al. 2008). Overexpression of p110 δ induces strong phosphorylation of AKT at T308 and S473 and of the downstream targets S6K, 4E-BP and GSK3 β (glycogen and synthase kinase 3 β). FOXO1 (forkhead box transcription factor O1) becomes undetectable in these cells. This signaling pattern closely resembles that of the p110 α mutant H1047R. Expression of p110 β and p110 γ induces much lower levels of phosphorylation of AKT, S6K, 4E-BP, and GSK3 β . The levels of FOXO1 are not significantly reduced in these cells. The contrasting properties of p110 δ vs. p110 β and p110 γ are also seen in their responses to activation by RAS and to inhibition of the MAP kinase pathway. Introducing point mutations into the RAS binding domain that are designed to abolish RAS binding interferes with oncogenic transformation and signaling induced by p110 β and p110 γ , but does not affect p110 δ . The activities of the mutated p110 β and p110 γ proteins can be restored by adding a myristylation signal to the N-terminus of the proteins, suggesting that the interaction with RAS mediates recruitment to the plasma membrane. The apparent independence of p110 δ from RAS requires further examination and confirmation. Although the mutated residue in the RAS-binding domain of p110 δ is conserved

among the isoforms and is known to control RAS binding in p110 α and in p110 γ (Pirola et al. 2001; Rodriguez-Viciana et al. 1996), the effect of this mutation in p110 δ on RAS binding has not been verified. Sensitivity to inhibitors also sets p110 β and p110 γ apart from p110 δ . The MEK1/2 inhibitor U0126 effectively interferes with cellular transformation induced by p110 β and p110 γ , but does not affect the oncogenic activity of p110 δ and of the H1047R mutant of p110 α . The inhibitor of RAF, BAY 43-9006, shows a similar preferential effect on p110 β and p110 γ , but does not interfere with cellular transformation caused by p110 δ . These observations single out p110 δ as an exceptionally potent signaling protein, resistant to MAP kinase inhibition and capable of functioning preferentially through AKT. The similarities between p110 β and p110 γ are unexpected because these isoforms respond to different sets of upstream signaling: p110 β has been linked to receptor tyrosine kinases and G-protein coupled receptors and p110 γ exclusively to G-protein coupled receptors (Roche et al. 1998; Guillermet-Guibert et al. 2008). The similarities between p110 β and p110 γ revealed by the studies on oncogenicity and signaling in chicken embryo fibroblasts may extend to mammalian cells. An important role of non-alpha isoforms of PI3K in cancer is also emerging from new animal models and from studies in cell culture (Ciraolo et al. 2008; Jia et al. 2008; Torbett et al. 2008; Wee et al. 2008). These reveal isoform-specific enzymatic and nonenzymatic functions of p110 β and are discussed in other chapters of this volume.

5 Class II and III PI3Ks

The family of PI3Ks encompasses three distinct classes that differ in structure and function (Vanhaesebroeck et al. 1997; Vanhaesebroeck and Waterfield 1999). One of the defining criteria for each class of PI3Ks is substrate recognition and hence the spectrum of products. Class I PI3Ks can utilize the nonphosphorylated phosphatidylinositol (PI), the monophosphate (PI(4)P), and the bisphosphate (PI(4,5)P₂) phosphatidylinositols, giving rise to PI(3)P, PI(3,4)P₂ and PI(3,4,5)P₃, also referred to as PIP, PIP₂ and PIP₃, respectively. Class II PI3Ks recognize PI and PI(4)P, but not PI(4,5)P₂ as substrates to produce PIP and PIP₂. Class III PI3Ks can use only PI and convert it to PIP (Pirola et al. 2001). In addition, class I PI3Ks can function as serine protein kinases (Dhand et al. 1994). One of the protein substrates of class I PI3Ks is the regulatory subunit p85. This phosphorylation represents an autoregulatory mechanism (Foukas et al. 2004). The protein kinase activity of p110 is, however, not sufficient for oncogenic transformation induced by class I p110 mutants and isoforms (Denley et al. 2009; Kang et al. 2006). In the canonical PI3K signaling pathway, PIP₂ and PIP₃ are recognized by the pleckstrin homology domains of PDK-1 (phosphoinositide-dependent kinase) and of AKT (Alessi et al. 1996a, b; Klippel et al. 1997; Nicholson and Anderson 2002). These interactions recruit PDK-1 and AKT to the plasma membrane, resulting in the phosphorylation of

AKT by PDK-1, catalytic activation of AKT, and phosphorylation of downstream targets (Alessi et al. 1997; Currie et al. 1999; Filippa et al. 2000; McManus et al. 2004; Milburn et al. 2003; Vanhaesebroeck and Alessi 2000). The structure of the AKT pleckstrin homology domain bound to IP₄, the headgroup of PIP₃, shows critical ionic interactions between basic pleckstrin homology domain residues and the phosphates at the D-3 and D-4 positions of IP₄ (Milburn et al. 2003). The phosphate at the D-5 position does not participate in the interaction with the AKT pleckstrin homology domain. Hence, AKT has a lower affinity for PIP₂ (PI(4,5)P₂) than for PIP₃.

So far, only class I PI3Ks have been firmly involved in cancer, although there are some observations that suggest class II may also play a role (Low et al. 2008). The defining characteristic of class I PI3Ks is the generation of PIP₃ (Vanhaesebroeck et al. 1997; Vanhaesebroeck and Waterfield 1999). This ability may therefore constitute a prerequisite for the oncogenicity of lipid kinases. Emerging evidence supports this suggestion. A short sequence in the activation loop of PI3Ks determines substrate recognition and product specificity (Bondeva et al. 1998; Pirola et al. 2001). Substitution of this sequence in class I PI3K with the corresponding sequence of class II or of class III generates enzymes that produce PIP and PI(3,4)P₂ or PIP, respectively, but fail to make PIP₃. These constructs do not induce oncogenic transformation in cell culture and show greatly reduced signaling through AKT. Expression of the wild-type or the myristylated form of hVps34 class III PI3K fails to induce oncogenic transformation in cultures of chicken embryo fibroblasts and does not increase the phosphorylation status of Akt, p70 S6 kinase, 4E-BP, and glycogen synthase kinase-3 β or cause a change in the level of FoxO1 (Denley et al. 2009). The production of PIP₃ and PI3K signaling are reduced in the presence of the PIPP phosphatase which removes the phosphate from the D-5 position of phosphatidylinositol. Expression of PIPP also interferes with oncogenic transformation induced by the four isoforms of class I PI3K, reduces the levels of AKT phosphorylation and attenuates the degradation of FOXO1 (Denley et al. 2009). These observations support the conclusion that the ability to produce PIP₃ is essential for the oncogenic activity of PI3K.

6 PI3K-Driven Oncogenic Transformation: Mechanistic Considerations

PI3K signaling affects numerous downstream targets, not all will be essential for oncogenic transformation. The canonical signaling cascade proceeds through AKT, the TSC (tuberous sclerosis complex), RHEB (Ras homolog enriched in brain) to TOR and from there to additional targets. In this pathway, two components stand out as particularly significant for oncogenicity: AKT and TOR. AKT is an important signal branching point that can direct PI3K signals into many different directions; TOR is of importance because it functions as integrator, receiving input from

multiple sources. Thus, AKT and TOR link the canonical PI3K pathway to other regulatory activities in the cell.

In the canonical pathway, AKT phosphorylates and thereby inhibits TSC2 (Dan et al. 2002; Inoki et al. 2002; Manning et al. 2002). The TSC complex functions as GTPase-activating protein for RHEB (Castro et al. 2003; Garami et al. 2003; Inoki et al. 2003; Tee et al. 2003; Zhang et al. 2003b); reduction of GTPase activation by AKT activates RHEB. The GTP-bound RHEB then directly interacts with TOR and activates this target. AKT and RHEB are oncogenic when constitutively activated (Ahmed et al. 1993; Aoki et al. 1998; Jiang and Vogt 2008). TOR is a PI 3-kinase related protein kinase (PIKK) (Abraham 2004) that fulfills numerous tasks in cell growth and metabolism.

TOR functions in two distinct multiprotein complexes, TORC1 and TORC2 (Jacinto et al. 2004). TORC1 contains the proteins RAPTOR, LST8 and PRAS40 (Kim et al. 2002). TORC2 consists of LST8, RICTOR and SIN1 (Sarbasov et al. 2004). TORC1 and TORC2 are differentially regulated and have distinct functions. TORC1 can be activated by AKT-dependent and AKT-independent signals. AKT stimulates TORC1 by inducing an inhibition of the GTPase-activating protein activity of the TSC complex that targets RHEB and by phosphorylating and thereby inactivating PRAS40, a negative regulator of TORC1. AKT-independent regulation of TOR can also be mediated by the TSC complex. TSC is a sensor and integrator of signals that originate from energy deprivation, hypoxia or stimulation of growth. For instance, AMPK (AMP kinase), activated by an increase of cellular AMP, activates TSC2 and thereby inhibits TOR. Rag (Ras-related small GTP-binding proteins) activate TORC1 in response to the availability of amino acids (Sancak et al. 2008). Another AKT-independent pathway to TORC1 has been identified in glioblastoma and is mediated by PKC α (protein kinase C α) (Fan et al. 2009). The principal downstream targets of TORC1 are 4E-BP1 (eukaryotic initiation factor 4E-binding protein) and S6K1 (p70 ribosomal protein S6 kinase). They will be considered below.

In contrast to the various AKT-dependent and AKT-independent ways that have been identified for the regulation of TORC1 (Cheng et al. 2009; Memmott and Dennis 2009; Vasudevan et al. 2009), the regulation of TORC2 is not fully understood. A distinguishing mark of TORC2 activation is the requirement for an active TSC complex, one that is not phosphorylated by AKT and opposite to the requirement for TORC1 activation (Huang and Manning 2009). Several targets have been identified for TORC2 (Huang et al. 2009). Among these, the phosphorylation of AKT at S473 appears potentially relevant to PI3K signaling (Sarbasov et al. 2005). This phosphorylation event achieves maximal activation of AKT, and it also expands the spectrum of AKT targets to include PRAS40 and FOXO (Guertin et al. 2006). However, it is doubtful whether the additional targets that can be addressed by the S473-phosphorylated AKT play an important role in transformation (see below).

TOR is essential for the oncogenicity of PI3K and AKT (Jiang et al. 2000; Neshat et al. 2001; Podsypanina et al. 2001). The TOR inhibitor rapamycin strongly

and specifically interferes with PI3K- and AKT-induced cellular transformation; yet it does not reduce transformation caused by 14 other oncogenes (Aoki et al. 2001). Exposure to resistance-inducing concentrations of rapamycin does not significantly affect cell replication. Whereas short-term treatment with rapamycin selectively inhibits TORC1, cells treated over prolonged periods of time (24 h or more) also show inhibition of TORC2 (Sarbasov et al. 2006). Since interference with oncogenic transformation by rapamycin results from long-term treatment with the drug, both TORC1 and TORC2 would be affected and could play an essential role in oncogenicity. However, at least one TORC2 activity, the phosphorylation of AKT on S473, appears to be dispensable for oncogenic transformation (Aoki et al. 1998).

The available data are compatible with the idea that TORC1, but not TORC2, plays the predominant role in oncogenesis (Guertin and Sabatini 2007). TORC1 functions as a positive regulator of protein synthesis. It phosphorylates and thereby activates S6K. It also phosphorylates 4E-BP1, releasing eIF4E (eukaryotic initiation factor 4E, the cap-binding protein) to become available for the assembly of the translation initiation complex. The TOR-dependent stimulation of protein synthesis preferentially affects mRNAs that have complex secondary structures in their 5' untranslated regions (Culjkovic et al. 2005). These secondary structures require unwinding performed by the eIF4A (eukaryotic initiation factor 4A) helicase that together with the eukaryotic initiation factors eIF4E and eIF4G forms the eIF4F initiation complex. Numerous mRNAs that encode growth-promoting proteins are characterized by 5' untranslated sequences with complex secondary structures, and their efficient translation is highly dependent on an abundance of eIF4E and the eIF4A helicase (Culjkovic et al. 2006). The enhancement of this activity by TORC1 could be a critical factor in the transformation process.

The importance of high efficiency translational initiation in PI3K- and AKT-induced oncogenicity is also documented by the effects of the YB-1 (Y Box binding protein) on the transformation process. The YB-1 protein is highly conserved in evolution with close relatives throughout prokaryotic and eukaryotic forms of life. It is abundantly and ubiquitously expressed, and with its cold shock domain, it binds both DNA and RNA, affecting transcription and translation (Evdokimova et al. 2006a, 2009; Izumi et al. 2001; MacDonald et al. 1995; Mertens et al. 1997; Zasedateleva et al. 2002). By binding to mRNA, YB-1 has the capacity to inhibit translation (Evdokimova et al. 2006a). In cells transformed by PI3K or AKT, YB-1 is downregulated transcriptionally and posttranscriptionally (Bader et al. 2003; Bader and Vogt 2005, 2008; Evdokimova et al. 2006b; Sutherland et al. 2005). This downregulation appears to be a necessary facilitator of transformation, because re-expression of YB-1 causes a strong and specific cellular resistance to PI3K- and AKT-induced oncogenic transformation, yet does not interfere with transformation induced by other oncogenes (Bader et al. 2003). These YB-1-expressing cells do not show a detectable reduction in the rate of replication. Phenotypically, YB-1 therefore acts like a rapamycin mimic, but it intervenes in transformation downstream of TORC1, at a level of mRNA (Bader and Vogt 2008).

Interference with transformation depends on cytoplasmic localization of YB-1 and on the ability of YB-1 to bind to RNA (Bader and Vogt 2005). The interaction of YB-1 with RNA is not sequence-specific, and YB-1 can bind to multiple sites on the mRNA. However, for the inhibition of protein synthesis, binding at or near the cap structure of mRNA is essential. According to a recent model, YB-1 then interferes with binding of eIF4G (eukaryotic initiation factor 4G) to mRNA and thus competes with the assembly of the eIF4F initiation complex on the mRNA (Svitkin et al. 2009).

The specific sensitivity of PI3K-induced transformation to rapamycin and to expressed YB-1 supports the conclusion that the oncogenic effects of PI3K are mediated by TORC1 and that they involve stimulation of protein synthesis. This activity of TOR appears necessary for transformation, but it is probably not sufficient. A gain of function in TOR alone has so far not been found to transform cells. Cells lacking TSC1 or TSC2 show constitutive activation of TOR, but are not transformed (Kwiatkowski et al. 2002; Zhang et al. 2003a). Patients with heritable loss of function in the TSC complex develop hamartomas, but aggressive cancers are rare (Al-Saleem et al. 1998; Kwiatkowski and Manning 2005; Marcotte and Crino 2006). However, in rodent model systems, inactivating mutations of either TSC1 or TSC2 increases cancer incidence, possibly due to secondary mutations (Everitt et al. 1992; Kobayashi et al. 1999; Kwiatkowski et al. 2002).

If gain of function in TOR is necessary but not sufficient for transformation, what then are the other necessary, complementing oncogenic activities that originate with PI3K signaling? There likely will be several. A possible candidate is one of the multiple targets of AKT: NF κ B. The transcriptional activity of NF κ B is upregulated in AKT-transformed cells. This increased function is dependent on AKT and is abolished by small molecule inhibitors of AKT and by a dominant negative mutant of AKT (Bai et al. 2009). In AKT-transformed cells, the total amount of I κ B inhibitor protein is dramatically decreased. Blocking NF κ B activity with the super-repressor of NF κ B (I κ BSR) induces a cellular resistance that is selective for PI3K- and AKT-induced transformation. Thus, NF κ B activity is essential for the oncogenicity of PI3K and AKT. Although there is no general agreement on how AKT communicates with NF κ B, the balance of the evidence supports the idea of a phosphorylation cascade that connects the two proteins. AKT can phosphorylate IKK α (I κ B kinase) *in vivo* (Ozes et al. 1999), and the activated IKK complex then phosphorylates the p65 subunit of NF κ B (Sakurai et al. 1999), enhancing its transcriptional activity. It is, however, possible that the essential requirement for NF κ B in PI3K-induced transformation can be satisfied with a basal level of activity and that the AKT-mediated gain of function represents a secondary consequence of transformation.

The identification of essential components in the oncogenic pathway is important for therapeutic considerations and helps define suitable drug targets. In this regard, the catalytic subunit p110 of PI3K remains a strong candidate, but more understanding of isoform-specific functions in various cancers and cell types and on different contributing genetic backgrounds (e.g., gain of function in receptor

tyrosine kinases, loss of function in PTEN) is needed (Garcia-Echeverria and Sellers 2008; Kong and Yamori 2008; Maira et al. 2008; Wymann and Schreiber 2008; Yap et al. 2008). TOR emerges as another promising drug target (Guertin and Sabatini 2009). ATP-competitive inhibitors of TOR have recently been identified and are being characterized. They affect rapamycin-resistant functions of TOR (Feldman et al. 2009; García-Martínez et al. 2009; Guertin and Sabatini 2009; Malagu et al. 2009; Nowak et al. 2009; Thoreen et al. 2009; Yu et al. 2009; Zask et al. 2009). The situation with AKT is more complex, because not all PI3K-driven tumors show AKT dependence (Vasudevan et al. 2009). Our understanding of the oncogenic signals emanating from PI3K is still evolving. New pathways and feedbacks are being characterized and novel interacting proteins discovered. Tissue- and cell-type specific differences in PI3K signaling are being defined. All these advances will eventually result in the recognition of new drug targets.

7 Conclusion

PI3Ks have oncogenic potential. The requisite gain of function can be achieved by mutation or by differential expression. Oncogenicity is mainly associated with class I PI3Ks and correlated with the ability to produce PIP₃. PIP₃ is the critical PI3K product that links lipid kinase activity to a network of downstream signals originating in AKT. In sensitive experimental systems, gain of function in a single PI3K isoform is sufficient to induce oncogenic transformation. These systems can be used for quantitative determination of oncogenicity and serve as models for investigations of transformation-associated changes in the cellular phenotype. Transformed focus assays in cell culture remain the gold standard for measuring and comparing oncogenic activity; such assays include all four isoforms of class I PI3K and can yield valuable data on the antioncogenic potency of drug candidates. The experimental systems of PI3K-induced oncogenic transformation also allow determination of specific changes in signaling pathways, cell behavior and metabolism.

The PI3K pathway is deregulated in the majority of human cancers. In sporadic tumors and in cancer cell lines, there are numerous other genetic and epigenetic changes that have been extensively documented by the human cancer genome project (Cancer Genome Atlas Research Network 2008; He et al. 2008; Jones et al. 2008; Parsons et al. 2008; Wood et al. 2007). In these situations, PI3K signaling can be expected to make a contribution to the oncogenic phenotype of the cell, but rarely will it function as the sole or dominant transforming event. The oncogenic phenotype of human cancer is the composite of all genetic and epigenetic changes. Experience with inhibitors of PI3K reflects this complexity. The growth of cells experimentally transformed by PI3K is generally highly sensitive to PI3K inhibitors. Few human cancer cell lines show such sensitivity. However, combinations of inhibitors that target critical nodes in cellular signaling or single inhibitors that target a critical combination of oncoproteins can be very effective

(Cheng et al. 2009; Fan et al. 2006, 2007, 2009; Fan and Weiss 2006; Jaiswal et al. 2009b; Nelander et al. 2008). These observations are detailed in other chapters of this book.

The greatest challenge in the area of PI3K oncogenicity remains the identification of mutant-specific inhibitors that have drug-like properties. The highly targeted therapeutic potential of such inhibitors justifies intense efforts by industry and in academic laboratories.

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References

- Abraham RT (2004) PI 3 kinase related kinases: 'big' players in stress induced signaling pathways. *DNA Repair (Amst)* 3:883 887
- Ahmed NN, Franke TF, Bellacosa A, Datta K, Gonzalez Portal ME, Taguchi T, Testa JR, Tsichlis PN (1993) The proteins encoded by c akt and v akt differ in post translational modification, subcellular localization and oncogenic potential. *Oncogene* 8:1957 1963
- Alcazar I, Marques M, Kumar A, Hirsch E, Wymann M, Carrera AC, Barber DF (2007) Phosphoinositide 3 kinase gamma participates in T cell receptor induced T cell activation. *J Exp Med* 204:2977 2987
- Alessi DR, Andjelkovic M, Caudwell B, Cron P, Morrice N, Cohen P, Hemmings BA (1996a) Mechanism of activation of protein kinase B by insulin and IGF 1. *EMBO J* 15: 6541 6551
- Alessi DR, Caudwell FB, Andjelkovic M, Hemmings BA, Cohen P (1996b) Molecular basis for the substrate specificity of protein kinase B; comparison with MAPKAP kinase 1 and p70 S6 kinase. *FEBS Lett* 399:333 338
- Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PR, Reese CB, Cohen P (1997) Characterization of a 3 phosphoinositide dependent protein kinase which phosphorylates and activates protein kinase Balph. *Curr Biol* 7:261 269
- Ali K, Bilancio A, Thomas M, Pearce W, Gilfillan AM, Tkaczyk C, Kuehn N, Gray A, Giddings J, Peskett E, Fox R, Bruce I, Walker C, Sawyer C, Okkenhaug K, Finan P, Vanhaesebroeck B (2004) Essential role for the p110delta phosphoinositide 3 kinase in the allergic response. *Nature* 431:1007 1011
- Ali K, Camps M, Pearce WP, Ji H, Rückle T, Kuehn N, Pasquali C, Chabert C, Rommel C, Vanhaesebroeck B (2008) Isoform specific functions of phosphoinositide 3 kinases: p110 delta but not p110 gamma promotes optimal allergic responses in vivo. *J Immunol* 180:2538 2544
- Al Saleem T, Wessner LL, Scheithauer BW, Patterson K, Roach ES, Dreyer SJ, Fujikawa K, Bjornsson J, Bernstein J, Henske EP (1998) Malignant tumors of the kidney, brain, and soft tissues in children and young adults with the tuberous sclerosis complex. *Cancer* 83:2208 2216
- Amzel LM, Huang CH, Mandelker D, Lengauer C, Gabelli SB, Vogelstein B (2008) Structural comparisons of class I phosphoinositide 3 kinases. *Nat Rev Cancer* 8:665 669
- Aoki M, Batista O, Bellacosa A, Tsichlis P, Vogt PK (1998) The akt kinase: molecular determinants of oncogenicity. *Proc Natl Acad Sci USA* 95:14950 14955
- Aoki M, Schetter C, Himly M, Batista O, Chang HW, Vogt PK (2000) The catalytic subunit of phosphoinositide 3 kinase: requirements for oncogenicity. *J Biol Chem* 275:6267 6275

- Aoki M, Blazek E, Vogt P (2001) A role of the kinase mTOR in cellular transformation induced by the oncoproteins P3k and Akt. *Proc Natl Acad Sci USA* 98:136 141
- Bader AG, Vogt PK (2005) Inhibition of protein synthesis by Y box binding protein 1 blocks oncogenic cell transformation. *Mol Cell Biol* 25:2095 2106
- Bader AG, Vogt P (2008) Phosphorylation by Akt disables the anti oncogenic activity of YB 1. *Oncogene* 27:1179 1182
- Bader AG, Felts KA, Jiang N, Chang HW, Vogt PK (2003) Y box binding protein 1 induces resistance to oncogenic transformation by the phosphatidylinositol 3 kinase pathway. *Proc Natl Acad Sci USA* 100:12384 12389
- Bader AG, Kang S, Vogt PK (2006) Cancer specific mutations in PIK3CA are oncogenic in vivo. *Proc Natl Acad Sci USA* 103:1475 1479
- Bai D, Ueno L, Vogt PK (2009) Akt mediated regulation of NFκB and the essentialness of NFκB for the oncogenicity of PI3K and Akt. *Int J Cancer* 125:2863 2870
- Bénistant C, Chapuis H, Roche S (2000) A specific function for phosphatidylinositol 3 kinase alpha (p85alpha p110alpha) in cell survival and for phosphatidylinositol 3 kinase beta (p85alpha p110beta) in de novo DNA synthesis of human colon carcinoma cells. *Oncogene* 19:5083 5090
- Bi L, Okabe I, Bernard DJ, Wynshaw Boris A, Nussbaum RL (1999) Proliferative defect and embryonic lethality in mice homozygous for a deletion in the p110alpha subunit of phosphoinositide 3 kinase. *J Biol Chem* 274:10963 10968
- Bi L, Okabe I, Bernard DJ, Nussbaum RL (2002) Early embryonic lethality in mice deficient in the p110beta catalytic subunit of PI 3 kinase. *Mamm Genome* 13:169 172
- Bondeva T, Pirola L, Bulgarelli Leva G, Rubio I, Wetzker R, Wymann MP (1998) Bifurcation of lipid and protein kinase signals of PI3Kgamma to the protein kinases PKB and MAPK. *Science* 282:293 296
- Brachmann S, Fritsch C, Maira SM, Garcia Echeverria C (2009) PI3K and mTOR inhibitors: a new generation of targeted anticancer agents. *Curr Opin Cell Biol* 21:194 198
- Cancer Genome Atlas Research Network (2008) Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 455:1061 1068
- Cantley LC (2002) The phosphoinositide 3 kinase pathway. *Science* 296:1655 1657
- Carpenter CL, Duckworth BC, Auger KR, Cohen B, Schaffhausen BS, Cantley LC (1990) Purification and characterization of phosphoinositide 3 kinase from rat liver. *J Biol Chem* 265:19704 19711
- Carson JD, Van Aller G, Lehr R, Sinnamon RH, Kirkpatrick RB, Auger KR, Dhanak D, Copeland RA, Gontarek RR, Tummino PJ, Luo L (2008) Effects of oncogenic p110alpha subunit mutations on the lipid kinase activity of phosphoinositide 3 kinase. *Biochem J* 409:519 524
- Castro AF, Rebhun JF, Clark GJ, Quilliam LA (2003) Rheb binds tuberous sclerosis complex 2 (TSC2) and promotes S6 kinase activation in a rapamycin and farnesylation dependent manner. *J Biol Chem* 278:32493 32496
- Chang HW, Aoki M, Fruman D, Auger KR, Bellacosa A, Tsichlis PN, Cantley LC, Roberts TM, Vogt PK (1997) Transformation of chicken cells by the gene encoding the catalytic subunit of PI 3 kinase. *Science* 276:1848 1850
- Chaussade C, Cho K, Mawson C, Rewcastle GW, Shepherd PR (2009) Functional differences between two classes of oncogenic mutation in the PIK3CA gene. *Biochem Biophys Res Commun* 381:577 581
- Cheng CK, Fan QW, Weiss WA (2009) PI3K signaling in glioma animal models and therapeutic challenges. *Brain Pathol* 19:112 120
- Ciraolo E, Iezzi M, Marone R, Marengo S, Curcio C, Costa C, Azzolino O, Gonella C, Rubinetto C, Wu H, Dastrù W, Martin EL, Silengo L, Altruda F, Turco E, Lanzetti L, Musiani P, Rückle T, Rommel C, Backer JM, Forni G, Wymann MP, Hirsch E (2008) Phosphoinositide 3 kinase p110beta activity: key role in metabolism and mammary gland cancer but not development. *Sci Signal* 1:ra3

- Clayton E, Bardi G, Bell SE, Chantry D, Downes CP, Gray A, Humphries LA, Rawlings D, Reynolds H, Vigorito E, Turner M (2002) A crucial role for the p110delta subunit of phosphatidylinositol 3 kinase in B cell development and activation. *J Exp Med* 196:753-763
- Courtneidge SA, Smith AE (1984) The complex of polyoma virus middle T antigen and pp 60c src. *EMBO J* 3:585-591
- Cuevas BD, Lu Y, Mao M, Zhang J, LaPushin R, Siminovich K, Mills GB (2001) Tyrosine phosphorylation of p85 relieves its inhibitory activity on phosphatidylinositol 3 kinase. *J Biol Chem* 276:27455-27461
- Culjkovic B, Topisirovic I, Skrabanek L, Ruiz Gutierrez M, Borden KL (2005) eIF4E promotes nuclear export of cyclin D1 mRNAs via an element in the 3'UTR. *J Cell Biol* 169:245-256
- Culjkovic B, Topisirovic I, Skrabanek L, Ruiz Gutierrez M, Borden KL (2006) eIF4E is a central node of an RNA regulon that governs cellular proliferation. *J Cell Biol* 175:415-426
- Currie R, Walker KS, Gray A, Deak M, Casamayor A, Downes CP, Cohen P, Alessi DR, Lucocq J (1999) Role of phosphatidylinositol 3, 4, 5 trisphosphate in regulating the activity and localization of 3-phosphoinositide dependent protein kinase 1. *Biochem J* 337(pt 3):575-583
- Dan HC, Sun M, Yang L, Feldman RI, Sui XM, Ou CC, Nellist M, Yeung RS, Halley DJ, Nicosia SV, Pledger WJ, Cheng JQ (2002) Phosphatidylinositol 3 kinase/Akt pathway regulates tuberous sclerosis tumor suppressor complex by phosphorylation of tuberin. *J Biol Chem* 277:35364-35370
- Deane JA, Fruman DA (2004) Phosphoinositide 3 kinase: diverse roles in immune cell activation. *Annu Rev Immunol* 22:563-598
- Denley A, Kang S, Karst U, Vogt P (2008) Oncogenic signaling of class I PI3K isoforms. *Oncogene* 27:2561-2574
- Denley A, Gymnopoulos M, Kang S, Mitchell C, Vogt PK (2009) Requirement of phosphatidylinositol(3, 4, 5)trisphosphate in phosphatidylinositol 3 kinase induced oncogenic transformation. *Mol Cancer Res* 7:1132-1138
- Dhand R, Hiles I, Panayotou G, Roche S, Fry MJ, Gout I, Totty NF, Truong O, Vicendo P, Yonezawa K et al (1994) PI 3 kinase is a dual specificity enzyme: autoregulation by an intrinsic protein serine kinase activity. *EMBO J* 13:522-533
- Di Cristofano A, Pandolfi PP (2000) The multiple roles of PTEN in tumor suppression. *Cell* 100:387-390
- Engelman JA, Luo J, Cantley LC (2006) The evolution of phosphatidylinositol 3 kinases as regulators of growth and metabolism. *Nat Rev Genet* 7:606-619
- Engelman JA, Chen L, Tan X, Crosby K, Guimaraes AR, Upadhyay R, Maira M, McNamara K, Perera SA, Song Y, Chirieac LR, Kaur R, Lightbown A, Simendinger J, Li T, Padera RF, Garcia Echeverria C, Weissleder R, Mahmood U, Cantley L, Wong KK (2008) Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. *Nat Med* 14:1351-1356
- Evdokimova V, Ovchinnikov LP, Sorensen PH (2006a) Y box binding protein 1: providing a new angle on translational regulation. *Cell Cycle* 5:1143-1147
- Evdokimova V, Ruzanov P, Anglesio MS, Sorokin A, Ovchinnikov L, Buckley J, Triche TJ, Sonenberg N, Sorensen P (2006b) Akt mediated YB 1 phosphorylation activates translation of silent mRNA species. *Mol Cell Biol* 26:277-292
- Evdokimova V, Tognon C, Ng T, Ruzanov P, Melnyk N, Fink D, Sorokin A, Ovchinnikov LP, Davicioni E, Triche TJ, Sorensen PH (2009) Translational activation of snail1 and other developmentally regulated transcription factors by YB 1 promotes an epithelial mesenchymal transition. *Cancer Cell* 15:402-415
- Everitt JI, Goldsworthy TL, Wolf DC, Walker CL (1992) Hereditary renal cell carcinoma in the Eker rat: a rodent familial cancer syndrome. *J Urol* 148:1932-1936
- Fan QW, Weiss WA (2006) Isoform specific inhibitors of PI3 kinase in glioma. *Cell Cycle* 5:2301-2305

- Fan QW, Knight ZA, Goldenberg DD, Yu W, Mostov KE, Stokoe D, Shokat KM, Weiss WA (2006) A dual PI3 kinase/mTOR inhibitor reveals emergent efficacy in glioma. *Cancer Cell* 9:341 349
- Fan QW, Cheng CK, Nicolaidis TP, Hackett CS, Knight ZA, Shokat KM, Weiss WA (2007) A dual phosphoinositide 3 kinase alpha/mTOR inhibitor cooperates with blockade of epidermal growth factor receptor in PTEN mutant glioma. *Cancer Res* 67:7960 7965
- Fan QW, Cheng C, Knight ZA, Haas Kogan D, Stokoe D, James CD, McCormick F, Shokat KM, Weiss WA (2009) EGFR Signals to mTOR Through PKC and Independently of Akt in Glioma. *Science Signal* 2:ra4
- Feldman ME, Apsel B, Uotila A, Loewith R, Knight ZA, Ruggero D, Shokat KM (2009) Active site inhibitors of mTOR target rapamycin resistant outputs of mTORC1 and mTORC2. *PLoS Biol.* 7:e38
- Filippa N, Sable CL, Hemmings BA, Van Obberghen E (2000) Effect of phosphoinositide dependent kinase 1 on protein kinase B translocation and its subsequent activation. *Mol Cell Biol* 20:5712 5721
- Foukas LC, Beeton CA, Jensen J, Phillips WA, Shepherd PR (2004) Regulation of phosphoinositide 3 kinase by its intrinsic serine kinase activity in vivo. *Mol Cell Biol* 24:966 975
- Fruman DA, Bismuth G (2009) Fine tuning the immune response with PI3K. *Immunol Rev* 228:253 272
- Fruman DA, Meyers RE, Cantley L (1998) Phosphoinositide kinases. *Annu Rev Biochem* 67:481 507
- Garami A, Zwartkruis FJ, Nobukuni T, Joaquin M, Rocco M, Stocker H, Kozma SC, Hafen E, Bos JL, Thomas G (2003) Insulin activation of Rheb, a mediator of mTOR/S6K/4E BP signaling, is inhibited by TSC1 and 2. *Mol Cell* 11:1457 1466
- Garcia Echeverria C, Sellers WR (2008) Drug discovery approaches targeting the PI3K/Akt pathway in cancer. *Oncogene* 27:5511 5526
- García Martínez JM, Moran J, Clarke RG, Gray A, Cosulich SC, Chresta CM, Alessi DR (2009) Ku 0063794 is a specific inhibitor of the mammalian target of rapamycin (mTOR). *Biochem J* 421:29 42
- Geering B, Cutillas PR, Nock G, Gharbi SI, Vanhaesebroeck B (2007) Class IA phosphoinositide 3 kinases are obligate p85 p110 heterodimers. *Proc Natl Acad Sci USA* 104:7809 7814
- Ghigo A, Hirsch E (2008) Isoform selective phosphoinositide 3 kinase gamma and delta inhibitors and their therapeutic potential. *Recent Pat Inflamm Allergy Drug Discov* 2:1 10
- Graupera M, Guillermet Guibert J, Foukas LC, Phng LK, Cain RJ, Salpekar A, Pearce W, Meek S, Millan J, Cutillas PR, Smith AJ, Ridley AJ, Ruhrberg C, Gerhardt H, Vanhaesebroeck B (2008) Angiogenesis selectively requires the p110alpha isoform of PI3K to control endothelial cell migration. *Nature* 453:662 666
- Guertin DA, Sabatini DM (2007) Defining the role of mTOR in cancer. *Cancer Cell* 12:9 22
- Guertin DA, Sabatini DM (2009) The pharmacology of mTOR inhibition. *Science Signal* 2:pe24
- Guertin DA, Stevens DM, Thoreen CC, Burds AA, Kalaany NY, Moffat J, Brown M, Fitzgerald KJ, Sabatini DM (2006) Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signaling to Akt FOXO and PKCalpha, but not S6K1. *Dev Cell* 11:859 871
- Guillermet Guibert J, Bjorklof K, Salpekar A, Gonella C, Ramadani F, Bilancio A, Meek S, Smith AJ, Okkenhaug K, Vanhaesebroeck B (2008) The p110beta isoform of phosphoinositide 3 kinase signals downstream of G protein coupled receptors and is functionally redundant with p110gamma. *Proc Natl Acad Sci USA* 105:8292 8297
- Gymnopoulos M, Elsliger MA, Vogt PK (2007) Rare cancer specific mutations in PIK3CA show gain of function. *Proc Natl Acad Sci USA* 104:5569 5574
- Hawkins PT, Anderson KE, Davidson K, Stephens LR (2006) Signalling through Class I PI3Ks in mammalian cells. *Biochem Soc Trans* 34:647 662

- He X, Zhu Z, Johnson C, Stoops J, Eaker A, Bowen W, Defrances M (2008) PIK3IP1, a negative regulator of PI3K, suppresses the development of hepatocellular carcinoma. *Cancer Res* 68:5591-5598
- Hickey FB, Cotter TG (2006) BCR ABL regulates phosphatidylinositol 3 kinase p110gamma transcription and activation and is required for proliferation and drug resistance. *J Biol Chem* 281:2441-2450
- Hirsch E, Katanaev VL, Garlanda C, Azzolino O, Pirola L, Silengo L, Sozzani S, Mantovani A, Altruda F, Wymann MP (2000) Central role for G protein coupled phosphoinositide 3 kinase gamma in inflammation. *Science* 287:1049-1053
- Huang J, Manning BD (2009) A complex interplay between Akt, TSC2 and the two mTOR complexes. *Biochem Soc Trans* 37:217-222
- Huang CH, Mandelker D, Schmidt Kitterer O, Samuels Y, Velculescu VE, Kinzler KW, Vogelstein B, Gabelli SB, Amzel LM (2007) The structure of a human p110alpha/p85alpha complex elucidates the effects of oncogenic PI3Kalpha mutations. *Science* 318:1744-1748
- Huang CH, Mandelker D, Gabelli SB, Amzel LM (2008) Insights into the oncogenic effects of PIK3CA mutations from the structure of p110alpha/p85alpha. *Cell Cycle* 7:1151-1156
- Huang J, Wu S, Wu CL, Manning BD (2009) Signaling events downstream of mammalian target of rapamycin complex 2 are attenuated in cells and tumors deficient for the tuberous sclerosis complex tumor suppressors. *Cancer Res* 69:6107-6114
- Ikenoue T, Kanai F, Hikiba Y, Obata T, Tanaka Y, Imamura J, Ohta M, Jazag A, Guleng B, Tateishi K, Asaoka Y, Matsumura M, Kawabe T, Omata M (2005) Functional analysis of PIK3CA gene mutations in human colorectal cancer. *Cancer Res* 65:4562-4567
- Inoki K, Li Y, Zhu T, Wu J, Guan KL (2002) TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nat Cell Biol* 4:648-657
- Inoki K, Li Y, Xu T, Guan KL (2003) Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes Dev* 17:1829-1834
- Isakoff SJ, Engelman JA, Irie HY, Luo J, Brachmann SM, Pearlman RV, Cantley LC, Brugge JS (2005) Breast cancer associated PIK3CA mutations are oncogenic in mammary epithelial cells. *Cancer Res* 65:10992-11000
- Izumi H, Imamura T, Nagatani G, Ise T, Murakami T, Uramoto H, Torigoe T, Ishiguchi H, Yoshida Y, Nomoto M, Okamoto T, Uchiyama T, Kuwano M, Funa K, Kohno K (2001) Y box binding protein 1 binds preferentially to single stranded nucleic acids and exhibits 3' to 5' exonuclease activity. *Nucleic Acids Res* 29:1200-1207
- Jacinto E, Loewith R, Schmidt A, Lin S, Ruegg MA, Hall A, Hall MN (2004) Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat Cell Biol* 6:1122-1128
- Jaiswal BS, Janakiraman V, Kljavin NM, Chaudhuri S, Stern HM, Wang W, Kan Z, Dbouk HA, Peters BA, Waring P, Dela Vega T, Kenski DM, Bowman KK, Lorenzo M, Li H, Wu J, Modrusan Z, Stinson J, Eby M, Yue P, Kaminker JS, de Sauvage FJ, Backer JM, Seshagiri S (2009a) Somatic mutations in p85alpha promote tumorigenesis through class IA PI3K activation. *Cancer Cell* 16:463-474
- Jaiswal BS, Janakiraman V, Kljavin NM, Eastham Anderson J, Cupp JE, Liang Y, Davis DP, Hoefflich KP, Seshagiri S (2009b) Combined targeting of BRAF and CRAF or BRAF and PI3K effector pathways is required for efficacy in NRAS mutant tumors. *PLoS ONE* 4:e5717
- Ji H, Rintelen F, Waltzinger C, Bertschy Meier D, Bilancio A, Pearce W, Hirsch E, Wymann MP, Rückle T, Camps M, Vanhaesebroeck B, Okkenhaug K, Rommel C (2007) Inactivation of PI3Kgamma and PI3Kdelta distorts T cell development and causes multiple organ inflammation. *Blood* 110:2940-2947
- Jia S, Liu Z, Zhang S, Liu P, Zhang L, Lee SH, Zhang J, Signoretti S, Loda M, Roberts TM, Zhao JJ (2008) Essential roles of PI(3)K p110beta in cell growth, metabolism and tumorigenesis. *Nature* 454:776-779
- Jiang H, Vogt PK (2008) Constitutively active Rheb induces oncogenic transformation. *Oncogene* 27:5729-5740

- Jiang B, Zheng JZ, Aoki M, Vogt PK (2000) Phosphatidylinositol 3 kinase signaling mediates angiogenesis and expression of vascular endothelial growth factor in endothelial cells. *Proc Natl Acad Sci USA* 97:1749 1753
- Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, Hong SM, Fu B, Lin MT, Calhoun ES, Kamiyama M, Walter K, Nikolskaya T, Nikolsky Y, Hartigan J, Smith DR, Hidalgo M, Leach SD, Klein AP, Jaffee EM, Goggins M, Maitra A, Jacobuzio Donahue C, Eshleman JR, Kern SE, Hruban RH, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW (2008) Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* 321:1801 1806
- Jou ST, Carpino N, Takahashi Y, Piekorz R, Chao JR, Carpino N, Wang D, Ihle JN (2002) Essential, nonredundant role for the phosphoinositide 3 kinase p110delta in signaling by the B cell receptor complex. *Mol Cell Biol* 22:8580 8591
- Kang S, Bader AG, Vogt PK (2005) Phosphatidylinositol 3 kinase mutations identified in human cancer are oncogenic. *Proc Natl Acad Sci USA* 102:802 807
- Kang S, Denley A, Vanhaesebroeck B, Vogt PK (2006) Oncogenic transformation induced by the p110beta, gamma, and delta isoforms of class I phosphoinositide 3 kinase. *Proc Natl Acad Sci USA* 103:1289 1294
- Kaplan DR, Pallas DC, Morgan W, Schaffhausen B, Roberts TM (1989) Mechanisms of transformation by polyoma virus middle T antigen. *Biochim Biophys Acta* 948:345 364
- Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument Bromage H, Tempst P, Sabatini DM (2002) mTOR interacts with raptor to form a nutrient sensitive complex that signals to the cell growth machinery. *Cell* 110:163 175
- Klippel A, Kavanaugh WM, Pot D, Williams LT (1997) A specific product of phosphatidylinositol 3 kinase directly activates the protein kinase Akt through its pleckstrin homology domain. *Mol Cell Biol* 17:338 344
- Knobbe CB, Trampe Kieslich A, Reifenberger G (2005) Genetic alteration and expression of the phosphoinositide 3 kinase/Akt pathway genes PIK3CA and PIKE in human glioblastomas. *Neuropathol Appl Neurobiol* 31:486 490
- Kobayashi T, Minowa O, Kuno J, Mitani H, Hino O, Noda T (1999) Renal carcinogenesis, hepatic hemangiomatosis, and embryonic lethality caused by a germ line Tsc2 mutation in mice. *Cancer Res* 59:1206 1211
- Kong D, Yamori T (2008) Phosphatidylinositol 3 kinase inhibitors: promising drug candidates for cancer therapy. *Cancer Sci* 99:1734 1740
- Kwiatkowski DJ, Manning BD (2005) Tuberous sclerosis: a GAP at the crossroads of multiple signaling pathways. *Hum Mol Genet* 14 Spec No. 2:R251 R258
- Kwiatkowski DJ, Zhang H, Bandura JL, Heiberger KM, Glogauer M, el Hashemite N, Onda H (2002) A mouse model of TSC1 reveals sex dependent lethality from liver hemangiomas, and up regulation of p70S6 kinase activity in Tsc1 null cells. *Hum Mol Genet* 11:525 534
- Laffargue M, Calvez R, Finan P, Trifilieff A, Barbier M, Altruda F, Hirsch E, Wymann MP (2002) Phosphoinositide 3 kinase gamma is an essential amplifier of mast cell function. *Immunity* 16:441 451
- Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliareis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R (1997) PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* 275:1943 1947
- Liu Z, Roberts TM (2006) Human tumor mutants in the p110alpha subunit of PI3K. *Cell Cycle* 5:675 677
- Low S, Vougioukas VI, Hielscher T, Schmidt U, Unterberg A, Halatsch ME (2008) Pathogenetic pathways leading to glioblastoma multiforme: association between gene expressions and resistance to erlotinib. *Anticancer Res* 28:3729 3732
- MacDonald GH, Itoh Lindstrom Y, Ting JP (1995) The transcriptional regulatory protein, YB 1, promotes single stranded regions in the DRA promoter. *J Biol Chem* 270:3527 3533

- Maehama T, Dixon JE (1998) The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3, 4, 5 trisphosphate. *J Biol Chem* 273: 13375-13378
- Maira S, Voliva C, García Echeverría C (2008) Class IA phosphatidylinositol 3 kinase: from their biologic implication in human cancers to drug discovery. *Expert Opin Ther Targets* 12:223-238
- Malagu K, Duggan H, Menear K, Hummersone M, Gomez S, Bailey C, Edwards P, Drzewiecki J, Leroux F, Quesada MJ, Hermann G, Maine S, Molyneaux CA, Le Gall A, Pullen J, Hickson I, Smith L, Maguire S, Martin N, Smith G, Pass M (2009) The discovery and optimisation of pyrido[2,3-d]pyrimidine 2,4-diamines as potent and selective inhibitors of mTOR kinase. *Bioorg Med Chem Lett* 19:5950-5953
- Manning BD, Tee AR, Logsdon MN, Blenis J, Cantley LC (2002) Identification of the tuberous sclerosis complex 2 tumor suppressor gene product tuberlin as a target of the phosphoinositide 3 kinase/akt pathway. *Mol Cell* 10:151-162
- Marcotte L, Crino PB (2006) The neurobiology of the tuberous sclerosis complex. *Neuromolecular Med* 8:531-546
- Marone R, Cmiljanovic V, Giese B, Wymann MP (2008) Targeting phosphoinositide 3 kinase: moving towards therapy. *Biochim Biophys Acta* 1784:159-185
- McManus EJ, Collins BJ, Ashby PR, Prescott AR, Murray Tait V, Armit LJ, Arthur JS, Alessi DR (2004) The in vivo role of PtdIns(3,4,5)P₃ binding to PDK1 PH domain defined by knockin mutation. *EMBO J* 23:2071-2082
- Memmott RM, Dennis PA (2009) Akt dependent and independent mechanisms of mTOR regulation in cancer. *Cell Signal* 21:656-664
- Mertens PR, Harendza S, Pollock AS, Lovett DH (1997) Glomerular mesangial cell specific transactivation of matrix metalloproteinase 2 transcription is mediated by YB 1. *J Biol Chem* 272:22905-22912
- Milburn CC, Deak M, Kelly SM, Price NC, Alessi DR, van Aalten DM (2003) Binding of phosphatidylinositol 3, 4, 5 trisphosphate to the pleckstrin homology domain of protein kinase B induces a conformational change. *Biochem J* 375:531-538
- Miled N, Yan Y, Hon WC, Perisic O, Zvelebil M, Inbar Y, Schneidman Duhovny D, Wolfson HJ, Backer JM, Williams RL (2007) Mechanism of two classes of cancer mutations in the phosphoinositide 3 kinase catalytic subunit. *Science* 317:239-242
- Mizoguchi M, Nutt CL, Mohapatra G, Louis DN (2004) Genetic alterations of phosphoinositide 3 kinase subunit genes in human glioblastomas. *Brain Pathol* 14:372-377
- Nelander S, Wang W, Nilsson B, She QB, Pratilas C, Rosen N, Gennemark P, Sander C (2008) Models from experiments: combinatorial drug perturbations of cancer cells. *Mol Syst Biol* 4:216
- Neshat MS, Mellinghoff IK, Tran C, Stiles B, Thomas G, Petersen R, Frost P, Gibbons JJ, Wu H, Sawyers CL (2001) Enhanced sensitivity of PTEN deficient tumors to inhibition of FRAP/mTOR. *Proc Natl Acad Sci USA* 98:10314-10319
- Nicholson KM, Anderson NG (2002) The protein kinase B/Akt signalling pathway in human malignancy. *Cell Signal* 14:381-395
- Nowak P, Cole DC, Brooijmans N, Bursavich MG, Curran KJ, Ellingboe JW, Gibbons JJ, Hollander I, Hu Y, Kaplan J, Malwitz DJ, Toral Barza L, Verheijen JC, Zask A, Zhang WG, Yu K (2009) Discovery of potent and selective inhibitors of the mammalian target of rapamycin (mTOR) kinase. *J Med Chem* 52:7081-7089
- Okkenhaug K, Bilancio A, Farjot G, Priddle H, Sancho S, Peskett E, Pearce W, Meek SE, Salpekar A, Waterfield MD, Smith AJ, Vanhaesebroeck B (2002) Impaired B and T cell antigen receptor signaling in p110delta PI 3 kinase mutant mice. *Science* 297:1031-1034
- Okkenhaug K, Bilancio A, Emery JL, Vanhaesebroeck B (2004) Phosphoinositide 3 kinase in T cell activation and survival. *Biochem Soc Trans* 32:332-335
- Ozes ON, Mayo LD, Gustin JA, Pfeffer SR, Pfeffer LM, Donner DB (1999) NF kappaB activation by tumour necrosis factor requires the Akt serine threonine kinase. *Nature* 401:82-85
- Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, Olivi A, McLendon R, Rasheed BA, Keir S, Nikolskaya T, Nikolsky Y, Busam DA, Tekleab H, Diaz LA Jr, Hartigan J, Smith DR, Strausberg RL, Marie SK, Shinjo SM, Yan H,

- Riggins GJ, Bigner DD, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW (2008) An integrated genomic analysis of human glioblastoma multiforme. *Science* 321:1807 1812
- Patton DT, Garçon F, Okkenhaug K (2007) The PI3K p110delta controls T cell development, differentiation and regulation. *Biochem Soc Trans* 35:167 171
- Pirola L, Zvelebil MJ, Bulgarelli Leva G, Van Obberghen E, Waterfield MD, Wymann MP (2001) Activation loop sequences confer substrate specificity to phosphoinositide 3 kinase alpha (PI3Kalpha). Functions of lipid kinase deficient PI3Kalpha in signaling. *J Biol Chem* 276:21544 21554
- Podsypanina K, Lee RT, Politis C, Hennessy I, Crane A, Puc J, Neshat M, Wang H, Yang L, Gibbons J, Frost P, Dreisbach V, Blenis J, Gaciong Z, Fisher P, Sawyers C, Hedrick Ellenson L, Parsons R (2001) An inhibitor of mTOR reduces neoplasia and normalizes p70/S6 kinase activity in Pten+/- mice. *Proc Natl Acad Sci USA* 98:10320 10325
- Roche S, Downward J, Raynal P, Courtneidge SA (1998) A function for phosphatidylinositol 3 kinase beta (p85alpha p110beta) in fibroblasts during mitogenesis: requirement for insulin and lysophosphatidic acid mediated signal transduction. *Mol Cell Biol* 18:7119 7129
- Rodriguez Borlado L, Barber DF, Hernandez C, Rodriguez Marcos MA, Sanchez A, Hirsch E, Wymann M, Martinez AC, Carrera AC (2003) Phosphatidylinositol 3 kinase regulates the CD4/CD8 T cell differentiation ratio. *J Immunol* 170:4475 4482
- Rodriguez Viciano P, Warne PH, Vanhaesebroeck B, Waterfield MD, Downward J (1996) Activation of phosphoinositide 3 kinase by interaction with Ras and by point mutation. *EMBO J* 15:2442 2451
- Sakurai H, Chiba H, Miyoshi H, Sugita T, Toriumi W (1999) I kappaB kinases phosphorylate NF kappaB p65 subunit on serine 536 in the transactivation domain. *J Biol Chem* 274:30353 30356
- Samuels Y, Velculescu VE (2004) Oncogenic mutations of PIK3CA in human cancers. *Cell Cycle* 3:1221 1224
- Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell SM, Riggins GJ, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Velculescu VE (2004) High frequency of mutations of the PIK3CA gene in human cancers. *Science* 304:554
- Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar Peled L, Sabatini DM (2008) The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science* 320:1496 1501
- Sarbassov DD, Ali SM, Kim DH, Guertin DA, Latek RR, Erdjument Bromage H, Tempst P, Sabatini DM (2004) Rictor, a novel binding partner of mTOR, defines a rapamycin insensitive and raptor independent pathway that regulates the cytoskeleton. *Curr Biol* 14:1296 1302
- Sarbassov DD, Guertin DA, Ali SM, Sabatini DM (2005) Phosphorylation and regulation of Akt/PKB by the rictor mTOR complex. *Science* 307:1098 1101
- Sarbassov DD, Ali SM, Sengupta S, Sheen JH, Hsu PP, Bagley AF, Markhard AL, Sabatini DM (2006) Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Mol Cell* 22:159 168
- Sasaki T, Irie Sasaki J, Jones RG, Oliveira dos Santos AJ, Stanford WL, Bolon B, Wakeham A, Itie A, Bouchard D, Kozieradzki I, Joza N, Mak TW, Ohashi PS, Suzuki A, Penninger JM (2000) Function of PI3Kgamma in thymocyte development, T cell activation, and neutrophil migration. *Science* 287:1040 1046
- Schaffhausen BS, Roberts TM (2009) Lessons from polyoma middle T antigen on signaling and transformation: a DNA tumor virus contribution to the war on cancer. *Virology* 384:304 316
- Shekar SC, Wu H, Fu Z, Yip SC, Nagajyothi CSM, Girvin ME, Backer JM (2005) Mechanism of constitutive phosphoinositide 3 kinase activation by oncogenic mutants of the p85 regulatory subunit. *J Biol Chem* 280:27850 27855
- Stephens L, Hawkins PT, Eguinoa A, Cooke F (1996) A heterotrimeric GTPase regulated isoform of PI3K and the regulation of its potential effectors. *Philos Trans R Soc Lond B Biol Sci* 351:211 215

- Stephens LR, Eguinoa A, Erdjument Bromage H, Lui M, Cooke F, Coadwell J, Smrcka AS, Thelen M, Cadwallader K, Tempst P, Hawkins PT (1997) The G beta gamma sensitivity of a PI3K is dependent upon a tightly associated adaptor, p101. *Cell* 89:105-114
- Stephens L, Williams R, Hawkins P (2005) Phosphoinositide 3 kinases as drug targets in cancer. *Curr Opin Pharmacol* 5:357-365
- Stoyanov B, Volinia S, Hanck T, Rubio I, Loubtchenkov M, Malek D, Stoyanova S, Vanhaesebroeck B, Dhand R, Nürnberg B (1995) Cloning and characterization of a G protein activated human phosphoinositide 3 kinase. *Science* 269:690-693
- Sugimoto Y, Whitman M, Cantley L, Erikson RL (1984) Evidence that the Rous sarcoma virus transforming gene product phosphorylates phosphatidylinositol and diacylglycerol. *Proc Natl Acad Sci USA* 81:2117-2121
- Sugita H, Dan S, Kong D, Tomida A, Yamori T (2008) A new evaluation method for quantifying PI3K activity by HTRF assay. *Biochem Biophys Res Commun* 377:941-945
- Sujobert P, Bardet V, Cornillet Lefebvre P, Hayflick JS, Prie N, Verdier F, Vanhaesebroeck B, Muller O, Pesce F, Ifrah N, Hunault Berger M, Berthou C, Villemagne B, Jourdan E, Audhuy B, Solary E, Witz B, Harousseau JL, Himmerlin C, Lamy T, Lioure B, Cahn JY, Dreyfus F, Mayeux P, Lacombe C, Bouscary D (2005) Essential role for the p110delta isoform in phosphoinositide 3 kinase activation and cell proliferation in acute myeloid leukemia. *Blood* 106:1063-1066
- Sutherland BW, Kucab J, Wu J, Lee C, Cheang MC, Yorida E, Turbin D, Dedhar S, Nelson C, Pollak M, Leighton Grimes H, Miller K, Badve S, Huntsman D, Blake Gilks C, Chen M, Pallen CJ, Dunn SE (2005) Akt phosphorylates the Y box binding protein 1 at Ser102 located in the cold shock domain and affects the anchorage independent growth of breast cancer cells. *Oncogene* 24:4281-4292
- Svitkin YV, Evdokimova VM, Brasey A, Pestova TV, Fantus D, Yanagiya A, Imataka H, Skabkin MA, Ovchinnikov LP, Merrick WC, Sonenberg N (2009) General RNA binding proteins have a function in poly(A) binding protein dependent translation. *EMBO J* 28:58-68
- Tee AR, Manning BD, Roux PP, Cantley LC, Blenis J (2003) Tuberous sclerosis complex gene products, Tuberin and Hamartin, control mTOR signaling by acting as a GTPase activating protein complex toward Rheb. *Curr Biol* 13:1259-1268
- Thoreen CC, Kang SA, Chang JW, Liu Q, Zhang J, Gao Y, Reichling LJ, Sim T, Sabatini DM, Gray NS (2009) An ATP competitive mammalian target of rapamycin inhibitor reveals rapamycin resistant functions of mTORC1. *J Biol Chem* 284:8023-8032
- Torbett NE, Luna Moran A, Knight ZA, Houk A, Moasser M, Weiss W, Shokat KM, Stokoe D (2008) A chemical screen in diverse breast cancer cell lines reveals genetic enhancers and suppressors of sensitivity to PI3K isoform selective inhibition. *Biochem J* 415:97-110
- Utermark T, Schaffhausen BS, Roberts TM, Zhao JJ (2007) The p110alpha isoform of phosphatidylinositol 3 kinase is essential for polyomavirus middle T antigen mediated transformation. *J Virol* 81:7069-7076
- Vanhaesebroeck B, Alessi DR (2000) The PI3K/PDK1 connection: more than just a road to PKB. *Biochem J* 346(pt 3):561-576
- Vanhaesebroeck B, Waterfield MD (1999) Signaling by distinct classes of phosphoinositide 3 kinases. *Exp Cell Res* 253:239-254
- Vanhaesebroeck B, Leevers SJ, Panayotou G, Waterfield MD (1997) Phosphoinositide 3 kinases: a conserved family of signal transducers. *Trends Biochem Sci* 22:267-272
- Vanhaesebroeck B, Leevers SJ, Ahmadi K, Timms J, Katso R, Driscoll PC, Woscholski R, Parker PJ, Waterfield MD (2001) Synthesis and function of 3 phosphorylated inositol lipids. *Annu Rev Biochem* 70:535-602
- Vanhaesebroeck B, Ali K, Bilancio A, Geering B, Foukas LC (2005) Signalling by PI3K isoforms: insights from gene targeted mice. *Trends Biochem Sci* 30:194-204
- Vasudevan KM, Barbie DA, Davies MA, Rabinovsky R, McNear CJ, Kim JJ, Hennessy BT, Tseng H, Pochanard P, Kim SY, Dunn IF, Schinzel AC, Sandy P, Hoersch S, Sheng Q, Gupta PB, Boehm JS, Reiling JH, Silver S, Lu Y, Stemke Hale K, Dutta B, Joy C, Sahin AA, Gonzalez Angulo AM, Lluch A, Rameh LE, Jacks T, Root DE, Lander ES, Mills GB, Hahn WC, Sellers WR, Garraway LA (2009) AKT independent signaling downstream of oncogenic PIK3CA mutations in human cancer. *Cancer Cell* 16:21-32

- Walker EH, Perisic O, Ried C, Stephens L, Williams RL (1999) Structural insights into phosphoinositide 3 kinase catalysis and signalling. *Nature* 402:313–320
- Wee S, Wiederschain D, Maira SM, Loo A, Miller C, deBeaumont R, Stegmeier F, Yao YM, Lengauer C (2008) PTEN deficient cancers depend on PIK3CB. *Proc Natl Acad Sci USA* 105:13057–13062
- Whitman M, Kaplan DR, Schaffhausen B, Cantley L, Roberts TM (1985) Association of phosphatidylinositol kinase activity with polyoma middle T competent for transformation. *Nature* 315:239–242
- Wood LD, Parsons DW, Jones S, Lin J, Sjoblom T, Leary RJ, Shen D, Boca SM, Barber T, Ptak J, Silliman N, Szabo S, Dezso Z, Ustyanksky V, Nikolskaya T, Nikolsky Y, Karchin R, Wilson PA, Kaminker JS, Zhang Z, Croshaw R, Willis J, Dawson D, Shipitsin M, Willson JK, Sukumar S, Polyak K, Park BH, Pethiyagoda CL, Pant PV, Ballinger DG, Sparks AB, Hartigan J, Smith DR, Suh E, Papadopoulos N, Buckhaults P, Markowitz SD, Parmigiani G, Kinzler KW, Velculescu VE, Vogelstein B (2007) The genomic landscapes of human breast and colorectal cancers. *Science* 318:1108–1113
- Wu H, Shekar SC, Flinn RJ, El Sibai M, Jaiswal BS, Sen KI, Janakiraman V, Seshagiri S, Gerfen GJ, Girvin ME, Backer JM (2009) Regulation of Class IA PI 3 kinases: C2 domain iSH2 domain contacts inhibit p85/p110alpha and are disrupted in oncogenic p85 mutants. *Proc Natl Acad Sci USA*. 106:20258–20263
- Wymann MP, Schneider R (2008) Lipid signalling in disease. *Nat Rev Mol Cell Biol* 9:162–176
- Yap T, Garrett M, Walton M, Raynaud F, Debono J, Workman P (2008) Targeting the PI3K/AKT/mTOR pathway: progress, pitfalls, and promises. *Curr Opin Pharmacol* 8:393–412
- Yart A, Chap H, Raynal P (2002) Phosphoinositide 3 kinases in lysophosphatidic acid signaling: regulation and cross talk with the Ras/mitogen activated protein kinase pathway. *Biochim Biophys Acta* 1582:107–111
- Yu K, Toral Barza L, Shi C, Zhang WG, Lucas J, Shor B, Kim J, Verheijen J, Curran K, Malwitz DJ, Cole DC, Ellingboe J, Ayril Kaloustian S, Mansour TS, Gibbons JJ, Abraham RT, Nowak P, Zask A (2009) Biochemical, cellular, and in vivo activity of novel ATP competitive and selective inhibitors of the mammalian target of rapamycin. *Cancer Res* 69:6232–6240
- Yuan TL, Cantley LC (2008) PI3K pathway alterations in cancer: variations on a theme. *Oncogene* 27:5497–5510
- Zasedateleva OA, Krylov AS, Prokopenko DV, Skabkin MA, Ovchinnikov LP, Kolchinsky A, Mirzabekov AD (2002) Specificity of mammalian Y box binding protein p50 in interaction with ss and ds DNA analyzed with generic oligonucleotide microchip. *J Mol Biol* 324:73–87
- Zask A, Verheijen JC, Curran K, Kaplan J, Richard DJ, Nowak P, Malwitz DJ, Brooijmans N, Bard J, Svenson K, Lucas J, Toral Barza L, Zhang WG, Hollander I, Gibbons JJ, Abraham RT, Ayril Kaloustian S, Mansour TS, Yu K (2009) ATP Competitive Inhibitors of the Mammalian Target of Rapamycin: Design and Synthesis of Highly Potent and Selective Pyrazolopyrimidines. *J Med Chem* 52:5013–5016
- Zhang H, Cicchetti G, Onda H, Koon HB, Asrican K, Bajraszewski N, Vazquez F, Carpenter CL, Kwiatkowski DJ (2003a) Loss of Tsc1/Tsc2 activates mTOR and disrupts PI3K/Akt signaling through downregulation of PDGFR. *J Clin Invest* 112:1223–1233
- Zhang Y, Gao X, Saucedo LJ, Ru B, Edgar BA, Pan D (2003b) Rheb is a direct target of the tuberous sclerosis tumour suppressor proteins. *Nat Cell Biol* 5:578–581
- Zhang H, Liu G, Dziubinski M, Yang Z, Ethier SP, Wu G (2008) Comprehensive analysis of oncogenic effects of PIK3CA mutations in human mammary epithelial cells. *Breast Cancer Res Treat* 112:217–227
- Zhao JJ, Roberts TM (2006) PI3 kinases in cancer: from oncogene artifact to leading cancer target. *Sci STKE* 2006:pe52
- Zhao L, Vogt P (2008a) Class I PI3K in oncogenic cellular transformation. *Oncogene* 27:5486–5496

- Zhao L, Vogt PK (2008b) Helical domain and kinase domain mutations in p110alpha of phosphatidylinositol 3 kinase induce gain of function by different mechanisms. *Proc Natl Acad Sci USA* 105:2652-2657
- Zhao JJ, Liu Z, Wang L, Shin E, Loda MF, Roberts TM (2005) The oncogenic properties of mutant p110alpha and p110beta phosphatidylinositol 3 kinases in human mammary epithelial cells. *Proc Natl Acad Sci USA* 102:18443-18448

AKT Signaling in Physiology and Disease

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Abstract The serine/threonine kinase AKT functions as a critical mediator of signaling downstream of PI3 kinase. Studies over the last two decades have firmly established the importance of AKT in the regulation of cell survival, proliferation, and insulin-dependent metabolic cell responses. AKT executes these diverse tasks through phosphorylation of numerous cellular substrates. Substantial progress has been made in understanding the regulation of AKT activity by upstream kinases and elucidating downstream mechanisms that mediate its myriad cellular effects. Here, we present an overview of AKT regulation and function in physiological and pathological settings. An emphasis is placed on the involvement of aberrant AKT signaling in human diseases ranging from diabetes to cancer and neurological diseases.

1 Introduction

The phosphatidylinositol-3-kinase (PI3K) signaling pathway underpins many aspects of cell growth, survival, and metabolism (Engelman et al. 2006; Hennessy et al. 2005). As described in other chapters, activated PI3Ks phosphorylate the 3'-OH position in inositol phospholipids, generating 3'-phosphoinositides (PIs). Products of the PI3Ks include phosphatidylinositol 3,4-bisphosphate (PI-3,4P2) and phosphatidylinositol 3,4,5-trisphosphate (PI-3,4,5P3 or PIP3). These PIs function as second messengers that activate downstream pathways regulating many cell growth, survival, and metabolic processes (Vivanco and Sawyers 2002). Steady-state PIP3 levels are tightly controlled by the combined effects of stringent PI3K regulation and the action of several PIP3 phosphatases, including PTEN, SHIP1 and SHIP2 (Cantley and Neel 1999; Engelman et al. 2006).

Upon generation at the plasma membrane, PI-3,4P2 and PIP3 bind a subset of pleckstrin-homology (PH) and other lipid-binding domains present in target effector proteins to recruit them to activation sites within the plasma membrane. Among these, the serine/threonine kinase AKT has been most extensively studied as a critical PI3K effector. This chapter provides an overview of AKT and its cellular functions downstream of PI3K in biology and disease.

2 AKT Kinases

AKT, also known as protein kinase B (PKB), is a ubiquitously expressed serine/threonine kinase with a PH domain that selectively binds 3-phosphoinositides. AKT is so-named because it represents the proto-oncogene of the v-AKT murine thymoma virus (Staal et al. 1977). Nearly two decades ago, three groups independently identified and cloned the first AKT isoform (Bellacosa et al. 1991; Coffey and Woodgett 1991; Jones et al. 1991). AKT is now well established as the predominant PI3K effector in many cell types. Indeed, phosphorylation and activation of AKT is often used as a surrogate readout of PI3K activity, because direct measurement of 3-phosphoinositide levels is technically more difficult.

2.1 Isoforms

There are three members of AKT gene family, designated as *AKT1* (PKB α), *AKT2* (PKB β) and *AKT3* (PKB γ). In humans, these genes are located at chromosomes 14q32, 19q13, and 1q44, respectively. The AKT family belongs to the more general class of AGC kinases (related to AMP/GMP kinase and protein kinase C). While AKT1 and AKT2 are ubiquitously expressed, AKT3 displays a more restricted tissue distribution and is expressed most abundantly in neuronal tissues (Bellacosa et al. 2004). Each AKT member is activated by similar mechanisms during PI3K signaling (Bellacosa et al. 2005).

2.2 Domain Structure

Each AKT isoform contains an amino terminal PH domain, a short alpha-helical linker, a kinase domain and a carboxyl-terminal regulatory domain (Fig. 1). PH domains are structurally well-characterized modules of approximately 120 amino acids. As noted above, this domain binds phosphatidylinositide products of PI3K. The PH domain also contains a sequence motif defined by a pattern of basic residues centered on the loop between two β -strands (Isakoff et al. 1998). This so-called “headgroup” interacts with a well-defined binding pocket, and multiple

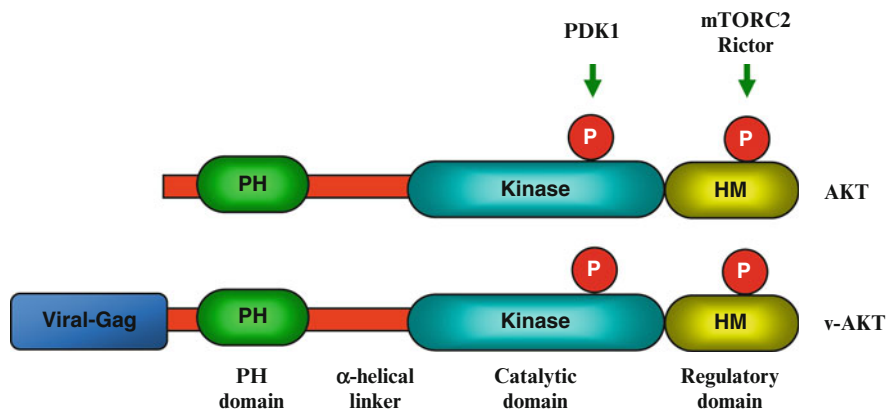


Fig. 1 Domain structure of AKT. All the family members of AKT contains three distinct functional domains namely PH (Pleckstrin Homology) domain at the N terminus, a kinase domain and c terminal regulatory domain that contains hydrophobic motif (HM). The key phosphorylation sites necessary for activation have been depicted. v AKT is the viral form of AKT and is a fusion between the viral Gag and mouse AKT1

hydrogen bonds are formed with available phosphate groups (Lemmon 2003). In general, the AKT PH domain interacts with phosphatidylinositol (3,4,5) triphosphate (PIP3) and phosphatidylinositol (3,4) diphosphate (PI(3,4)P2) with similar affinity (Bellacosa et al. 2005), although some *in vitro* studies suggest that it may interact with PIP2 more strongly than PIP3 (Franke et al. 1997).

The kinase domain of AKT shares high similarity with other AGC kinases such as Protein Kinase A (PKA), Protein Kinase C (PKC), p70 S6 kinase (S6K), Serum and Glucocorticoid-regulated Kinase (SGK), and p90 Ribosomal S6 kinase (RSK). The sequence identity among AKT isoforms exceeds 80% in the kinase domain (Bellacosa et al. 2004). The C-terminus of the AKT kinase domain contains a regulatory hydrophobic motif (HM), a hallmark of all AGC kinases.

3 Mechanisms of AKT Activation

AKT is typically activated upon engagement of receptor tyrosine kinases (RTKs) by peptide growth factors and cytokines. In this case, the critical upstream step required for activation is RTK engagement of PI3K in response to growth factor stimulation. AKT may also be activated by other extracellular stimuli such as oxidative stress. Full AKT activation requires both membrane translocation and phosphorylation. To accomplish this, the AKT PH domain first interacts with 3' phosphoinositides (PI(3,4)P2 or PIP3), thereby directing its recruitment to the plasma membrane. Subsequently, AKT undergoes a conformational change that exposes two crucial amino acids as substrates for phosphorylation and ensuing kinase activation. One of these residues is a threonine at position 308 in AKT1

(Fig. 1) is located in the kinase domain. Thr-308 is phosphorylated by phosphoinositide-dependent kinase 1 (PDK1), which stabilizes the activation loop. The other key residue a serine at position 473 in AKT1 resides in the hydrophobic C-terminal domain (Fig. 1). Ser-473 is phosphorylated primarily by the TORC2 (mTOR-Rictor) complex, although DNA dependent protein kinase (DNA-PK) may also mediate Ser-473 phosphorylation in some cases (Fig. 2). Phosphorylation of

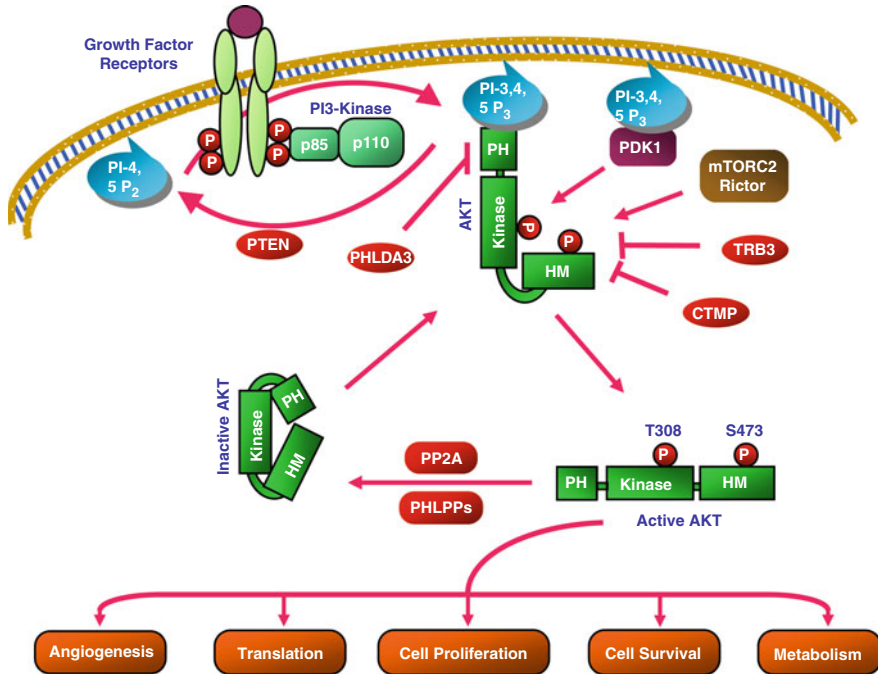


Fig. 2 Model for AKT regulation by growth factors. Activation of cell surface growth factor receptors by ligands such as epidermal growth factor and platelet derived growth factor leads to auto phosphorylation of specific tyrosine residues on the intracellular portion of the receptor through their intrinsic receptor tyrosine kinase (RTK) activity. PI3 kinase then recruited to the plasma membrane, via binding of the SH2 domains of the regulatory subunit (p85) to the phosphorytyrosine residues on the receptor, which leads to a conformational change in the kinase (p110), and consequently to activation. Activated PI3 kinase catalyzes the 3' phosphorylation of phosphoinositides PtdIns(4,5)P2 (PIP2) in the membrane resulting increased production of PtdIns (3,4,5)P3 (PIP3), a key signaling intermediate of the PI3 kinase signaling. PIP3 then mediate the membrane recruitment AKT from the cytosol to the plasma membrane via its PH domain, and change the kinase from an inactive to an active state. AKT is then phosphorylated on Thr 308 and Ser 473 by PDK1, and by mTORC2 Rictor, respectively. Activated AKT mediates its multiple cellular functions by phosphorylating specific substrates (Table 1), and then becomes inactivated by the action of phosphatases such as PP2A and PHLPP1/2 which dephosphorylate at Thr 308 and Ser 473 and return AKT to its inactive conformation in the cytosol. PTEN, a lipid phosphatase, limits AKT activation by dephosphorylating PIP3 covering back to PtdIns(4,5)P2 (PIP2). PHLDA3 is PH domain only protein that interferes with AKT activation by directly competing for the available PIP3 at the membrane. TRB3 and CTMP1 negatively regulate AKT activation by directly interacting with AKT and preventing its phosphorylation

both Thr-308 and Ser-473 is necessary for full AKT activation (Hennessy et al. 2005; Sarbassov et al. 2005); these events are described in detail below.

3.1 *PDK1-Dependent AKT Phosphorylation*

PDK1 is a Ser/Thr kinase ubiquitously expressed in human tissues. This protein consists of an N-terminal kinase domain and a C-terminal PH domain. PDK1 was first identified by its ability to phosphorylate Thr-308 of AKT *in vitro* (Alessi et al. 1997; Stephens et al. 1998; Stokoe et al. 1997). As this activity was absolutely dependent on the inclusion of PtdIns(3,4)P₂ or PtdIns(3,4,5)P₃ in the reaction mixture, this kinase was given the name 3'-phosphoinositide-dependent kinase-1 (Vanhaesebroeck and Alessi 2000). The PDK1 PH domain affinity for PIs appears to be significantly higher than that of AKT. PDK1 exists in an active, phosphorylated confirmation under basal conditions that is largely refractory to additional activation by agonists that up-regulate PI3K. On the other hand, membrane translocation of PDK1 is strongly augmented by PI3K signaling (Anderson et al. 1998). The PH domain-dependent membrane recruitment of AKT and PDK1 upon PI3K activation results in a conformational shift by AKT that facilitates subsequent phosphorylation by PDK1 on Thr-308 (Fig. 2). This event is critical for AKT activation.

3.2 *Hydrophobic Motif Phosphorylation*

As noted above, concomitant phosphorylation at both the HM and the PDK1 site is required for full AKT activation. Prior phosphorylation of Ser-473 may potentiate PDK1-dependent phosphorylation at Thr-308; however, several lines of evidence suggest that Thr-308 phosphorylation may occur independently of Ser-473 (Alessi et al. 1996; Vanhaesebroeck and Alessi 2000). Nonetheless, Ser-473 phosphorylation is itself dynamically regulated in response to growth factor stimulation and PI3K activation, although the detailed mechanisms governing this activation step remain incompletely understood.

In contrast to PDK1, characterization of the AKT Ser-473 kinase remained elusive for several years. As purified or recombinant PDK1 only phosphorylated Thr-308 of AKT and not Ser-473, it stood to reason that Ser-473 phosphorylation would be catalyzed by a distinct kinase, provisionally termed "PDK2". Several lines of evidence have established mTORC2 (the rapamycin-insensitive component of mTOR) and DNA-PK as critical mediators of Ser-473 phosphorylation. Indeed, AKT is phosphorylated by mTORC2 at Ser-473 following various types of growth factor and mitogen stimulation (Sarbassov et al. 2005). Not only does mTORC2 comprise the dominant AKT Ser473 kinase, AKT appears to be the dominant TORC2 substrate. Thus, mTOR present within the mTORC2 complex acts as an AKT activator, whereas the mTORC1 complex (the rapamycin-sensitive

mTOR component) receives stimulatory input downstream of AKT (Bhaskar and Hay 2007).

Under certain conditions of cellular stress such as DNA damage, DNA-dependent protein kinase (DNA-PK) also appears to gain importance as an AKT Ser-473 kinase (Boehme et al. 2008; Bozulic et al. 2008; Feng et al. 2004; Park et al. 2009). Toward this end, AKT translocates to the nucleus under certain conditions, thereby bringing it into proximity with components of the DNA damage response. However, DNA-PK does not appear to play a role in growth factor or insulin-promoted activation of AKT (Bozulic and Hemmings 2009).

Thr-308 phosphorylation stimulates AKT enzymatic activity by at least 100-fold, whereas phosphorylation of Ser-473 provides an additional 7- to 10-fold augmentation of AKT activity (Alessi et al. 1996). Hence, phosphorylation at both residues results in ~1,000-fold increase in AKT kinase activity (Bellacosa et al. 2005).

3.3 Phosphorylation of Other AKT Residues

The contribution of other AKT phosphorylation sites to its activation state is less well characterized. Phosphorylation of certain tyrosine residues (Tyr-315 and Tyr-326 in AKT1) appears to be required for full activation of AKT by EGF (Chen et al. 2001). These sites are phosphorylated by the c-Src tyrosine kinase *in vitro* and *in vivo*, mediated by the interaction of the c-Src SH3 domain with an AKT carboxyl-terminal PXXP motif (Jiang and Qiu 2003). Also, c-Jun N-terminal kinase (JNK) may enable reactivation of AKT after ischemic injury through phosphorylation at Thr-450. Thr-450 phosphorylation following resolution of hypoxia appears to prime AKT for subsequent phosphorylation by PDK1 (Shao et al. 2006). JNK can also negatively regulate AKT signaling through modulation of IRS1 and IRS2 signaling. In this case, phosphorylation of IRS1 on Ser-307 by JNK1 inhibits insulin signaling by decreasing the interaction between IRS1 and the insulin receptor (Aguirre et al. 2002). JNK pathway activation by oxidative stress, cytokines and endoplasmic reticulum (ER) stress dynamically modulates both AKT signaling and β -cell apoptosis (Kaneto et al. 2005).

4 Negative Regulation of AKT Signaling

4.1 Lipid Phosphatases

In contrast to the detailed understanding of the kinase circuitry that activates AKT, less is known about counteracting AKT inactivation. Overall, PI3K/AKT signaling is directly inactivated by the PTEN tumor suppressor protein. PTEN functions as a lipid phosphatase that converts 3'-phosphorylated phosphoinositides to the 3' unphosphorylated form. However, the precise mechanisms by which PTEN is

inactivated upon initiation of PI3K signaling are not completely elucidated. The SHIP inositol 5'phosphatases hydrolyze the conversion of PI(3,4,5)P3 to PI(3,4)P2 and can also negatively regulate AKT activity. SHIP1 is mostly found in hematopoietic cells, while SHIP2 is widely expressed in non-hematopoietic cells (Habib et al. 1998; Ishihara et al. 1999; Kandel and Hay 1999). Overexpression of SHIP1 inhibits AKT activity, and SHIP1-null cells exhibit prolonged activation of AKT following growth factor stimulation (Aman et al. 1998; Liu et al. 1999). The relative roles of PTEN and SHIP1/2 in the regulation of PIP3 levels and AKT activation may be tissue and cell-type specific.

4.2 *AKT-Specific Protein Phosphatases*

AKT signaling is also acutely terminated through direct dephosphorylation. For example, the *PPTR-1* gene, which encodes a member of the B56 family of protein phosphatase 2A (PP2A) regulatory subunits, has been shown to down-regulate AKT activation in *Caenorhabditis elegans* by decreasing its phosphorylation at the activation loop (Padmanabhan et al. 2009). In accordance with this observation, mammalian B56 beta was also shown to regulate AKT phosphorylation at Thr-308 in mammalian cells. PP2A-dependent AKT regulation may be counterbalanced by heat shock protein-90 (HSP90), which protects AKT from dephosphorylation by PP2A (Sato et al. 2000).

PH domain-containing protein phosphatases such as the leucine-rich repeat protein phosphatase PHLPP1 and PHLPP2 have recently been identified as key negative regulators of AKT signaling. PHLPPs specifically dephosphorylate the HM of AKT (Ser-473 in AKT1), resulting in decreased AKT activation (Brognard et al. 2007; Gao et al. 2005). Interestingly, these PHLPPs show selective action against different AKT isoforms. PHLPP1 dephosphorylates only AKT2 and AKT3, while PHLPP2 dephosphorylates AKT1 and AKT3 (Brognard et al. 2007). However, the phosphatase activity of PHLPPs is not limited to AKT, as both enzymes also dephosphorylate the HMs of PKC isoforms (Brognard and Newton 2008).

4.3 *AKT Inhibition by Interacting Proteins*

AKT is also negatively regulated by carboxy-terminal modulator protein (CTMP), which decreases its HM phosphorylation (Maira et al. 2001). AKT may also be inhibited through direct binding of TRB3, a human homolog of the *Drosophila* Tribbles protein (Du et al. 2003). TRB3 expression is induced in the liver under fasting conditions, and this protein disrupts insulin signaling by binding directly to AKT and blocking its activation (Du et al. 2003). Keratin K10, a keratinocyte specific protein, has also been shown to negatively regulate AKT activation by direct interaction (Brazil et al. 2002).

4.4 Lipid Binding PH Domain-Only Proteins

The p53 target gene PHLDA3, which encodes a PH domain-only protein, has been found to negatively regulate AKT signaling through direct competition with the AKT PH domain for binding of membrane lipids. This competition results in the inhibition of AKT membrane translocation (Kawase et al. 2009).

4.5 Feedback Regulation of AKT Signaling

As described above, the primary means of PI3K/AKT pathway activation involves RTKs such as the insulin and insulin-like growth factor I (IGF-I) receptors. However, physiologic activation of these receptors also results in feedback down-regulation of the pathway, mediated in part by loss of insulin receptor substrate-1 (IRS-1) expression (Haruta et al. 2000; Shah and Hunter 2004; Um et al. 2004). IRS-1 and its counterpart IRS-2 are adapter proteins that represent the major substrates of the IGF-1 and insulin receptors responsible for propagation of the signal induced by ligand binding. As described in other chapters, AKT signaling results in downstream activation of mTOR and S6 kinase, which in turn phosphorylate and down-regulate IRS-1, leading to feedback inhibition of the PI3K/AKT activation (O'Reilly et al. 2006). Undoubtedly, negative feedback regulation of AKT signaling occurs by other means as well. Elucidation of detailed negative feedback mechanisms represents an important ongoing research area.

5 AKT Substrates

AKT kinases mediate a wide range of cellular functions, including cell proliferation, survival, modulation of metabolism, and angiogenesis. This plethora of effects is the consequence of phosphorylation of numerous substrate effector proteins by AKT. The minimal consensus site for AKT phosphorylation consists of RXXRXXS/T-B, where X represents any amino acid and B represents a bulky hydrophobic residue (Manning and Cantley 2007). Phosphorylation of many AKT substrates results in altered subcellular localization; indeed, regulation of substrate compartmentalization by AKT appears to be a consequence of phosphorylation near nuclear localization or nuclear export sequences. In some cases, binding of AKT-phosphorylated substrates to 14.3.3 protein may also affect subcellular localization. Over 50 well-validated AKT substrate proteins have been identified so far, and many additional AKT substrates continue to be discovered. A list of the best-characterized AKT substrates and their cellular roles is presented in Table 1. In the following section, a detailed account of AKT substrates is provided with respect to their specific physiological functions in the context of the normal and diseased cell.

Table 1 Key AKT substrates

Substrate protein	Phosphorylation site(s)	Effect of phosphorylation
<i>Glucose homeostatis and metabolism</i>		
AS160	Ser 588 and Thr 642	Stimulates AS160 and GLUT4 trafficking
ATP citrate lyase	Ser 454	Stimulate the enzyme activity
GSK3 α	Ser 21	Inhibits of GSK3 kinase activity
GSK3 β	Ser 9	Inhibits of GSK3 kinase activity
NADPH oxidase	Ser 304 and Ser 328	Increases enzymatic activity
PIKfyve kinase	Ser 318	Stimulates the kinase /GLUT4 trafficking
PFK 2	Ser 466 and Ser 483	Activates of PFK 2
PGC 1 α	Ser 570	Inhibits its transcriptional co function
Phosphodiesterase 3B	Ser 273	Increases enzymatic activity
PTP1B	Ser 50	Inhibits its phosphatase activity
<i>Cell proliferation</i>		
Androgen receptor	Ser 210 and Ser 790	Inhibits of its transcriptional activity
BRCA1	Thr 509	Prevents nuclear accumulation/activity?
EZH2	Ser 21	Suppresses its methyltransferase activity
Mdm2 (Hdm2)	Ser 166 and Ser 186	Prevention of Mdm2 degradation
p21	Thr 145	Nuclear exclusion and cell cycle entry
p27	Thr 157	Nuclear exclusion and cell cycle entry
WNK1	Thr 60	Induces mitogenesis by insulin
<i>Cell survival</i>		
ARK5	Ser 600	Increased activity/apoptosis prevention
ASK1	Ser 83	Prevents apoptosis induced by ASK1
BAD	Ser 136	Prevents apoptosis induced by BAD
CHK1	Ser 280	Inhibits its function/nuclear exclusion
Caspase 9	Ser 196	Prevents Caspase 9 activation
FoxO1	Thr 24, Ser 256, and Ser 319	Inhibits its transcriptional activity
FoxO3A	Thr 32, Ser 253, and Ser 315	Inhibits its transcriptional activity
FoxO4	Thr 28, Ser 193, and Ser 258	Inhibits its transcriptional activity
Par 4	Ser 249 (Rat Par 4 only)	Inhibits apoptosis by Par 4
PED/PEA 15	Ser 116	Prevents its degradation/promotes survival
SEK1/MKMKK4	Ser 78	SEK1 inactivation/apoptosis prevention
Nurr 77	Ser 350	Suppresses its apoptotic activity
<i>Cell growth and protein translation</i>		
mTORC1 Raptor	Ser 2448	Activates mTORC1 kinase activity
PRAS40	Thr 246	Relieves its inhibitory action on mTORC1
TSC2	Ser 939 and Thr 1462	Relieves its inhibitory action on mTORC1
<i>Cell migration and invasion</i>		
c Raf	Ser 259	Inactivates of Raf MAPK signaling
b Raf	Ser 364, Ser 428, and Thr 439	Inactivates of Raf MAPK signaling
EDG 1	Thr 236	increased endothelial cell migration
Rac 1	Ser 71	Inhibits of Rac 1 GTP binding
<i>Angiogenesis</i>		
eNOS	Ser 1177	Nitric oxide accumulation/vasodilation
<i>Immunity</i>		
IKK α	Thr 23	Activates IKK α and NF κ B
Tpl 2 (Cot)	Ser 400	Activates IKK and NF κ B

(continued)

Table 1 (continued)

Substrate protein	Phosphorylation site(s)	Effect of phosphorylation
<i>Neurological function</i>		
Arfaptin 2	Ser 260	Prevents neuronal cell apoptosis
Ataxin 1	Ser 776	Increased protein accumulation/apoptosis
CREB	Ser 133	Increased binding with co activators
GABA(A)R	Ser 410	Increased surface expression at synapses
Huntingtin	Ser 421	Prevents apoptosis by Huntingtin

6 AKT Signaling in Physiology

6.1 Glucose Homeostasis and Metabolism

One of the best known functions of AKT is its crucial role in glucose metabolism. Of the three AKT isoforms, AKT2 is most strongly associated with regulation of glucose homeostasis and represents the predominant isoform expressed in insulin responsive tissues. AKT2 null mice are characterized by defective insulin-stimulated glucose uptake in muscle and adipose tissue, as well as failure to suppress glucose output (Cho et al. 2001). As a result, these mice exhibit glucose intolerance, insulin resistance, and ultimately the development of severe diabetes accompanied by pancreatic β -cell failure. In contrast, both *AKT1* and *AKT3* null mice show normal glucose homeostasis (Cho et al. 2001; Dummler et al. 2006; Easton et al. 2005).

At the cellular level, insulin increases glucose uptake into muscle and fat cells by initiating translocation of the glucose transporter GLUT4 from intracellular storage vesicles to the cell surface. The specific effects of AKT on GLUT4 translocation are mediated at least in part by the AKT substrate AS160 (AKT substrate 160 kDa). AS160 contains a GAP (GTPase-activating protein) domain for Rab proteins, and phosphorylation of AS160 by AKT inhibits its GAP activity. (The Rab protein which is regulated by AS160 has yet to be identified.) Rab proteins are involved in the regulation of membrane trafficking and therefore provide a likely link between GLUT4 translocation and AS160 phosphorylation (Watson and Pessin 2006; Dummler and Hemmings 2007). PIKfyve kinase is another AKT substrate implicated in GLUT4 translocation. PIKfyve may promote sorting of GLUT4 from internalized endosomes into GLUT4 storage vesicles (Karlsson et al. 2005). AKT activation also leads to increased expression of another glucose transporter GLUT1. GLUT1 is the main glucose transporter in most cell types and is primarily regulated at the level of gene transcription. Activation of mTORC1 through AKT results in HIF1 α dependent transcription of GLUT1 and cap-dependent translation of GLUT1 mRNA (Barthel et al. 1999; Taha et al. 1999).

In addition to the regulation of glucose uptake, AKT signaling regulates glucose and lipid metabolism at other levels. For example, the AKT substrate GSK3 β , which is inactivated by phosphorylation, regulates glycogen synthesis (GSK3 β inactivation leads to increased glycogen accumulation). AKT phosphorylation

and inhibition of GSK3 β also promotes Sterol Regulatory Element Binding Proteins (SREBPs), which induces transcription of genes involved in cholesterol and fatty acid biosynthesis (Manning and Cantley 2007). Similarly, AKT phosphorylation of phosphofructokinase stimulates glycolysis. Further, AKT activation enhances the association of hexokinase isoforms with the mitochondria, where they more readily phosphorylate glucose (Gottlob et al. 2001). Glucose 6-phosphate can be stored by conversion to glycogen or catabolized to produce cellular energy through glycolysis; AKT signaling regulates both these processes (Manning and Cantley 2007). Moreover, through regulation of the Forkhead transcription factor FOXO1, AKT inhibits hepatic glucose production. In hepatocytes, AKT inhibits gluconeogenesis and fatty acid oxidation through direct phosphorylation of PGC-1 α , a co-activator of FOXO1 (Li et al. 2007). Thus AKT regulates many facets of glucose and lipid metabolism.

6.2 Cell Proliferation

Consistent with the original observation that v-AKT is a transforming oncoprotein, AKT regulates cell proliferation through multiple mechanisms. For example, AKT prevents degradation of cyclin D1 by phosphorylating and inhibiting GSK3 β . AKT also promotes translation of cyclin D1 and D3 mRNAs (Muise-Helmericks et al. 1998). Also, AKT directly inhibits the cell cycle inhibitors p21^{waf1} and p27^{kip1} by phosphorylating these proteins near the nuclear localization signal, thereby promoting their cytoplasmic retention. In contrast, phosphorylation of Mdm2 (HDM2 in humans) by AKT is necessary for its nuclear localization (where Mdm2 forms a complex with p53 to promote its ubiquitination and proteosomal degradation) (Mayo and Donner 2001). Inhibition of p53 function is particularly relevant in the control of cell cycle check points induced by DNA damage (Bellacosa et al. 2004). Thus, multiple tumor suppressors and negative regulators of the cell cycle are inhibited by AKT.

Interestingly, AKT also has been shown to phosphorylate the reverse transcriptase subunit of telomerase, which may promote unlimited cell replication (Kang et al. 1999). Further, AKT can regulate DNA methylation and gene expression through regulation of Enhancer of Zeste homolog 2 (EZH2). EZH2 is a methyltransferase that regulates many biological processes through trimethylation of lysine 27 in histone H3. AKT phosphorylates EZH2 at Ser 21 and suppresses its methyltransferase activity by impeding EZH2 binding to histone H3, thereby causing derepression of genes that may influence oncogenesis (Cha et al. 2005).

6.3 Cell Survival

AKT provides survival (or anti-apoptotic) signals that prevent programmed cell death by a variety of mechanisms. AKT phosphorylates the pro-apoptotic protein

BAD, thereby preventing the release of cytochrome c from mitochondria, an event that activates caspases and the ensuing programmed cell death response. Survival factors, such as PDGF and IGF-1 stimulate AKT-mediated phosphorylation of BAD on Ser-136. This creates a binding site for 14-3-3 proteins, which trigger the release of BAD from its target proteins. AKT also phosphorylates pro-caspase 9 (on Ser-196), which decreases its protease activity and helps to prevent the apoptotic cascade (Cardone et al. 1998). AKT phosphorylation of PED/PEA15 (a cytosolic inhibitor of caspase-3) stabilizes this protein, resulting in protection from apoptosis.

Similar to the effects on p21 and p27 described above, AKT phosphorylation restricts the nuclear entry of transcription factors from the Forkhead family. In particular, AKT phosphorylates FOXO1 on Thr-24, Ser-256 and Ser-319; FOXO3 α and FOXO4 are phosphorylated at three equivalent sites. AKT phosphorylation of FOXO proteins promotes their interaction with 14-3-3 and triggers their export from the nucleus. As a result, pro-apoptotic genes such as BIM, Fas ligand, TRAIL and TRADD fail to be transcribed. In contrast, AKT promotes the nuclear translocation of NF- κ B by phosphorylating and activating I κ B kinase (IKK). NF- κ B induces transcription of several anti-apoptotic genes, including cIAP1, cIAP2, A1, and BFL1 (Altomare and Testa 2005). AKT also appears to phosphorylate Par-4, a pro-apoptotic protein, which inhibits its apoptotic potential (Goswami et al. 2006). In aggregate, the multi-faceted down-regulation of the apoptotic machinery by AKT potentiates a robust cell growth and survival signal that is broadly relevant in normal physiology but also becomes perturbed in disease states such as cancer, as described below.

6.4 Cell Migration and Invasion

Numerous studies suggest that AKT positively regulates cell migratory processes. Overexpression of myristolated AKT (Myr-AKT) enhances fibroblast motility by phosphorylating Girdin, an actin binding protein that promotes stress fiber formation and lamellapodia (Enomoto et al. 2005). AKT1 overexpression also leads to increased matrix metalloproteinase-2 (MMP2) activity in mouse mammary epithelial cells, thereby enhancing invasion (Park et al. 2001).

Paradoxically, other studies have revealed a surprising anti-migratory role for AKT1 in human epithelial breast cancer cells. Toward this end, multiple groups have demonstrated that AKT1 activation may limit breast cancer cell invasion in some contexts. This inhibitory effect is mediated through complex mechanisms involving proteosomal degradation of the transcription factor NF-AT (Nuclear Factor for Activated T-Cells) (Yoeli-Lerner et al. 2005), attenuation of extracellular signal regulated kinase/mitogen-activated protein kinase (ERK/MAPK) activity (Irie et al. 2005), and inhibition of Rho-GTPase activity with concomitant degradation of TSC2 (Liu et al. 2006). AKT1 may also suppress the epithelial-mesenchymal transition (EMT) in breast epithelial cells (Irie et al. 2005). These studies suggest that AKT1 may play a dual role in tumorigenesis, acting not only pro-oncogenically

by suppressing apoptosis but also anti-oncogenically by suppressing invasion and metastasis.

On the other hand, AKT2 has been shown to enhance migration and invasion of breast cancer cells. AKT2, but not AKT1 and AKT3, can upregulate β 1 integrins to promote adhesion and invasion of breast cancer cells *in vitro*, as well as metastasis *in vivo* (Arboleda et al. 2003). Unlike AKT1, AKT2 localizes adjacent to the collagen IV matrix during cellular attachment. However, in fibroblasts the effects of AKT1 and AKT2 on cell migration appears to be opposite to the breast epithelial cell phenotype (Zhou et al. 2006). Thus, AKT1 and AKT2 may function in an opposing manner in the regulation of cell migration and invasion.

6.5 Cell Growth and Protein Translation

AKT has been shown regulate cell size and protein translation mainly through the TSC-2/mTORC1 pathway. TSC1 and TSC2 are encoded by tumor-suppressor genes that are mutated in the tuberous sclerosis disease complex. These proteins form a heterodimeric complex that acts as a functional unit to suppress mTORC1 activity. TSC2 contains a GAP (GTPase-activating protein) domain that stimulates the intrinsic GTPase activity of the small G-protein Rheb, thereby enhancing the conversion of Rheb into its GDP-bound inactive state. The active version of Rheb (GTP-bound form) is a potent activator of mTORC1 (Manning and Cantley 2007).

AKT directly phosphorylates TSC2 at multiple sites. These phosphorylation events relieve the inhibitory effects of the TSC1-TSC2 complex on Rheb, thereby activating mTORC1 in response to growth factors. More recently, the proline-rich AKT substrate of 40 kDa (PRAS40) was found to be an additional downstream effector that mediates mTORC1 activation (Kovacina et al. 2003). AKT directly phosphorylates PRAS40 on Thr-246, triggering binding to 14-3-3 protein. In the steady-state, PRAS40 is a negative regulator of mTORC1 through direct protein-protein interaction, but AKT phosphorylation appears to relieve the PRAS40 inhibitory action on mTORC1. Thus, AKT activates mTORC1 via parallel mechanisms involving both TSC2 and PRAS40.

Activation of mTORC1 by AKT promotes cell growth by regulating ribosomal biogenesis and protein translation. This activation also regulates the cellular response to nutrients. Specifically, mTORC1 stimulates protein synthesis by phosphorylating proteins such as p70 S6 kinase (p70S6K) and eIF4E binding proteins 1, 2, and 3 (4E-BPs). In turn, p70S6K phosphorylates the ribosomal protein S6 to increase translation of mRNAs with 5'-terminal oligopolypyrimidine (5'TOP) tracts. Phosphorylation of 4E-BPs releases the initiation factor eIF4E to promote cap-dependent translation of mRNAs for cyclin D1, c-Myc, and vascular endothelial growth factor (VEGF), among others (Bjornsti and Houghton 2004; Ruggero and Pandolfi 2003).

6.6 Angiogenesis

Several studies indicate that AKT signaling may promote angiogenesis. AKT1 is the predominant isoform expressed in endothelial cells, where it activates endothelial nitric oxide synthase (eNOS) through direct phosphorylation of Ser-1177 (Dimmeler et al. 1999; Fulton et al. 1999). The release of nitric oxide produced by activated eNOS enhances vasodilation, vascular remodeling, and angiogenesis. AKT also regulates multiple aspects of VEGF-mediated angiogenesis in both physiological and pathological contexts. In endothelial cells, AKT phosphorylates the substrate protein Girdin (described above), thereby promoting the VEGF-dependent cell migratory process that is necessary for vessel sprouting, formation and branching during angiogenesis (Kitamura et al. 2008). In addition, VEGF enhances human endothelial cell survival through AKT signaling. In turn, AKT activation promotes VEGF expression in endothelial cells and tumor cells through HIF1 α (Gordan and Simon 2007; Jiang et al. 2000). These reciprocal interactions between AKT and VEGF provide a positive feedback loop that facilitates the formation of neo-vasculature during tumor progression.

Despite these pro-angiogenic roles of AKT, some evidence suggests that the effects of AKT1 may vary during distinct stages of angiogenesis. Paradoxically, the long term effects of AKT activation may inhibit angiogenesis; as genetic ablation of AKT1 was associated with enhanced angiogenesis in some contexts (Chen et al. 2005a). A number of endothelial cell responses involved in angiogenesis, including capillary formation *ex vivo*, endothelial cell proliferation and endothelial cell migration in response to VEGF *in vitro*, are impaired in *AKT1*^{-/-} endothelial cells, but the permeability of an endothelial monolayer is increased, which may promote a proangiogenic effect (Chen et al. 2005a). Furthermore, chronic but not short-term overexpression of AKT1 in cardiac tissues suppresses angiogenesis (Shiojima et al. 2005). Thus, the overall effect of AKT1 on angiogenesis may depend on the nature and chronicity of activation.

6.7 Apoptosis and Senescence Induction

In contrast to the well-established cell growth and survival properties described above, AKT activation is not always advantageous for cellular proliferation. Indeed, strong AKT activation may increase oxidative stress and render cells susceptible to reactive oxygen species (ROS)-triggered damage (Nogueira et al. 2008). A pro-apoptotic activity of AKT has also been reported in studies investigating the mechanism of action of apoptin, a viral protein that selectively kills cancer cells upon nuclear translocation (Maddika et al. 2009). Furthermore, nuclear but not cytoplasmic AKT interacts with Ebp1 (an inhibitor of caspase-activated DNase-dependent DNA fragmentation) and modulates its anti-apoptotic action (Ahn et al. 2006).

In addition to apoptosis induction, hyperactive AKT can also induce cellular senescence (a state of permanent cell cycle arrest). Conversely, AKT-deficient cells are resistant to ROS-induced senescence. Importantly, acute *PTEN* inactivation induces growth arrest through an AKT-mediated and p53-dependent cellular senescence pathway both *in vitro* and *in vivo*, which can be fully rescued by concomitant loss of *p53* (Chen et al. 2005b). Thus, under some circumstances, AKT signaling may mediate both ROS-dependent and p53-dependent cellular senescence.

At first glance, the pro-apoptotic and senescent functions of AKT seem to be at odds with its well-established roles in the maintenance of cell survival and energy regulation. These differential effects may relate in part to the magnitude and acuity of AKT activation. Conceivably, at lower levels of AKT activation cells may detoxify ROS, thereby enabling the AKT signal to effect normal growth and development. In contrast, the aforementioned anti-proliferative effects may be triggered in response to sudden, strong AKT activity, ensuing increased ROS production, and sustained inhibition of FoxO, possibly as a feedback mechanism to prevent uncontrolled cellular proliferation (Nogueira et al. 2008).

6.8 Immunity

AKT signaling is crucial to direct the optimal response of immune cells to antigens. In both T and B cells, AKT becomes activated following antigen engagement by the immune receptor, the levels of which are greatly boosted by co-stimulation (Fruman 2004). Numerous cytokines also activate AKT (Cantrell 2002). Constitutive membrane-targeting of AKT in T cells is sufficient to cause lymphoma in mouse models, and is associated with altered lymphocyte homeostasis and autoimmunity (Jones et al. 2000; Rathmell et al. 2003). A similar phenotype is observed in mice that are heterozygous for *PTEN*, where the AKT pathway is also constitutively active (Di Cristofano et al. 1999). At the molecular level, expression of activated AKT in T cells correlates with augmented NF- κ B function, including upregulation of Bcl- X_L and diminished FasL-mediated lymphocyte apoptosis (Jones et al. 2000). Membrane-targeted AKT also restores antigen-mediated production of IL-2 and interferon-gamma to T cells lacking the essential co-stimulatory molecule CD28 (Kane et al. 2001). On the other hand, no lymphocyte phenotypes have been reported in mice lacking individual AKT isoforms, perhaps because of functional redundancy among this family (Fruman 2004).

6.9 Brain Development, Neuronal Differentiation, and Function

Gene knockout studies in mice have shown that AKT3 is essential for the attainment of normal brain size. *AKT3*^{-/-} mice have a 20% decreased brain size with smaller and fewer neuronal cells. mTORC1 signaling is attenuated in the brains of

AKT3^{-/-} but not *AKT1*^{-/-} mice, suggesting an isoform-specific regulation of central nervous system cell growth (Easton et al. 2005). Developing neurons that do not make correct synaptic connections die by apoptosis; in the fully developed brain, post-mitotic neurons become dependent on neurotrophic factors and neurotransmitters for survival. The AKT pathway, which is activated by neurotrophins, has emerged as an important survival pathway in this regard. AKT signaling has also been implicated in neuronal differentiation, and several aspects of neurite outgrowth including elongation, caliber, and branching are regulated by AKT.

Known AKT substrates or downstream effectors that are implicated in neuronal differentiation include GSK3 β , mTORC1, cyclic AMP response element binding protein (CREB), Peripherin, and β -catenin. In addition, a physical interaction between AKT and Hsp27, another protein linked to neurite outgrowth, may also contribute (Read and Gorman 2009). Moreover, AKT is implicated in synaptogenesis and synaptic transmission in the nervous system. AKT phosphorylates the type A gamma-aminobutyric acid receptor (GABA(A)R) both *in vitro* and *in vivo*; this is the principal receptor mediating fast inhibitory synaptic transmission in the mammalian brain. AKT-dependent phosphorylation increases the number of GABA(A)Rs on the plasma membrane surface, thereby increasing the receptor-mediated synaptic transmission in neurons (Wang et al. 2003).

7 Roles of the AKT Signaling Pathway in Human Disease

Dysregulated AKT signaling is the underlying cause of several life-threatening diseases, including diabetes, neurodegenerative syndromes and various types of cancers. A detailed description of the roles of aberrant AKT signaling in the development of these disease conditions is presented below.

7.1 Diabetes

The capacity of β -cells to expand in response to insulin resistance is critical for maintenance of glucose homeostasis; failure to achieve this contributes to the pathogenesis of type 2 diabetes. The IRS2/PI3K/AKT axis plays a critical role in the regulation of pancreatic β -cell mass by stimulating proliferation and survival of β cells (Elghazi et al. 2007). As mentioned above, AKT2 is the crucial regulator of glucose homeostasis. *AKT2*^{-/-} mice develop diabetes due to reduction in insulin-stimulated glucose uptake in peripheral tissues and β -cell failure, a phenotype reminiscent of type 2 diabetes in humans (Cho et al. 2001). In humans, an *AKT2* mutation has been identified in a family with severe hyperinsulinemia and diabetes. This mutation creates an R274H amino acid substitution in the catalytic domain of AKT2 that abolishes kinase activity and results in defective insulin signaling. This

AKT2(R274H) variant also appears to exert a dominant negative effect on the remaining wild type AKT2 allele (George et al. 2004). This germline event thus provides an instructive example of a monogenic, inherited defect in post-receptor insulin signaling that leads to insulin resistance and diabetes mellitus. Genetic variants in *IRS1* and *PIK3RI* have also been reported in subjects with insulin resistance and/or type 2 diabetes mellitus (George et al. 2004; Pedersen 1999). A genetic variant in *TRB3*, a negative regulator of AKT, has also been linked to insulin resistance (Prudente et al. 2005). Conversely, *TRB3* is over-expressed in some murine models of diabetes (Du et al. 2003).

7.2 Neurological Diseases

Diminished AKT signaling contributes importantly to the pathogenesis of Huntington's disease (HD), a fatal neurodegenerative disorder. AKT phosphorylates the causative protein, polyQ-Huntingtin, thereby minimizing its toxic properties (Humbert et al. 2002). AKT also acts on other downstream effector(s) to prevent neuronal death in HD. For example, AKT phosphorylates the ADP-ribosylation factor-interacting protein Arfaptin 2, which also results in a neuroprotective effect (Rangone et al. 2005). Arfaptin 2 levels are increased in HD patients; this increase is thought to contribute to HD pathogenicity. During the late stages of the HD, AKT is cleaved into an inactive form by caspase-3. Thus, the neuroprotective effects of AKT signaling are lost during HD progression (Colin et al. 2005).

In addition to HD, AKT also exerts a neuroprotective effect that becomes impaired in other neurodegenerative conditions, including Alzheimer's disease and Parkinson's disease (Burke 2007). However, consistent with its paradoxical effects in other physiological contexts (as described above), AKT activation might also promote neurodegeneration in some settings. In spinocerebellar ataxia type 1, an autosomal-dominant neurodegenerative disorder characterized by ataxia and progressive motor deterioration, phosphorylation of a mutated form of Ataxin-1 by AKT at Ser-776 residue enables its interaction with 14-3-3. This interaction facilitates the accumulation of Ataxin-1 and ultimately triggers apoptosis and neurodegeneration (Chen et al. 2003).

7.3 Cancer

Aberrant AKT activation occurs in a wide variety of human cancers. In several tumor types, AKT activation has been shown to correlate with advanced disease and/or poor prognosis. Gene mutations and or other genetic alterations affecting the components of PI3K signaling pathway are largely responsible for the dysregulated AKT signaling in human cancers, as described below.

7.3.1 Genetic Alterations in the Upstream RTK Signaling Axis

Many cancer genetic alterations that deregulate cell signaling pathways exert their oncogenic effects at least in part through PI3K/AKT pathway. For example, activating mutations in RTKs such as EGFR, PDGFR, c-KIT, and ABL kinase result oncogenic PI3K signaling characterized by elevated AKT activation. Tumors harboring such mutations frequently exhibit oncogenic “addiction” to PI3K/AKT signaling; that is, a critical dependency on this pathway for tumorigenesis. Pharmacologic agents targeting these receptors silence both RTK and downstream PI3K/AKT signaling to achieve clinical responses. Conversely, resistance to such therapeutics is associated with refractory AKT signaling.

7.3.2 Inactivating Mutations of PTEN

PTEN deletion and other inactivating mutations occur in a wide spectrum of human cancers. Loss of *PTEN* function also occurs through transcriptional silencing or protein stability (Georgescu et al. 1999; Vasudevan et al. 2004, 2007). Germline *PTEN* mutations underlie the pathogenesis of Cowden disease and Bannayan Zonana syndrome, two related hereditary cancer predisposition disorders associated with enhanced risk for breast and thyroid cancer (Liaw et al. 1997; Marsh et al. 1997). Somatic mutations and biallelic deletion involving *PTEN* occur commonly in advanced glioblastoma, prostate cancers and endometrial cancers (Sansal and Sellers 2004). Loss of *PTEN* almost invariably results in strong AKT activation; moreover, potent AKT signaling is typically required for the survival and tumorigenesis of *PTEN*-null cancers.

7.3.3 Activating Mutations of PI3K

PIK3CA, the gene that encodes the catalytic p110 α subunit of PI3K, is also commonly mutated in many human cancers (Samuels et al. 2004; Samuels and Ericson 2006). For example, gain-of-function *PIK3CA* point mutations occur in 30% of breast, colon, and endometrial cancers, and at a lower frequency in several other tumor types. The most common tumor-associated *PIK3CA* mutations (>80% of cases) involve either the helical domain (exon 9; e.g., E542K and E545K) or the kinase domain (exon 20; e.g., H1047R) of p110 α (Samuels et al. 2004, 2005), both of which are capable of up-regulating its lipid kinase activity and transforming various types of immortalized cell models *in vitro* (Ikenoue et al. 2005; Kang et al. 2005; Samuels et al. 2004). Mutant-expressing cancer cells and human tumors frequently but not invariably show elevated downstream signaling through AKT, S6K, 4EBP and GSK3 β . The regulatory p85 subunit of PI3K is also mutated in human cancers (Philp et al. 2001). In this case, p85 structural alterations are thought to release the p85-p110 complex from negative regulation, thereby

bypassing the normal role of RTK signaling in PI3K activation (Vivanco and Sawyers 2002).

7.3.4 Activating Mutations of AKT

AKT1 mutations have also been discovered in human cancers, albeit rarely. In particular, an activating E17K mutation located within the PH domain *AKT1* has been identified in human breast (up to 8%), colon (6%), ovarian (2%) and endometrial cancers (2%) (Carpten et al. 2007; Shoji et al. 2009). The E17K mutation potentiates PI3K-independent membrane translocation and activation. Like myristoylated AKT, membrane targeting of this variant appears critical for its transforming activity (Carpten et al. 2007). A similar mutation in the PH domain of AKT3 has occasionally been observed in human melanomas (Davies et al. 2008).

7.3.5 Mouse Tumor Models of AKT Activation

Various transgenic mice that overexpress a constitutively active form of AKT (driven by different tissue specific promoters) have been generated to evaluate the importance of AKT activation in tumorigenesis. In aggregate, these transgenic mouse models have suggested that AKT activation is not sufficient by itself to induce tumor formation in several epithelial tissues. For example, constitutive AKT activation in mammary glands markedly accelerates tumor induction when co-expressed with an *ErbB2* transgene, but cannot induce tumors when present as a single lesion (Hutchinson et al. 2004). Similarly, transgenic mice expressing Myr-AKT1 in the ventral prostate under the control of rat probasin promoter develop PIN-like lesions by 8 weeks of age (Majumder et al. 2003), but these mice do not form invasive cancer, even at an advanced age. In the prostate model, AKT overexpression leads to mTOR activation, and inhibition of mTOR pathway completely reverses the hyperplastic phenotype (Majumder et al. 2004). On the other hand, prostate-specific deletion of PTEN in one mouse model results in development of invasive and metastatic cancer in about 2 months. Thus, loss of PTEN may also activate AKT-independent downstream oncogenic pathways, or the magnitude and dynamics of AKT activation may differ importantly between these models.

In non-epithelial transgenic mouse models, the outcome is somewhat different. Expression of Myr-AKT1 in thymocytes using the *lck*-driven promoter causes aggressive lymphomas to emerge within 10–20 weeks (Rathmell et al. 2003). In contrast, *PTEN*^{+/-} heterozygous mice develop a wide variety of tumors at an early age, with a higher tumor incidence in the endometrium, prostate, thyroid, adrenal medulla, intestine and mammary glands (Podsypanina et al. 1999; Suzuki et al. 1998). Crossing of *PTEN*^{+/-} mice with AKT1-deficient mice results in a marked decrease in the tumor incidence and development compared to *PTEN*^{+/-} mice in many tissues, with the most effective tumor inhibition observed in the prostate, endometrium and small intestine (Chen et al. 2006).

8 AKT Independent Signaling by PI3K

The foregoing discussion makes it clear that AKT transduces the predominant PI3K signal in many physiologic and pathologic contexts. At the same time, increasing evidence suggests that AKT-independent PI3K signaling might also contribute to the full spectrum of biology elaborated by this cellular pathway. It has long been recognized that gain- or loss-of-function mutations in PI3K versus analogous mutations in AKT result in non-overlapping phenotypes in several model systems, including transgenic and knockout mice; this indicates that these genes are not purely epistatic. In addition, PI3K and PIP3 can activate multiple signaling modules in addition to AKT (Vivanco and Sawyers 2002). Other PH domain containing proteins that are activated by PIP3 include the small GTP binding protein Rac1, the ADP ribosylation factor 6 (ARF6); and protein tyrosine kinases such as BTK (Bruton tyrosine kinase) and other Tec kinase family members. Further, PI3K-activated PDK1 can phosphorylate and activate multiple key protein kinases, including p70 S6-kinase, serum glucocorticoid-inducible kinase (SGKs) and protein kinase C zeta (PKC ζ) (Cantley 2002).

The SGK family of protein kinases have received increasing attention because of their high homology to AKT and similar functional effects on survival signaling pathways (Scheid and Woodgett 2001). However, the mechanism of PI3K-driven SGK activation differs from activation of AKT, because SGKs do not contain a PH domain (Vivanco and Sawyers 2002). Like AKT, the SGK family is encoded by three genes in mammalian genomes (*SGK1*, *SGK2*, and *SGK3*), whose catalytic domains share 80% sequence identity. *SGK1* mRNA is upregulated by glucocorticoids and other stimuli such as serum, aldosterone, extracellular osmolarity, transforming growth factor- β (TGF- β), and hyperosmotic stress (Tessier and Woodgett 2006). The SGK kinase domain shares 55% identity with the AKT kinase domain. All three SGK isoforms appear to require PI3K activation for function and are also direct substrates of PDK1 (Tessier and Woodgett 2006).

SGK3 differs from its sister isoforms in that it possesses a distinct type of phospholipid-binding domain known as the Phox homology (PX) domain. The PX domain binds the monophosphorylated lipid known as phosphatidylinositol 3' phosphate (PI(3)P), thereby directing *SGK3* to endosomal membranes (Virbasius et al. 2001). In addition, the PX domain modulates protein kinase activity. *SGK3*-null mice are viable and fertile, and display a defect in post-natal hair follicle development due to defects in cell proliferation (Alonso et al. 2005; McCormick et al. 2004). Recently, *SGK3* was implicated as an AKT-independent effector in some *PIK3CA* mutant cancer cells and tumors (Vasudevan et al. 2009).

In addition to SGKs, other kinases have also been postulated to mediate AKT-independent oncogenic signaling downstream of PI3K. For example, JNK kinase can be activated independently of AKT in tumors with PTEN inactivation (Vivanco et al. 2007). PTEN null cells exhibit increased JNK activity, and genetic studies show that JNK functions in parallel to and independently of AKT (Vivanco et al. 2007). Tec family tyrosine kinases may also mediate AKT-independent

signaling by PI3K. Toward this end, Bruton's agammaglobulinemia tyrosine kinase (BTK), a prototype Tec isoform, is a cytoplasmic tyrosine kinase required for B-lymphocyte development, differentiation, and proper B cell receptor-dependent signaling. Germline mutations in the *BTK* gene lead to X-linked agammaglobulinemia (XLA) in humans and X-linked immunodeficiency (Xid) in mice (Mohamed et al. 2009) BMX (ETK), the newest member of BTK tyrosine kinase family, also contains a PH domain and becomes activated by PI3K. Unlike other Tec kinases which are mostly hemopoietic cell-specific, BMX is widely expressed in epithelial and endothelial cells. PI3K-BMX signaling, independent of AKT pathway, has been implicated in prostate cancer (Dai et al. 2006; Qiu et al. 1998).

9 Conclusions

The AKT family of serine-threonine kinases provides a major effector mechanism for PI3 kinase signaling. The numerous AKT substrates direct a wide range of physiological processes that become deranged in several disease states. Exactly how these AKT substrates are differentially regulated by the three AKT isoforms remains incompletely understood. The extent to which PI3K-independent mechanisms contribute to AKT is also poorly characterized.

Given its prominent role in human disease and inherent 'druggability', AKT is considered an attractive potential therapeutic target. Specific inhibitors of AKT might be useful for cancer therapy, whereas drugs that restore AKT signaling might conceivably be useful for treatment of diabetes and degenerative diseases. As the roles of AKT in biology and disease continue to be resolved, the possibility exists that modulating these effects may provide considerable clinical benefit.

References

- Aguirre V, Werner ED, Giraud J, Lee YH, Shoelson SE, White MF (2002) Phosphorylation of Ser307 in insulin receptor substrate 1 blocks interactions with the insulin receptor and inhibits insulin action. *J Biol Chem* 277:1531–1537
- Ahn JY, Liu X, Liu Z, Pereira L, Cheng D, Peng J, Wade PA, Hamburger AW, Ye K (2006) Nuclear Akt associates with PKC phosphorylated Ebp1, preventing DNA fragmentation by inhibition of caspase activated DNase. *EMBO J* 25:2083–2095
- Alessi DR, Andjelkovic M, Caudwell B, Cron P, Morrice N, Cohen P, Hemmings BA (1996) Mechanism of activation of protein kinase B by insulin and IGF 1. *EMBO J* 15:6541–6551
- Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PR, Reese CB, Cohen P (1997) Characterization of a 3 phosphoinositide dependent protein kinase which phosphorylates and activates protein kinase Balpha. *Curr Biol* 7:261–269
- Alonso L, Okada H, Pasolli HA, Wakeham A, You Ten AI, Mak TW, Fuchs E (2005) Sgk3 links growth factor signaling to maintenance of progenitor cells in the hair follicle. *J Cell Biol* 170:559–570

- Altomare DA, Testa JR (2005) Perturbations of the AKT signaling pathway in human cancer. *Oncogene* 24:7455-7464
- Aman MJ, Lamkin TD, Okada H, Kurosaki T, Ravichandran KS (1998) The inositol phosphatase SHIP inhibits Akt/PKB activation in B cells. *J Biol Chem* 273:33922-33928
- Anderson KE, Coadwell J, Stephens LR, Hawkins PT (1998) Translocation of PDK 1 to the plasma membrane is important in allowing PDK 1 to activate protein kinase B. *Curr Biol* 8:684-691
- Arboleda MJ, Lyons JF, Kabbinnar FF, Bray MR, Snow BE, Ayala R, Danino M, Karlan BY, Slamon DJ (2003) Overexpression of AKT2/protein kinase B leads to up regulation of beta1 integrins, increased invasion, and metastasis of human breast and ovarian cancer cells. *Cancer Res* 63:196-206
- Barthel A, Okino ST, Liao J, Nakatani K, Li J, Whitlock JP Jr, Roth RA (1999) Regulation of GLUT1 gene transcription by the serine/threonine kinase Akt1. *J Biol Chem* 274:20281-20286
- Bellacosa A, Testa JR, Staal SP, Tsichlis PN (1991) A retroviral oncogene, akt, encoding a serine threonine kinase containing an SH2 like region. *Science* 254:274-277
- Bellacosa A, Testa JR, Moore R, Larue L (2004) A portrait of AKT kinases: human cancer and animal models depict a family with strong individualities. *Cancer Biol Ther* 3:268-275
- Bellacosa A, Kumar CC, Di Cristofano A, Testa JR (2005) Activation of AKT kinases in cancer: implications for therapeutic targeting. *Adv Cancer Res* 94:29-86
- Bhaskar PT, Hay N (2007) The two TORCs and Akt. *Dev Cell* 12:487-502
- Bjornsti MA, Houghton PJ (2004) The TOR pathway: a target for cancer therapy. *Nat Rev Cancer* 4:335-348
- Boehme KA, Kulikov R, Blattner C (2008) p53 stabilization in response to DNA damage requires Akt/PKB and DNA PK. *Proc Natl Acad Sci USA* 105:7785-7790
- Bozulic L, Hemmings BA (2009) PIKKing on PKB: regulation of PKB activity by phosphorylation. *Curr Opin Cell Biol* 21:256-261
- Bozulic L, Surucu B, Hynx D, Hemmings BA (2008) PKBalpha/Akt1 acts downstream of DNA PK in the DNA double strand break response and promotes survival. *Mol Cell* 30:203-213
- Brazil DP, Park J, Hemmings BA (2002) PKB binding proteins. Getting in on the Akt. *Cell* 111:293-303
- Brogna J, Newton AC (2008) PHLiPPing the switch on Akt and protein kinase C signaling. *Trends Endocrinol Metab* 19:223-230
- Brogna J, Sieracki E, Gao T, Newton AC (2007) PHLPP and a second isoform, PHLPP2, differentially attenuate the amplitude of Akt signaling by regulating distinct Akt isoforms. *Mol Cell* 25:917-931
- Burke RE (2007) Inhibition of mitogen activated protein kinase and stimulation of Akt kinase signaling pathways: Two approaches with therapeutic potential in the treatment of neurodegenerative disease. *Pharmacol Ther* 114:261-277
- Cantley LC (2002) The phosphoinositide 3 kinase pathway. *Science* 296:1655-1657
- Cantley LC, Neel BG (1999) New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3 kinase/AKT pathway. *Proc Natl Acad Sci USA* 96:4240-4245
- Cantrell D (2002) Protein kinase B (Akt) regulation and function in T lymphocytes. *Semin Immunol* 14:19-26
- Cardone MH, Roy N, Stennicke HR, Salvesen GS, Franke TF, Stanbridge E, Frisch S, Reed JC (1998) Regulation of cell death protease caspase 9 by phosphorylation. *Science* 282:1318-1321
- Carpten JD, Faber AL, Horn C, Donoho GP, Briggs SL, Robbins CM, Hostetter G, Boguslawski S, Moses TY, Savage S, Uhlik M, Lin A, Du J, Qian YW, Zeckner DJ, Tucker Kellogg G, Touchman J, Patel K, Mousses S, Bittner M, Schevitz R, Lai MH, Blanchard KL, Thomas JE (2007) A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. *Nature* 448:439-444

- Cha TL, Zhou BP, Xia W, Wu Y, Yang CC, Chen CT, Ping B, Otte AP, Hung MC (2005) Akt mediated phosphorylation of EZH2 suppresses methylation of lysine 27 in histone H3. *Science* 310:306-310
- Chen R, Kim O, Yang J, Sato K, Eisenmann KM, McCarthy J, Chen H, Qiu Y (2001) Regulation of Akt/PKB activation by tyrosine phosphorylation. *J Biol Chem* 276:31858-31862
- Chen HK, Fernandez Funez P, Acevedo SF, Lam YC, Kaytor MD, Fernandez MH, Aitken A, Skoulakis EM, Orr HT, Botas J, Zoghbi HY (2003) Interaction of Akt phosphorylated ataxin 1 with 14-3-3 mediates neurodegeneration in spinocerebellar ataxia type 1. *Cell* 113:457-468
- Chen J, Somanath PR, Razorenova O, Chen WS, Hay N, Bornstein P, Byzova TV (2005a) Akt1 regulates pathological angiogenesis, vascular maturation and permeability *in vivo*. *Nat Med* 11:1188-1196
- Chen Z, Trotman LC, Shaffer D, Lin HK, Dotan ZA, Niki M, Koutcher JA, Scher HI, Ludwig T, Gerald W, Cordon Cardo C, Pandolfi PP (2005b) Crucial role of p53 dependent cellular senescence in suppression of Pten deficient tumorigenesis. *Nature* 436:725-730
- Chen ML, Xu PZ, Peng XD, Chen WS, Guzman G, Yang X, Di Cristofano A, Pandolfi PP, Hay N (2006) The deficiency of Akt1 is sufficient to suppress tumor development in Pten^{+/-} mice. *Genes Dev* 20:1569-1574
- Cho H, Mu J, Kim JK, Thorvaldsen JL, Chu Q, Crenshaw EB III, Kaestner KH, Bartolomei MS, Shulman GI, Birnbaum MJ (2001) Insulin resistance and a diabetes mellitus like syndrome in mice lacking the protein kinase Akt2 (PKB beta). *Science* 292:1728-1731
- Coffer PJ, Woodgett JR (1991) Molecular cloning and characterisation of a novel putative protein serine kinase related to the cAMP dependent and protein kinase C families. *Eur J Biochem* 201:475-481
- Colin E, Regulier E, Perrin V, Durr A, Brice A, Aebischer P, Deglon N, Humbert S, Saudou F (2005) Akt is altered in an animal model of Huntington's disease and in patients. *Eur J Neurosci* 21:1478-1488
- Dai B, Kim O, Xie Y, Guo Z, Xu K, Wang B, Kong X, Melamed J, Chen H, Bieberich CJ, Borowsky AD, Kung HJ, Wei G, Ostrowski MC, Brodie A, Qiu Y (2006) Tyrosine kinase Etk/BMX is up regulated in human prostate cancer and its overexpression induces prostate intraepithelial neoplasia in mouse. *Cancer Res* 66:8058-8064
- Davies MA, Stemke-Hale K, Tellez C, Calderone TL, Deng W, Prieto VG, Lazar AJ, Gershenwald JE, Mills GB (2008) A novel AKT3 mutation in melanoma tumours and cell lines. *Br J Cancer* 99:1265-1268
- Di Cristofano A, Kotsi P, Peng YF, Cordon Cardo C, Elkon KB, Pandolfi PP (1999) Impaired Fas response and autoimmunity in Pten^{+/-} mice. *Science* 285:2122-2125
- Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM (1999) Activation of nitric oxide synthase in endothelial cells by Akt dependent phosphorylation. *Nature* 399:601-605
- Du K, Herzig S, Kulkarni RN, Montminy M (2003) TRB3: a tribbles homolog that inhibits Akt/PKB activation by insulin in liver. *Science* 300:1574-1577
- Dummler B, Hemmings BA (2007) Physiological roles of PKB/Akt isoforms in development and disease. *Biochem Soc Trans* 35:231-235
- Dummler B, Tschopp O, Hynx D, Yang ZZ, Dirnhofer S, Hemmings BA (2006) Life with a single isoform of Akt: mice lacking Akt2 and Akt3 are viable but display impaired glucose homeostasis and growth deficiencies. *Mol Cell Biol* 26:8042-8051
- Easton RM, Cho H, Roovers K, Shineman DW, Mizrahi M, Forman MS, Lee VM, Szabolcs M, de Jong R, Oltersdorf T, Ludwig T, Efstratiadis A, Birnbaum MJ (2005) Role for Akt3/protein kinase Bgamma in attainment of normal brain size. *Mol Cell Biol* 25:1869-1878
- Elghazi L, Rachdi L, Weiss AJ, Cras Meneur C, Bernal Mizrahi E (2007) Regulation of beta cell mass and function by the Akt/protein kinase B signalling pathway. *Diabetes Obes Metab* 9 (Suppl 2):147-157
- Engelman JA, Luo J, Cantley LC (2006) The evolution of phosphatidylinositol 3 kinases as regulators of growth and metabolism. *Nat Rev Genet* 7:606-619

- Enomoto A, Murakami H, Asai N, Morone N, Watanabe T, Kawai K, Murakumo Y, Usukura J, Kaibuchi K, Takahashi M (2005) Akt/PKB regulates actin organization and cell motility via Girdin/APE. *Dev Cell* 9:389 402
- Feng J, Park J, Cron P, Hess D, Hemmings BA (2004) Identification of a PKB/Akt hydrophobic motif Ser 473 kinase as DNA dependent protein kinase. *J Biol Chem* 279:41189 41196
- Franke TF, Kaplan DR, Cantley LC, Toker A (1997) Direct regulation of the Akt proto oncogene product by phosphatidylinositol 3, 4 bisphosphate. *Science* 275:665 668
- Fruman DA (2004) Phosphoinositide 3 kinase and its targets in B cell and T cell signaling. *Curr Opin Immunol* 16:314 320
- Fulton D, Gratton JP, McCabe TJ, Fontana J, Fujio Y, Walsh K, Franke TF, Papapetropoulos A, Sessa WC (1999) Regulation of endothelium derived nitric oxide production by the protein kinase Akt. *Nature* 399:597 601
- Gao T, Furnari F, Newton AC (2005) PHLPP: a phosphatase that directly dephosphorylates Akt, promotes apoptosis, and suppresses tumor growth. *Mol Cell* 18:13 24
- George S, Rochford JJ, Wolfrum C, Gray SL, Schinner S, Wilson JC, Soos MA, Murgatroyd PR, Williams RM, Acerini CL, Dunger DB, Barford D, Umpleby AM, Wareham NJ, Davies HA, Schafer AJ, Stoffel M, O'Rahilly S, Barroso I (2004) A family with severe insulin resistance and diabetes due to a mutation in AKT2. *Science* 304:1325 1328
- Georgescu MM, Kirsch KH, Akagi T, Shishido T, Hanafusa H (1999) The tumor suppressor activity of PTEN is regulated by its carboxyl terminal region. *Proc Natl Acad Sci USA* 96:10182 10187
- Gordan JD, Simon MC (2007) Hypoxia inducible factors: central regulators of the tumor phenotype. *Curr Opin Genet Dev* 17:71 77
- Goswami A, Ranganathan P, Rangnekar VM (2006) The phosphoinositide 3 kinase/Akt1/Par 4 axis: a cancer selective therapeutic target. *Cancer Res* 66:2889 2892
- Gottlob K, Majewski N, Kennedy S, Kandel E, Robey RB, Hay N (2001) Inhibition of early apoptotic events by Akt/PKB is dependent on the first committed step of glycolysis and mitochondrial hexokinase. *Genes Dev* 15:1406 1418
- Habib T, Hejna JA, Moses RE, Decker SJ (1998) Growth factors and insulin stimulate tyrosine phosphorylation of the 51C/SHIP2 protein. *J Biol Chem* 273:18605 18609
- Haruta T, Uno T, Kawahara J, Takano A, Egawa K, Sharma PM, Olefsky JM, Kobayashi M (2000) A rapamycin sensitive pathway down regulates insulin signaling via phosphorylation and proteasomal degradation of insulin receptor substrate 1. *Mol Endocrinol* 14:783 794
- Hennessy BT, Smith DL, Ram PT, Lu Y, Mills GB (2005) Exploiting the PI3K/AKT pathway for cancer drug discovery. *Nat Rev Drug Discov* 4:988 1004
- Humbert S, Bryson EA, Cordelieres FP, Connors NC, Datta SR, Finkbeiner S, Greenberg ME, Saudou F (2002) The IGF 1/Akt pathway is neuroprotective in Huntington's disease and involves Huntingtin phosphorylation by Akt. *Dev Cell* 2:831 837
- Hutchinson JN, Jin J, Cardiff RD, Woodgett JR, Muller WJ (2004) Activation of Akt 1 (PKB alpha) can accelerate ErbB 2 mediated mammary tumorigenesis but suppresses tumor invasion. *Cancer Res* 64:3171 3178
- Ikenoue T, Kanai F, Hikiba Y, Obata T, Tanaka Y, Imamura J, Ohta M, Jazag A, Guleng B, Tateishi K, Asaoka Y, Matsumura M, Kawabe T, Omata M (2005) Functional analysis of PIK3CA gene mutations in human colorectal cancer. *Cancer Res* 65:4562 4567
- Irie HY, Pearlman RV, Grueneberg D, Hsia M, Ravichandran P, Kothari N, Natesan S, Brugge JS (2005) Distinct roles of Akt1 and Akt2 in regulating cell migration and epithelial mesenchymal transition. *J Cell Biol* 171:1023 1034
- Isakoff SJ, Cardozo T, Andreev J, Li Z, Ferguson KM, Abagyan R, Lemmon MA, Aronheim A, Skolnik EY (1998) Identification and analysis of PH domain containing targets of phosphatidylinositol 3 kinase using a novel *in vivo* assay in yeast. *EMBO J* 17:5374 5387
- Ishihara H, Sasaoka T, Hori H, Wada T, Hirai H, Haruta T, Langlois WJ, Kobayashi M (1999) Molecular cloning of rat SH2 containing inositol phosphatase 2 (SHIP2) and its role in the regulation of insulin signaling. *Biochem Biophys Res Commun* 260:265 272

- Jiang T, Qiu Y (2003) Interaction between Src and a C terminal proline rich motif of Akt is required for Akt activation. *J Biol Chem* 278:15789-15793
- Jiang BH, Zheng JZ, Aoki M, Vogt PK (2000) Phosphatidylinositol 3 kinase signaling mediates angiogenesis and expression of vascular endothelial growth factor in endothelial cells. *Proc Natl Acad Sci USA* 97:1749-1753
- Jones PF, Jakubowicz T, Pitossi FJ, Maurer F, Hemmings BA (1991) Molecular cloning and identification of a serine/threonine protein kinase of the second messenger subfamily. *Proc Natl Acad Sci USA* 88:4171-4175
- Jones RG, Parsons M, Bonnard M, Chan VS, Yeh WC, Woodgett JR, Ohashi PS (2000) Protein kinase B regulates T lymphocyte survival, nuclear factor kappaB activation, and Bcl X(L) levels *in vivo*. *J Exp Med* 191:1721-1734
- Kandel ES, Hay N (1999) The regulation and activities of the multifunctional serine/threonine kinase Akt/PKB. *Exp Cell Res* 253:210-229
- Kane LP, Andres PG, Howland KC, Abbas AK, Weiss A (2001) Akt provides the CD28 costimulatory signal for up regulation of IL 2 and IFN gamma but not TH2 cytokines. *Nat Immunol* 2:37-44
- Kaneto H, Matsuoka TA, Nakatani Y, Kawamori D, Miyatsuka T, Matsuhisa M, Yamasaki Y (2005) Oxidative stress, ER stress, and the JNK pathway in type 2 diabetes. *J Mol Med* 83: 429-439
- Kang SS, Kwon T, Kwon DY, Do SI (1999) Akt protein kinase enhances human telomerase activity through phosphorylation of telomerase reverse transcriptase subunit. *J Biol Chem* 274:13085-13090
- Kang S, Bader AG, Vogt PK (2005) Phosphatidylinositol 3 kinase mutations identified in human cancer are oncogenic. *Proc Natl Acad Sci USA* 102:802-807
- Karlsson HK, Hallsten K, Bjornholm M, Tsuchida H, Chibalin AV, Virtanen KA, Heinonen OJ, Lonnqvist F, Nuutila P, Zierath JR (2005) Effects of metformin and rosiglitazone treatment on insulin signaling and glucose uptake in patients with newly diagnosed type 2 diabetes: a randomized controlled study. *Diabetes* 54:1459-1467
- Kawase T, Ohki R, Shibata T, Tsutsumi S, Kamimura N, Inazawa J, Ohta T, Ichikawa H, Aburatani H, Tashiro F, Taya Y (2009) PH domain only protein PHLDA3 is a p53 regulated repressor of Akt. *Cell* 136:535-550
- Kitamura T, Asai N, Enomoto A, Maeda K, Kato T, Ishida M, Jiang P, Watanabe T, Usukura J, Kondo T, Costantini F, Murohara T, Takahashi M (2008) Regulation of VEGF mediated angiogenesis by the Akt/PKB substrate Girdin. *Nat Cell Biol* 10:329-337
- Kovacina KS, Park GY, Bae SS, Guzzetta AW, Schaefer E, Birnbaum MJ, Roth RA (2003) Identification of a proline rich Akt substrate as a 14-3-3 binding partner. *J Biol Chem* 278:10189-10194
- Lemmon MA (2003) Phosphoinositide recognition domains. *Traffic* 4:201-213
- Li X, Monks B, Ge Q, Birnbaum MJ (2007) Akt/PKB regulates hepatic metabolism by directly inhibiting PGC-1alpha transcription coactivator. *Nature* 447:1012-1016
- Liaw D, Marsh DJ, Li J, Dahia PL, Wang SI, Zheng Z, Bose S, Call KM, Tsou HC, Peacocke M, Eng C, Parsons R (1997) Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nat Genet* 16:64-67
- Liu Q, Sasaki T, Kozieradzki I, Wakeham A, Itte A, Dumont DJ, Penninger JM (1999) SHIP is a negative regulator of growth factor receptor mediated PKB/Akt activation and myeloid cell survival. *Genes Dev* 13:786-791
- Liu H, Radisky DC, Nelson CM, Zhang H, Fata JE, Roth RA, Bissell MJ (2006) Mechanism of Akt1 inhibition of breast cancer cell invasion reveals a protumorigenic role for TSC2. *Proc Natl Acad Sci USA* 103:4134-4139
- Maddika S, Panigrahi S, Wiechec E, Wesselborg S, Fischer U, Schulze Osthoff K, Los M (2009) Unscheduled Akt triggered activation of cyclin dependent kinase 2 as a key effector mechanism of apoptin's anticancer toxicity. *Mol Cell Biol* 29:1235-1248
- Maira SM, Galetic I, Brazil DP, Kaech S, Ingley E, Thelen M, Hemmings BA (2001) Carboxyl terminal modulator protein (CTMP), a negative regulator of PKB/Akt and v-Akt at the plasma membrane. *Science* 294:374-380

- Majumder PK, Yeh JJ, George DJ, Febbo PG, Kum J, Xue Q, Bikoff R, Ma H, Kantoff PW, Golub TR, Loda M, Sellers WR (2003) Prostate intraepithelial neoplasia induced by prostate restricted Akt activation: the MPAKT model. *Proc Natl Acad Sci USA* 100:7841-7846
- Majumder PK, Febbo PG, Bikoff R, Berger R, Xue Q, McMahon LM, Manola J, Brugarolas J, McDonnell TJ, Golub TR, Loda M, Lane HA, Sellers WR (2004) mTOR inhibition reverses Akt dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF 1 dependent pathways. *Nat Med* 10:594-601
- Manning BD, Cantley LC (2007) AKT/PKB signaling: navigating downstream. *Cell* 129:1261-1274
- Marsh DJ, Dahia PL, Zheng Z, Liaw D, Parsons R, Gorlin RJ, Eng C (1997) Germline mutations in PTEN are present in Bannayan Zonana syndrome. *Nat Genet* 16:333-334
- Mayo LD, Donner DB (2001) A phosphatidylinositol 3 kinase/Akt pathway promotes translocation of Mdm2 from the cytoplasm to the nucleus. *Proc Natl Acad Sci USA* 98:11598-11603
- McCormick JA, Feng Y, Dawson K, Behne MJ, Yu B, Wang J, Wyatt AW, Henke G, Grahmmer F, Mauro TM, Lang F, Pearce D (2004) Targeted disruption of the protein kinase SGK3/CISK impairs postnatal hair follicle development. *Mol Biol Cell* 15:4278-4288
- Mohamed AJ, Yu L, Backesjo CM, Vargas L, Faryal R, Aints A, Christensson B, Berglof A, Vihinen M, Nore BF, Smith CI (2009) Bruton's tyrosine kinase (Btk): function, regulation, and transformation with special emphasis on the PH domain. *Immunol Rev* 228:58-73
- Muise Helmericks RC, Grimes HL, Bellacosa A, Malstrom SE, Tschlis PN, Rosen N (1998) Cyclin D expression is controlled post transcriptionally via a phosphatidylinositol 3 kinase/Akt dependent pathway. *J Biol Chem* 273:29864-29872
- Nogueira V, Park Y, Chen CC, Xu PZ, Chen ML, Tonic I, Unterman T, Hay N (2008) Akt determines replicative senescence and oxidative or oncogenic premature senescence and sensitizes cells to oxidative apoptosis. *Cancer Cell* 14:458-470
- O'Reilly KE, Rojo F, She QB, Solit D, Mills GB, Smith D, Lane H, Hofmann F, Hicklin DJ, Ludwig DL, Baselga J, Rosen N (2006) mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. *Cancer Res* 66:1500-1508
- Padmanabhan S, Mukhopadhyay A, Narasimhan SD, Tesz G, Czech MP, Tissenbaum HA (2009) A PP2A regulatory subunit regulates *C. elegans* insulin/IGF 1 signaling by modulating AKT 1 phosphorylation. *Cell* 136:939-951
- Park BK, Zeng X, Glazer RI (2001) Akt1 induces extracellular matrix invasion and matrix metalloproteinase 2 activity in mouse mammary epithelial cells. *Cancer Res* 61:7647-7653
- Park J, Feng J, Li Y, Hammarsten O, Brazil DP, Hemmings BA (2009) DNA dependent protein kinase mediated phosphorylation of protein kinase B requires a specific recognition sequence in the C terminal hydrophobic motif. *J Biol Chem* 284:6169-6174
- Pedersen O (1999) Genetics of insulin resistance. *Exp Clin Endocrinol Diabetes* 107:113-118
- Philp AJ, Campbell IG, Leet C, Vincan E, Rockman SP, Whitehead RH, Thomas RJ, Phillips WA (2001) The phosphatidylinositol 3' kinase p85alpha gene is an oncogene in human ovarian and colon tumors. *Cancer Res* 61:7426-7429
- Podsypanina K, Ellenson LH, Nemes A, Gu J, Tamura M, Yamada KM, Cordon Cardo C, Catoretti G, Fisher PE, Parsons R (1999) Mutation of Pten/Mmac1 in mice causes neoplasia in multiple organ systems. *Proc Natl Acad Sci USA* 96:1563-1568
- Prudente S, Hribal ML, Flex E, Turchi F, Morini E, De Cosmo S, Bacci S, Tassi V, Cardellini M, Lauro R, Sesti G, Dallapiccola B, Trischitta V (2005) The functional Q84R polymorphism of mammalian Tribbles homolog TRB3 is associated with insulin resistance and related cardiovascular risk in Caucasians from Italy. *Diabetes* 54:2807-2811
- Qiu Y, Robinson D, Pretlow TG, Kung HJ (1998) Etk/Bmx, a tyrosine kinase with a pleckstrin homology domain, is an effector of phosphatidylinositol 3' kinase and is involved in interleukin 6 induced neuroendocrine differentiation of prostate cancer cells. *Proc Natl Acad Sci USA* 95:3644-3649
- Rangone H, Pardo R, Colin E, Girault JA, Saudou F, Humbert S (2005) Phosphorylation of arfaptin 2 at Ser260 by Akt inhibits PolyQ huntingtin induced toxicity by rescuing proteasome impairment. *J Biol Chem* 280:22021-22028

- Rathmell JC, Elstrom RL, Cinalli RM, Thompson CB (2003) Activated Akt promotes increased resting T cell size, CD28 independent T cell growth, and development of autoimmunity and lymphoma. *Eur J Immunol* 33:2223-2232
- Read DE, Gorman AM (2009) Involvement of Akt in neurite outgrowth. *Cell Mol Life Sci* 66:2975-2984
- Ruggero D, Pandolfi PP (2003) Does the ribosome translate cancer? *Nat Rev Cancer* 3:179-192
- Samuels Y, Ericson K (2006) Oncogenic PI3K and its role in cancer. *Curr Opin Oncol* 18:77-82
- Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell SM, Riggins GJ, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Velculescu VE (2004) High frequency of mutations of the PIK3CA gene in human cancers. *Science* 304:554
- Samuels Y, Diaz LA Jr, Schmidt K, Kitterer O, Cummins JM, DeLong L, Cheong I, Rago C, Huso DL, Lengauer C, Kinzler KW, Vogelstein B, Velculescu VE (2005) Mutant PIK3CA promotes cell growth and invasion of human cancer cells. *Cancer Cell* 7:561-573
- Sansal I, Sellers WR (2004) The biology and clinical relevance of the PTEN tumor suppressor pathway. *J Clin Oncol* 22:2954-2963
- Sarbassov DD, Guertin DA, Ali SM, Sabatini DM (2005) Phosphorylation and regulation of Akt/PKB by the rictor mTOR complex. *Science* 307:1098-1101
- Sato S, Fujita N, Tsuruo T (2000) Modulation of Akt kinase activity by binding to Hsp90. *Proc Natl Acad Sci USA* 97:10832-10837
- Scheid MP, Woodgett JR (2001) PKB/AKT: functional insights from genetic models. *Nat Rev Mol Cell Biol* 2:760-768
- Shah OJ, Hunter T (2004) Critical role of T loop and H motif phosphorylation in the regulation of S6 kinase 1 by the tuberous sclerosis complex. *J Biol Chem* 279:20816-20823
- Shao Z, Bhattacharya K, Hsieh E, Park L, Walters B, Germann U, Wang YM, Kyriakis J, Mohanlal R, Kuida K, Namchuk M, Salituro F, Yao YM, Hou WM, Chen X, Aronovitz M, Tschlis PN, Bhattacharya S, Force T, Kilter H (2006) c-Jun N-terminal kinases mediate reactivation of Akt and cardiomyocyte survival after hypoxic injury *in vitro* and *in vivo*. *Circ Res* 98:111-118
- Shiojima I, Sato K, Izumiya Y, Schiekofer S, Ito M, Liao R, Colucci WS, Walsh K (2005) Disruption of coordinated cardiac hypertrophy and angiogenesis contributes to the transition to heart failure. *J Clin Invest* 115:2108-2118
- Shoji K, Oda K, Nakagawa S, Hosokawa S, Nagae G, Uehara Y, Sone K, Miyamoto Y, Hiraike H, Hiraike Wada O, Nei T, Kawana K, Kuramoto H, Aburatani H, Yano T, Taketani Y (2009) The oncogenic mutation in the pleckstrin homology domain of AKT1 in endometrial carcinomas. *Br J Cancer* 101:145-148
- Staal SP, Hartley JW, Rowe WP (1977) Isolation of transforming murine leukemia viruses from mice with a high incidence of spontaneous lymphoma. *Proc Natl Acad Sci USA* 74:3065-3067
- Stephens L, Anderson K, Stokoe D, Erdjument-Bromage H, Painter GF, Holmes AB, Gaffney PR, Reese CB, McCormick F, Tempst P, Coadwell J, Hawkins PT (1998) Protein kinase B kinases that mediate phosphatidylinositol 3, 4, 5 trisphosphate dependent activation of protein kinase B. *Science* 279:710-714
- Stokoe D, Stephens LR, Copeland T, Gaffney PR, Reese CB, Painter GF, Holmes AB, McCormick F, Hawkins PT (1997) Dual role of phosphatidylinositol 3, 4, 5 trisphosphate in the activation of protein kinase B. *Science* 277:567-570
- Suzuki A, de la Pompa JL, Stambolic V, Elia AJ, Sasaki T, del Barco Barrantes I, Ho A, Wakeham A, Itie A, Khoo W, Fukumoto M, Mak TW (1998) High cancer susceptibility and embryonic lethality associated with mutation of the PTEN tumor suppressor gene in mice. *Curr Biol* 8:1169-1178
- Taha C, Liu Z, Jin J, Al-Hasani H, Sonenberg N, Klip A (1999) Opposite translational control of GLUT1 and GLUT4 glucose transporter mRNAs in response to insulin. Role of mammalian target of rapamycin, protein kinase B, and phosphatidylinositol 3 kinase in GLUT1 mRNA translation. *J Biol Chem* 274:33085-33091
- Tessier M, Woodgett JR (2006) Serum and glucocorticoid regulated protein kinases: variations on a theme. *J Cell Biochem* 98:1391-1407

- Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M, Sticker M, Fumagalli S, Allegrini PR, Kozma SC, Auwerx J, Thomas G (2004) Absence of S6K1 protects against age and diet induced obesity while enhancing insulin sensitivity. *Nature* 431:200-205
- Vanhaesebroeck B, Alessi DR (2000) The PI3K/PDK1 connection: more than just a road to PKB. *Biochem J* 346(Pt 3):561-576
- Vasudevan KM, Gurumurthy S, Rangnekar VM (2004) Suppression of PTEN expression by NF- κ B prevents apoptosis. *Mol Cell Biol* 24:1007-1021
- Vasudevan KM, Burikhanov R, Goswami A, Rangnekar VM (2007) Suppression of PTEN expression is essential for antiapoptosis and cellular transformation by oncogenic Ras. *Cancer Res* 67:10343-10350
- Vasudevan KM, Barbie DA, Davies MA, Rabinovsky R, McNear CJ, Kim JJ, Hennessy BT, Tseng H, Pochanard P, Kim SY, Dunn IF, Schinzel AC, Sandy P, Hoersch S, Sheng Q, Gupta PB, Boehm JS, Reiling JH, Silver S, Lu Y, Stemke-Hale K, Dutta B, Joy C, Sahin AA, Gonzalez-Angulo AM, Lluch A, Rameh LE, Jacks T, Root DE, Lander ES, Mills GB, Hahn WC, Sellers WR, Garraway LA (2009) AKT independent signaling downstream of oncogenic PIK3CA mutations in human cancer. *Cancer Cell* 16:21-32
- Virbasius JV, Song X, Pomerleau DP, Zhan Y, Zhou GW, Czech MP (2001) Activation of the Akt related cytokine independent survival kinase requires interaction of its phospho domain with endosomal phosphatidylinositol 3 phosphate. *Proc Natl Acad Sci USA* 98:12908-12913
- Vivanco I, Sawyers CL (2002) The phosphatidylinositol 3 Kinase/AKT pathway in human cancer. *Nat Rev Cancer* 2:489-501
- Vivanco I, Palaskas N, Tran C, Finn SP, Getz G, Kennedy NJ, Jiao J, Rose J, Xie W, Loda M, Golub T, Mellinger IK, Davis RJ, Wu H, Sawyers CL (2007) Identification of the JNK signaling pathway as a functional target of the tumor suppressor PTEN. *Cancer Cell* 11:555-569
- Wang Q, Liu L, Pei L, Ju W, Ahmadian G, Lu J, Wang Y, Liu F, Wang YT (2003) Control of synaptic strength, a novel function of Akt. *Neuron* 38:915-928
- Watson RT, Pessin JE (2006) Bridging the GAP between insulin signaling and GLUT4 translocation. *Trends Biochem Sci* 31:215-222
- Yoeli Lerner M, Yiu GK, Rabinovitz I, Erhardt P, Jauliac S, Toker A (2005) Akt blocks breast cancer cell motility and invasion through the transcription factor NFAT. *Mol Cell* 20:539-550
- Zhou GL, Tucker DF, Bae SS, Bhatheja K, Birnbaum MJ, Field J (2006) Opposing roles for Akt1 and Akt2 in Rac/Pak signaling and cell migration. *J Biol Chem* 281:36443-36453

Faithfull Modeling of *PTEN* Loss Driven Diseases in the Mouse

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Abstract A decade of work has indisputably defined *PTEN* as a pivotal player in human health and disease. Above all, *PTEN* has been identified as one of the most commonly lost or mutated tumor suppressor genes in human cancers. For this reason, the generation of a multitude of mouse models has been an invaluable

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strategy to dissect the function and consequences-of-loss of this essential, evolutionary conserved lipid phosphatase in tumor initiation and progression.

In this chapter, we will summarize the mouse models that have allowed us to faithfully recapitulate features of human cancers and to highlight the network of connections between the *PTEN* signaling cascade and other oncogenic or tumor suppressive pathways.

Notably, *PTEN* represents one of the most extensively modeled genes involved in human cancer and exemplifies the strength of genetic mouse modeling as an approach to gain information aimed to improve our understanding of and ability to alleviate human disease.

1 Introduction

In 1997, *PTEN* (phosphatase and tensin homolog deleted on chromosome 10) was identified as the frequently lost tumor suppressor gene in a region of human chromosome 10 (10q23) that was known to be highly susceptible to deletion in malignant tumors of the prostate and the brain (Li et al. 1997; Steck et al. 1997).

Soon after its discovery, the work of Maehama and Dixon (1998) unveiled the biochemical function of PTEN as a plasma-membrane lipid phosphatase that hydrolyzes the 3-phosphate on the second-messenger molecule phosphatidylinositol-3,4,5-triphosphate (PIP₃) to generate phosphatidylinositol-4,5-bisphosphate (PIP₂). In the following years, several groups (Di Cristofano et al. 1998; Podsypanina et al. 1999; Stambolic et al. 1998) showed that PTEN exerts its function as a tumor suppressor at least in part through negative regulation of the crucial cell survival serine/threonine kinase AKT (PKB).

Since then, PTEN has been shown to affect pleiotropic cellular processes such as cell cycle progression, cell proliferation, senescence, chemotaxis, apoptosis, aging, muscle contractility, DNA damage response, angiogenesis, and cell polarity. In line with its role in multiple crucial cellular processes, PTEN has a role in the pathogenesis of numerous diseases such as diabetes, autism, and cancer.

Indeed, *PTEN* is one of the most frequently mutated, deleted, and silenced tumor suppressor genes in human cancer. The importance of *PTEN* as a tumor suppressor is supported by the observations that germline *PTEN* mutations in humans can result in autosomal dominant syndromes collectively referred to as the *PTEN* hamartomas tumor syndromes (PHTS), characterized by developmental defects, neurological deficits, multiple hamartomas in various tissues including skin, breast, intestine and brain, and an increased risk of breast, thyroid and endometrial cancers (Liaw et al. 1997; Marsh et al. 1997; Zhou et al. 2000).

The identification of *PTEN* as an important tumor suppressor gene led to a rapid outburst of several mouse models aimed at understanding the consequences of *Pten* loss. During this time, these mouse models have been further refined to study specific organs and specific cell lineages. This has allowed us to faithfully recapitulate some features of human cancers and to reconstruct the intricate connections

between the *PTEN* signaling cascade and other oncogenic or tumor suppressive pathways.

Overall, this chapter will focus on the role of *PTEN* as a critical player in human diseases and, specifically, on the faithful mouse models generated to dissect the roles of this phosphatase in tumor initiation and progression in different organs. Additionally, a particular relevance will be given to the work carried out *in vivo* in the mice to identify the network of signaling pathways enabling *PTEN* to exert its tumor suppressive function. Finally, emphasis will be given to the differential outcomes observed in different contexts as a consequence of loss of *Pten*.

2 Spectrum of Human Diseases Associated with Loss of *PTEN*

Over the last decade a multitude of important studies have identified *PTEN* gene mutations in a wide range of sporadic malignancies and at a high frequency in cancer-susceptibility syndromes.

Sequencing of the *PTEN* gene has revealed that this non-redundant, evolutionary conserved phosphatase is one of the most commonly mutated tumor suppressors in human malignancies (Cairns et al. 1998; Dahia et al. 1997; Duerr et al. 1998; Rasheed et al. 1997; Shao et al. 1998; Tashiro et al. 1997; Wang et al. 1997).

Genetic alterations of the *PTEN* gene include various types of abnormalities ranging from point mutations (encoding mostly unstable and/or catalytically inactive proteins) to large chromosomal deletions (Georgescu et al. 1999, 2000; Li et al. 1997; Steck et al. 1997). *PTEN* mutations can affect both alleles in various cancers with the following frequencies: endometrial (~50%), glioblastoma (~30%), melanoma (~12%), prostate (~10%), and breast (~5%) (Ali et al. 1999; Birck et al. 2000; Cairns et al. 1997; Celebi et al. 2000; Chiariello et al. 1998; Duerr et al. 1998; Haluska et al. 2006; Lin et al. 1998; Saal et al. 2005; Shao et al. 1998; Steck et al. 1997; Tashiro et al. 1997; Wang et al. 1997; Zhou et al. 2002). Loss of one *PTEN* allele is frequently observed in the following malignancies: glioma (~75%), breast (~40%), colon (~20%), lung (~37%), prostate (~42%) (Bose et al. 1998; Feilotter et al. 1998; Lin et al. 1998; Rubin et al. 2000; Teng et al. 1997).

Importantly, *PTEN* expression is regulated not only genetically but also at the transcriptional/translational level. DNA methylation, transcriptional repression, and microRNA-directed mRNA degradation and translational abrogation have been reported to be important mechanisms in reducing *PTEN* expression in several cancers (Wiencke et al. 2007; Yang et al. 2008; Poliseno et al. 2010).

Overall, these findings imply that loss of function of *PTEN* is a common event in cancer, which is accomplished through several layers of control and mechanisms.

Germline deletion/mutation of *PTEN* is associated with several autosomal dominant tumor predisposition syndromes including Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, Lhermitte-Duclos disease, Proteus syndrome, and Proteus-like syndrome (Liaw et al. 1997; Marsh et al. 1997; Zhou et al. 2000, 2001). These patients often suffer from hamartomas in multiple organs with the risk of

progression to malignant cancer transformation. In addition to hamartoma development, patients affected by Cowden syndrome and Bannayan Riley Ruvalcaba also develop macrocephaly (Buxbaum et al. 2007; Herman et al. 2007). This observation led to an association of PTEN mutation with autism, which is characterized by patients with macrocephaly (Butler et al. 2005). This observation expanded the role of PTEN to suppress human diseases of non-neoplastic nature. Recently, PTEN loss has also been associated with neurological diseases such as Parkinson's (Gasser 2007) and metabolic syndromes such as diabetes. The latter implication is supported by studies in animal that have demonstrated that *Pten* deletion causes an insulin sensitivity phenotype (Stiles et al. 2004, 2006). This finding supports the notion that patients affected by PHTS characterized by *Pten* mutations also present increased insulin sensitivity (Iida et al. 2000).

Together these data point to a role for PTEN as a key regulator of several cellular processes and that deregulation of its function can cause a wide spectrum of human diseases.

3 Modeling *PTEN* Loss in Specific Murine Organs

Extensive mouse modeling has been performed to elucidate the importance of PTEN and the consequences of its loss in human health and disease.

Homozygous deletion of *Pten* in the mouse embryo is lethal and is characterized by developmental defects in the mesoderm, endoderm and ectoderm (Di Cristofano et al. 1998). Heterozygous *Pten* mice develop multiple neoplasias in a wide spectrum of tissues including prostate, thyroid, colon, lymphatic system, mammary gland, and endometrium (Di Cristofano et al. 1998; Podsypanina et al. 1999; Stambolic et al. 2000; Suzuki et al. 1998). These mouse models also recapitulate some of the features of the PTEN-associated hamartoma syndromes in humans.

To further analyze the consequences of *Pten* loss in other organs, several tissue specific models of *Pten* deletion have been developed using a conditional gene-targeting approach (Table 1). In this chapter, we will examine several mouse models of *Pten* conditional inactivation as examples of the human tissues where loss of *Pten* is observed, such as brain, prostate, and breast.

3.1 Brain

The first three mouse models of tissue-specific inactivation of *Pten* were generated in 2001 (Backman et al. 2001; Groszer et al. 2001; Kwon et al. 2001) with the brain chosen as a target organ. The choice was most likely dictated by the fact that: (1) *PTEN* is very highly frequently mutated in glioblastoma (30%), the most aggressive primary brain tumor in humans (Knobbe et al. 2002) and (2) syndromes associated with germline mutation of *PTEN* are characterized by neurological abnormalities.

Table 1 Tissue-specific mouse models of *Pten* deletion

Promoter	Tissue	References	Phenotype
Adipose tissue			
<i>aP2Cre</i>	Adipocytes	Kurlawalla-Martinez et al. (2005)	No alterations in adiposity or plasma fatty acids. Increased systemic glucose tolerance and insulin sensitivity
Bone and cartilage			
<i>Col2a1Cre</i>	Osteo-chondro progenitors	Ford-Hutchinson et al. (2007)	Alterations in skeletal size and bone architecture. Metastatic osteosarcomas at very low penetrance
<i>OcCre</i>	Osteoblast	Liu et al. (2007)	Increase in bone mineral density throughout life. In vitro osteoblasts lacking <i>Pten</i> show more differentiation and reduced apoptosis
Bladder			
<i>FabpCre</i>	Urothelium of the bladder, kidney and ureter	Tsuruta et al. (2006), Yoo et al. (2006)	Urothelial hyperplasia in which component cells show enlarged nuclei and increased cell size. With time, 10% of mutant mice spontaneously develop pedicellate papillary transitional cell carcinomas (TCC) These mice develop also adenocarcinoma of prostate, seminal vesicles, and urethra; vaginal squamous cell carcinoma; adenocarcinoma of colon
Breast			
<i>MMTVCre</i>	Breast epithelium. Also prostate and skin are targeted	Backman et al. (2004), Li et al. (2002)	Breast development abnormalities. Breast tumors at 9–10 months. High-grade PIN that progresses to prostrate carcinoma. Mild epidermal hyperplasia

(continued)

Table 1 (continued)

Promoter	Tissue	References	Phenotype
Central nervous system			
<i>En2Cre</i>	Vermis	Marino et al. (2002)	No differences in cell differentiation. Mild defect in cell migration and decreased proliferation
<i>GfapCre</i>	Granule cells of cerebellum, dentate gyrus, cortical neurons, in a fraction of Bergmann-glia as well as astrocytes and oligodendrocytes	Backman et al. (2001), Kwon et al. (2001), Yue et al. (2005)	Premature death. Defects in neuronal migration and specialized subcellular structure. Increased cell size. Abnormalities phenocopying the ones observed in patients with Lhermitte–Duclos disease
<i>L7Cre</i>	Purkinje cells	Marino et al. (2002)	Subtle irregularities of Purkinje cell lining but no major architectural disturbances. Noticeable increase in cell size, including thickening of dendrites and descending axons
<i>NesCre</i>	Neural stem/progenitor cells	Groszer et al. (2001, 2006)	Enlarged, histoarchitecturally abnormal brains, which resulted from increased cell proliferation, decreased cell death, and enlarged cell size. Enhanced self-renewal capacity of neural stem cells
<i>NesCre</i>	Layers II–V of cerebral cortex, granular and polymorphic layers of dentate gyrus	Kwon et al. (2006)	Abnormal social interaction and exaggerated responses to sensory stimuli. Macrocephaly and neuronal hypertrophy, including hypertrophic and ectopic dendrites and axonal tracts with increased synapses. Abnormal behavior resembling autism spectrum disorder
<i>PomcCre</i>	Hypotalamic proopiomelanorin neurons	Plum et al. (2006)	Hyperphagia and sexually dimorphic diet-sensitive obesity. Neuronal membrane hyperpolarization and reduction in basal

<i>Endothelium</i>				firing rate due to increased ATP-sensitive potassium (KATP) channel activity
<i>Tie2Cre</i>	Endothelial and endocardial cells	Hamada et al. (2005), Suzuki et al. (2007)	Heterozygous mice display enhanced tumorigenesis due to an increase in angiogenesis driven by vascular growth factors. Homozygous mice die before embryonic day 11.5 (E11.5) due to bleeding and cardiac failure caused by impaired recruitment of pericytes and vascular smooth muscle cells to blood vessels, and of cardiomyocytes to the endocardium	
<i>Immune system</i>				
<i>CD19Cre</i>	B cells	Anzelon et al. (2003), Suzuki et al. (2003)	Increased serum autoantibodies and elevated numbers of B1a cells. Defects in immunoglobulin class switch recombination associated with impaired induction of activation-induced cytidine deaminase	
<i>LckCre</i>	T cells	Suzuki et al. (2001), Hagenbeek et al. (2004), Hagenbeek and Spits (2008)	T-cell lymphomas leading to a premature death. Increased proliferation and decreased apoptosis	
<i>LysMCre</i>	Myeloid lineage; granulocytes	Zhu et al. (2006), Subramanian et al. (2007)	Augmented chemoattractant-induced transwell migration and superoxide production. Enhanced recruitment of neutrophils to the inflamed peritoneal cavity	
<i>LysMCre</i>	Myeloid lineage; macrophages	Kuroda et al. (2008)	Enhanced susceptibility to Leishmania infection	
<i>Mx1Cre</i>	HSCs	Yilmaz et al. (2006), Zhang et al. (2006)	Myeloproliferative disease within days and transplantable leukaemias within weeks.	

(continued)

Table 1 (continued)

Promoter	Tissue	References	Phenotype
Liver			Enhanced hematopoietic stem cell (HSC) proliferation leading to HSC depletion via a cell-autonomous mechanism
<i>AlbCre</i>	Hepatocytes	Horie et al. (2004), Stiles et al. (2004)	Massive hepatomegaly and steatohepatitis with triglyceride accumulation. Insulin hypersensitivity. 100% incidence of hepatoadenomas and 66% of hepatocellular carcinomas
Lung			
<i>SpCre (doxycyclin-inducible)</i>	Lung epithelium	Yanagi et al. (2007)	Death caused by hypoxia soon after birth. Surviving mice develop spontaneous lung adenocarcinomas. Mice with postnatal deletion of <i>Pten</i> show lung tumors with high penetrance
Pancreas			
<i>RipCre</i>	Pancreatic β -cells and hypothalamus	Nguyen et al. (2006), Stiles et al. (2006)	Significant whole-body growth restriction and increased insulin sensitivity
<i>Pdx1Cre</i>	All pancreatic cell lineages	Stanger et al. (2005)	Increased islet mass without compromise of beta-cell function. Protection from developing streptozotocin-induced diabetes A fraction of mice develop ductal malignancy. Ductal metaplasia results from the expansion of centroacinar cells
Prostate			
<i>PbCre</i>	Prostatic epithelium	Trotman et al. (2003)	High-grade PIN that progresses to invasive prostate carcinoma
<i>PbCre4</i>	Prostatic epithelium	Wang et al. (2003), Trotman et al. (2003)	

<i>PsaCre</i>	Prostatic luminal epithelial cells	Ma et al. (2005c)	High grade PIN with progression to invasive adenocarcinoma and, only in Wang et al. model, lung metastasis Increased size of the luminal epithelial cells, large areas of hyperplasia, focal PIN that progresses to focal microinvasion and invasive prostate carcinoma
<i>PbCreER(T2)</i>	Prostatic epithelium	Luchman et al. (2008)	Low-grade PIN that progresses to overtly malignant lesions, characterized by high-grade PIN and microinvasive carcinoma
<i>PsaCreER(T2)</i>	Prostatic epithelium	Ratnacaram et al. (2008)	PIN lesions that progresses to adenocarcinoma
Reproductive system			
<i>Gdf9Cre</i>	Oocytes	Reddy et al. (2008)	Activation of the entire primordial follicle pool resulting in premature ovarian failure
<i>TnapCre</i>	Primordial germ cells	Kimura et al. (2003)	Bilateral testicular teratoma, characters. Primordial germ cells with greater proliferative capacity and enhanced pluripotent embryonic germ cell colony formation
Skeletal muscle and heart			
<i>MckCre</i>	Skeletal muscle cells and cardiac myocytes	Crackower et al. (2002), Wijesekara et al. (2005)	Hypertrophy, and decrease in cardiac contractility. Protection from insulin resistance and diabetes caused by high-fat feeding
Skin			
<i>K5Cre</i>	Keratinocytes	Suzuki et al. (2003)	Wrinkled skin because of epidermal hyperplasia and hyperkeratosis. Spontaneous tumors and acceleration in the onset of chemical-induced tumors
<i>DctCre</i>	Pigment producing cells: melanocytes, melanocyte stem	Inoue-Narita et al. (2008)	Half of the mice die shortly after birth with enlargements of the cerebral cortex and

(continued)

Table 1 (continued)

Promoter	Tissue	References	Phenotype
<i>Smooth muscle</i> <i>TaqI⁺Cre</i>	cells, retinal pigment epithelial cells, cells in brain (dentate gyrus of the hippocampus and the cortex)	Hernando et al. (2007)	hippocampus. Resistance to hair graying and susceptibility to carcinogen-induced melanomagenesis
<i>Thyroid gland</i> / <i>TpoCre</i>	Smooth muscle cells Thyroid epithelium	 Yeager et al. (2007)	Widespread smooth muscle cell hyperplasia and abdominal leiomyosarcomas, with a very rapid onset and elevated incidence (approximately 80%) Diffuse goiter characterized by extremely enlarged follicles. Increase in the thyrocyte proliferative index. Over two thirds of the mutant females develop follicular adenomas

For instance, patients with Lhermitte Duclos disease (LDD) develop dysplastic gangliocytoma, which is described clinically as a benign overgrowth of neurons in the cerebellum that causes increased intracranial pressure, ataxia and seizure (Zhou et al. 2003). Although patients affected by LDD have inherited only one normal copy of *PTEN*, the dysplastic cells have either completely lost *PTEN* expression or express only the mutant allele due to loss of heterozygosity (LOH); both events are characterized by an increase in the phosphorylation of AKT (Abel et al. 2005; Iida et al. 1998; Zhou et al. 2003). Two of these mouse models generated faithfully recapitulated the features of LDD. In these models, *Pten*^{flax/flax} mice were crossed with transgenic mice in which Cre recombinase expression is under the control of the glial fibrillary acidic protein (*Gfap*) promoter. In these mice, *Pten* is deleted late in the development of granule neurons of the cerebellum and results in a cell-autonomous loss of size regulation (Backman et al. 2001; Kwon et al. 2001). As a consequence, the size of *Pten*-deficient granule neurons progressively increases without evidence of abnormal proliferation. This observation is reminiscent of the focal lesions in LDD that rarely contain proliferative cells. Furthermore, LDD is characterized by dysplastic neurons ectopically placed in the molecular layer, which is similar to the ectopically positioned granule neurons resulting from a neuronal migration defect in mouse models (Abel et al. 2005; Backman et al. 2001; Kwon et al. 2001). Indeed, several studies have established that deletion of *Pten* in different neuronal types during development results in marked defects in migration and patterning in brain (Backman et al. 2001; Kwon et al. 2001; Marino et al. 2002; Yue et al. 2005). Another similarity between humans and the mouse models is that abnormalities in synaptic structure have been identified in LDD patients as well as in *Pten* conditional knockout mice (Fraser et al. 2008; Kwon et al. 2006). Overall, these mouse models suggest that the abnormalities observed in LDD can be attributed to key roles of *PTEN* in neuronal migration, size regulation, and specialized subcellular structure. Notably, this nonproliferative disease resulting from *PTEN* inactivation, although not malignant, is often associated with premature morbidity.

The third mouse model developed in 2001 utilized the neural stem cell specific nestin promoter (*NesCre*) to deliver Cre (Groszer et al. 2001) in neural stem cells, thereby resulting in *Pten* deletion throughout the entire brain. These mice succumb to an early postnatal death, presumably due to a continuous increase in brain size with individual cells being larger than those from wild-type mice brains. In contrast to the other two mouse models previously described, these mutant mice showed increased cell proliferation and decreased cell death. Using this model, Groszer et al. concluded that *Pten* most likely negatively regulates neural stem/progenitor cells self-renewal capability by modulating G₀ G₁ cell cycle entry (Groszer et al. 2006).

Other neurological abnormalities observed in patients with germline mutations of *PTEN* were also modeled in mice. One manifestation of inherited *PTEN* mutation includes macrocephaly; several studies have identified autism or autistic behaviors in macrocephalic PHTS patients (Butler et al. 2005; Goffin et al. 2001). Moreover, it is interesting to note that neurological phenotypes associated with

PHTS are very variable. As outlined above, the development of LDD is characterized by a second hit that inactivates the wild-type allele of *PTEN* in the lesions of the cerebellum. It is possible that other neurological deficits observed in PHTS patients, such as macrocephaly, mental retardation, and autism, are also associated with second hits that occur stochastically during development. In such a scenario, the timing and specific cell populations in which *PTEN* function is lost during development would determine the specific neurological outcome observed. For instance, a mouse model where *Pten* was deleted in subsets of differentiated neurons in the cerebral cortex and hippocampus showed anxiety-like behavior and decreased learning, that may recapitulate the autistic features of some PHTS patients (Kwon et al. 2006).

Although *PTEN* is frequently inactivated in malignant human brain tumors, PHTS is not associated with an increased incidence of brain tumors, and mice with heterozygous loss of *Pten* fail to develop brain tumors. Brain tumors are also not observed in conditional knockouts targeting *Pten* deletion in the brain, indicating that cooperating mutations in other genes are required for the neoplastic process (Backman et al. 2001; Fraser et al. 2004; Groszer et al. 2001; Kwon et al. 2001; Marino et al. 2002) (see following paragraphs).

3.2 Prostate

Prostate cancer and glioblastoma cell lines were the first cellular models where deletion of the chromosomal region containing *PTEN* was reported. These findings led to the identification of *PTEN* as a tumor suppressor gene (Li et al. 1997; Steck et al. 1997). It is reported that the majority of primary prostate cancers show loss of only one allele of *PTEN*, whereas homozygous inactivation of *PTEN* is generally associated with advanced cancer and metastasis (Gray et al. 1998).

Although human and mouse prostates are structurally dissimilar, prostate cancer progression in mice and humans is strikingly similar. In both species, epithelial hyperplasia is followed by low-grade prostatic intraepithelial neoplasia (PIN), which can progress to high-grade PIN. As the lesion becomes more neoplastic and aggressive, the prostate epithelium invades through the basement membrane into the surrounding stroma, thus establishing a localized yet invasive adenocarcinoma (De Marzo et al. 2003; Marandola et al. 2004).

In an effort to define the role of *PTEN* loss in prostate tumorigenesis, a series of *Pten* loss mouse models, the so called “hypomorphic *Pten* allelic series” (*Pten* heterozygous, *Pten* hypomorphic, and *Pten* conditional knock-out), have been generated (Trotman et al. 2003). Specific deletion of *Pten* in the prostate was achieved by crossing *Pten*^{loxP/loxP} mice with *Probasin-Cre* (*PB-Cre*) transgenic mice. *PB-Cre* transgenic mice express *Cre* recombinase under the control of the *ARR2 Probasin* promoter specifically in the prostate epithelium post-puberty (Wu

et al. 2001). The generation of the “hypomorphic *Pten* allelic series” has revealed that the prostatic epithelium is exquisitely vulnerable to subtle variations of PTEN expression levels. For instance, loss of one allele of *Pten* is associated with the development of high-grade PIN with incomplete penetrance after a long latency (9 months), whereas when the level of *Pten* is reduced to ~30% (hypomorphic mouse model), mice developed invasive prostatic adenocarcinoma albeit with incomplete penetrance (Trotman et al. 2003). Furthermore, complete loss of *Pten* results in the development of high-grade PIN (HG-PIN) as early as 8 weeks of age, together with the concomitant activation of cellular senescence response (see below) (Chen et al. 2005). HG-PIN lesions progress to invasive prostate cancer with complete penetrance at 6 months of age, once the senescence response has been evaded (Chen et al. 2005; Trotman et al. 2003). These analyses imply that (1) loss of PTEN is critical for prostate cancer initiation and that (2) the level of PTEN expression is inversely associated with prostate tumorigenesis.

Wang et al. also used *PB-Cre* transgenic mice to generate mice with conditional inactivation of *Pten* in the prostate, which also results in invasive prostate cancer (Wang et al. 2003). Additionally, these mice developed metastatic prostate cancer of the lymph nodes and lung, which is not observed in other mouse models of *Pten* conditional inactivation in the prostate (Abate-Shen et al. 2003; Chen et al. 2005; Wang et al. 2003). This may be due to the different genetic background strain of the mice, which is known to influence cancer susceptibility.

In a later report, by crossing *Pten*^{loxP/loxP} mice with *MMTV-Cre* transgenic mice, Backman et al. inactivated *Pten* in the prostate during development (Backman et al. 2004). Deletion of *Pten* in the prostate before puberty resulted in the onset of neoplastic lesions at a very early time point, with mice displaying high-grade PIN by the age of 2 weeks at complete penetrance that frequently progressed to invasive adenocarcinomas by 7–14 weeks (Backman et al. 2004). These data show that, if *Pten* has already been deleted in the prostate during development, the incidence, penetrance, and progression of neoplasia are much greater than if *Pten* is lost during or after puberty.

In 2005, yet another model of complete *Pten* inactivation in the prostate was generated using *Pten*^{loxP/loxP} mice crossed with prostate-specific antigen (PSA)-*Cre* transgenic mice (Ma et al. 2005c). The onset of prostatic neoplastic lesions is significantly delayed in these mice which show focal PIN at the age of 4–5 months. By 7–9 months, focal microinvasion was observed which progressed to frank invasive adenocarcinoma at 10–14 months (Ma et al. 2005c).

Recently, two groups have generated two mouse models where *Pten* deletion is temporally controlled through the use of *Pten*^{loxP/loxP} mice crossed with tamoxifen-inducible Cre recombinase transgenic mice (Luchman et al. 2008; Ratnacaram et al. 2008). Like other models before, deletion of *Pten* results in the development of PIN lesions that later progress to invasive adenocarcinoma.

Together, models of conditional *Pten* inactivation in the prostate clearly demonstrate the sensitivity of the prostatic epithelium to alterations of *Pten* and they recapitulate the sequential stages of the human disease from PIN to invasive

prostate cancer where time to progression is dictated solely by the developmental time of *Pten* excision and the remaining dose of functional Pten.

3.3 Breast

A characteristic feature of Cowden disease is the development of benign breast hamartomas that are accompanied by a higher risk of breast cancer. Although somatic *PTEN* mutations are detected only in a smaller fraction of breast cancer cases (Dahia 2000), LOH at the *PTEN* locus (10q23) is frequently found (40%) (Bose et al. 1998; Garcia et al. 1999). Furthermore, immunohistochemical studies suggest that loss of PTEN protein expression is a common event in breast cancer (33–48%), with strong correlation with lymph node metastasis, loss of estrogen receptor staining, and disease related death (Depowski et al. 2001; Perren et al. 1999). Thus, epigenetic mechanisms are hypothesized to be responsible for a number of cases in which PTEN levels are downregulated or even totally ablated in the absence of a detectable mutation.

The relevance of *Pten* in breast tumorigenesis was initially highlighted in the mouse model of *Pten* germline heterozygous loss generated by Stambolic et al. (2000). Female *Pten*^{+/-} developed mammary tumors at incomplete penetrance, with most of them having features of well-differentiated adenocarcinoma. Similar to CS patients (Schrager et al. 1998), breast lesions in *Pten*^{+/-} mice displayed marked proliferation of the stroma. The authors observed an increased penetrance of the breast tumors with age in *Pten*^{+/-} mice thereby suggesting a requirement for additional hits for tumor progression in this tissue.

After that, to fully understand the role of *Pten* in breast tumorigenesis, in 2002 Li et al. crossed *Pten*^{loxp/loxp} mice with transgenic mice expressing MMTV-Cre transgenes in order to achieve *Pten* deletion in the mammary epithelium (Li et al. 2002). The deletion of *Pten* in mammary epithelium triggered increased cell proliferation, hyper-branched ductal structure, precocious development, delayed involution and severely impaired apoptosis. *Pten*-deficient mammary epithelium also displayed remarkable neoplastic changes. Females with mammary-specific *Pten* deletion develop tumors as early as 2 months. Histological features of the tumors varied from benign fibroadenomas to pleiomorphic adenocarcinomas. Furthermore, immunohistochemistry analysis revealed up-regulation of cytokeratins 5 and 6 in these mice (Li et al. 2002). Interestingly, this finding nicely correlates with over-expression of these two cytokeratins in human breast tumors of the basal subtype (Sorlie et al. 2001). The basal subtype often occurs in patients with germline BRCA1 mutations and is associated with a poor prognosis. Importantly, it has been recently shown that heterozygous inactivation of *Pten* leads to the formation of basal-like mammary tumors in mice, and that loss of *PTEN* expression is significantly associated with this subtype of breast cancer in human sporadic and BRCA1-associated hereditary breast cancers (Saal et al. 2008). In addition, Saal and colleagues have identified frequent gross *PTEN* mutations, involving

intragenic chromosome breaks, inversions, deletions and micro copy number aberrations, specifically in BRCA1-deficient tumors (Saal et al. 2008).

It has recently been shown that even a subtle reduction in *Pten* dose determines breast cancer susceptibility (Alimonti et al. 2010). Indeed, *Pten* hypomorphic mice, expressing 80% normal levels of *Pten*, develop a spectrum of tumors, with breast occurring at the highest penetrance (Alimonti et al 2010). Overall, all these observations underscore the essential role of *PTEN* during normal mammary gland development and in suppressing breast cancer formation.

4 In Vivo Deconstruction of the PI3K-AKT-mTOR Axis

The numerous mouse models generated to study the PI3K-AKT-mTOR pathway have been valuable tools to shed light on the role of various components of the PI3K signaling cascade in disease and tumorigenesis. Overall, these mouse models have defined the mTOR pathway as a crucial converging node downstream PI3K-AKT signals required for oncogenic transformation driven by loss of *PTEN*.

4.1 PI3K-PDK-AKT

The PI3K (phosphatidylinositol-3-kinase) pathway starts at the plasma membrane where the binding of ligands to the growth factor receptor tyrosine kinases activate PI3K, which phosphorylates PIP2 to produce PIP3, thereby directly antagonizing PTEN (Klinghoffer et al. 1996).

Class I PI3K contains four p110 isoforms, α , β , γ , and δ . The association between p110 α and tumorigenesis is well established and has been corroborated by the occurrence of gain of function p110 α mutations in human cancer (Samuels and Velculescu 2004). Recently, the p110 β isoform has also been connected to oncogenesis (Ciraolo et al. 2008; Jia et al. 2008). With regards to *PTEN*-loss driven cancer, conditional inactivation of PIK3CB, the gene encoding p110 β , blocked prostate tumorigenesis mediated by loss of *PTEN* whereas prostate-specific knockout of the α -isoform did not alter tumor formation. The observations of Jia and co-workers identify a previously unknown role for p110 β in cancer, specifically in *PTEN* mutated tumors. These studies collectively suggest that p110 β may represent a potential “druggable” target, specially in *PTEN* null cancers.

PI3K signaling induces a series of growth-promoting events through the activation of the protein kinases PDK1 and AKT, which directly bind to and are activated by PIP3 (Alessi et al. 1997; Currie et al. 1999). Upon PIP3 binding, PDK1 induces AKT kinase activity 30-fold by phosphorylating it on residue T308 in addition to the phosphorylation of numerous other target proteins within the T loop (such as Serum and Glucocorticoid-regulated kinases, SGK) enabling their activation

(Alessi et al. 1997). AKT, in turn, phosphorylates multiple targets to activate the cell cycle, prevent apoptosis and trigger cellular growth (Manning and Cantley 2007). *In vivo* studies in the mice have genetically highlighted the important epistasis of *Pten* and *Pdk1* and *Akt*. Compound mutant mice have provided clear genetic evidence for the roles of *Akt* and *Pdk1* as mediators of cancer phenotypes identified upon heterozygous *Pten*-loss in mice. Specifically, *Akt1* deficiency suppresses tumor development in *Pten*^{+/-} mice (Chen et al. 2006). Similarly, the hypomorphic expression of *Pdk1* (levels that are 80-90% reduced compared with normal) inhibits tumor formation in *Pten*^{+/-} mice (Bayascas et al. 2005). Therefore, these mouse models have validated AKT1 and PDK1 as critical players in mediating tumorigenesis upon PTEN-loss.

4.2 TSC1/2-Rheb-mTOR

Among the many downstream targets of AKT, the mammalian target of rapamycin (mTOR) has been demonstrated to be an essential effector in promoting cell proliferation and susceptibility to oncogenic transformation. mTOR is a serine/threonine kinase that regulates protein synthesis, cell growth, and proliferation in response to pleiotropic inputs including growth factors, nutrients, energy, and stress (Wullschlegel et al. 2006). mTOR differentially regulates PI3K/AKT signaling by acting as a key component of two multiprotein complexes: mTOR complex 1 (mTORC1) which is activated downstream of AKT, and mTOR complex 2 (mTORC2) which has been demonstrated to phosphorylate AKT on Ser 473 (Sarbasov et al. 2005). This modification, in conjunction with the phosphorylation on Thr308 by PDK1 triggers full activation of AKT in response to mitogenic stimuli (Sarbasov et al. 2005). Moreover, in many cell types, mTORC1 has been reported to elicit a negative feedback regulation on the PI3K pathway through the ability of its downstream target ribosomal S6 kinase 1 (S6K1) to inhibit IRS-1 (reviewed in Guertin and Sabatini 2007). The elusive cross-talk between the AKT and mTOR pathways was uncovered by the finding that tuberous sclerosis complex 1 (TSC1) and 2 (TSC2) negatively regulates mTORC1 (Gao et al. 2002; Tapon et al. 2001). These studies demonstrated that AKT phosphorylates and inactivates the TSC1/TSC2 complex, and as a consequence results in mTORC1 activation (Jaeschke et al. 2002; Tee et al. 2002). Specifically, Ras homologue enriched in brain (Rheb), a small guanosine triphosphate (GTP)-binding protein, was discovered as a novel substrate for TSC2, which could also lead to the activation of mTOR (Garami et al. 2003; Inoki et al. 2003; Zhang et al. 2003). TSC2 was shown to display a GTPase activating protein (GAP) activity towards the Rheb GTPase; this event stimulates the intrinsic GTP-hydrolysis activity of Rheb to promote its transition from an active GTP-bound to an inactive guanosine diphosphate (GDP)-bound form (Garami et al. 2003; Zhang et al. 2003). Conversely, inactivation of the TSC1/TSC2 complex by AKT phosphorylation, results in GTP loading and activation of Rheb, which ultimately promotes the activation of mTORC1. AKT also promotes mTORC1 activity through phosphorylation of PRAS40, which prevents its inhibitory function

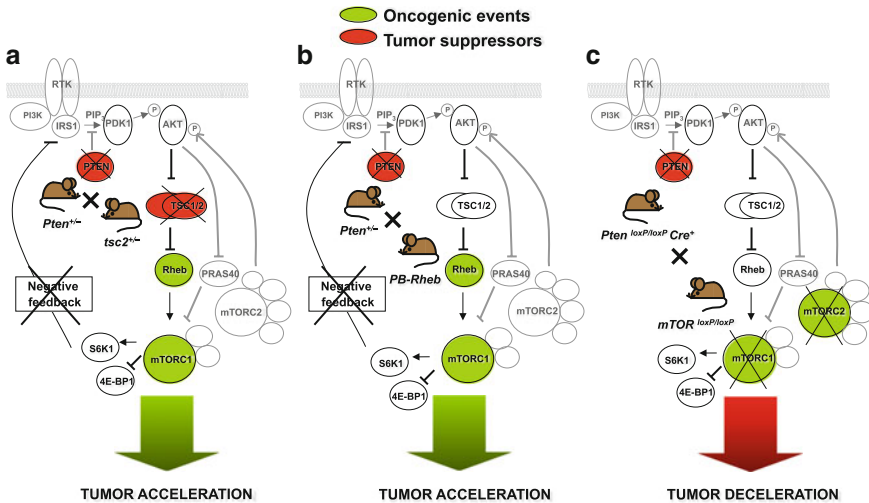


Fig. 1 Analysis of the contribution of the PI3K pathway to *Pten* loss driven disease, through the combination of genetic events in the mouse. *Pten* loss driven disease is accelerated by *Tsc2* heterozygous loss (a) as well as *Rheb* transgenic expression (b), whereas it is opposed by loss of *mTOR* (c)

on mTORC1 (reviewed in Guertin and Sabatini 2007). When active, mTORC1 promotes cell growth through phosphorylation of various regulators of translation including the well-characterized ribosomal S6K1 which activates the S6 ribosomal protein (S6), and the eukaryotic translation initiation factor 4E binding protein 1 (4EBP1) which leads to its uncoupling from the elongation initiation factor 4E (eIF4E; Wullschlegel et al. 2006).

Several mouse models have validated the importance of the mTOR pathway in further promoting tumorigenesis driven by *Pten* loss. For instance, oncogenic events proximal to mTOR activation such as *Tsc2* heterozygosity as well as *Rheb* overexpression cooperate with *Pten* haploinsufficiency to accelerate tumorigenesis (Ma et al. 2005b; Manning et al. 2005; Nardella et al. 2008) (Fig. 1a, b). A definitive proof of principle for a major role of mTOR in prostate tumorigenesis driven by *Pten*-loss was uncovered by the conditional inactivation of *mTOR* in *Pten* null prostates. In these prostates, *mTOR* deletion markedly suppressed the tumor initiation and progression observed in *Pten*-null mice (Nardella et al. 2009) (Fig. 1c). These findings are corroborated by the work of Guertin et al, which showed that Rictor, one of the components of the mTORC2 multiprotein complex, is also required for *Pten*-loss induced tumorigenesis in the mice (Guertin et al. 2009).

Collectively, these data have important therapeutic application in the treatment of cancer triggered by loss of PTEN, since mTOR is a kinase with activity that is amenable to pharmacological inhibition. Indeed, efforts have been placed to develop and improve upon drugs that inhibit mTOR activity. The first generation

of mTOR inhibitors directed solely against mTORC1, such as Rapamycin have had limited success in the clinic. We now await the development and testing of drugs targeting both mTORC1 and mTORC2 for the treatment of tumors triggered by PTEN deficiency and aberrant PI3K-AKT-mTOR signaling.

5 PTEN Network: Linking the PI3K Signaling Cascade to Other Oncogenic Pathways Through In Vivo Genetic Analysis

It is now well established that the PI3K pathway is intimately linked to several other oncogenic events including activated MAPK signaling, ETS-related gene (ERG) overexpression, and loss of the tumor suppressor gene p53. Below, we will describe the efforts to generate faithful mouse models of human cancers which recapitulate critical cooperative events identified in human cancer.

5.1 *PTEN-MAPK Pathway*

The RAS oncogene and the PTEN tumor suppressor are upstream of two of the most predominant oncogenic signaling pathways, MAPK and PI3K, respectively (Dhillon et al. 2007; Engelman et al. 2006). The signaling emanating from these two pathways is however complicated by a remarkable number of interconnections (Carracedo et al. 2008).

Ras is the upstream regulator of the MAPK pathway and frequently activated in cancer through mutations (G12D, G12V) which are sufficient to initiate cancer in the mouse (Fisher et al. 2001; Johnson et al. 2001). Ras mutations lead to hyperactivation of the MAPK pathway, which cross-talks with the PI3K cascade through the regulation of common targets such as BAD and TSC2 (Datta et al. 1996; Fang et al. 1999; Gupta et al. 2007; Ma et al. 2005a). In addition, activated Ras leads to loss of PTEN expression through c-Jun-mediated transcriptional events (Vasudevan et al. 2007). Consistent with this notion, mutations in RAS and PTEN in cancer tend to be mutually exclusive RAS mutations are prevalent in pancreatic, lung, and colon cancers, but not in glioblastomas whereas the opposite is true for PTEN mutation (Liu et al. 1997; Simpson and Parsons 2001).

Downstream of RAS, RAF (BRAF, CRAF, and ARAF) serine/threonine protein kinases regulate MAPK signaling (Balmanno and Cook 2009; Moodie et al. 1993). BRAF is also frequently found mutated in cancer (principally V600E mutation), most frequently in melanoma where BRAF mutation is observed in 50-70% of cell lines and tumors, and does not overlap with RAS mutations (Halilovic and Solit 2008). Unlike RAS, concomitant genetic alterations in PTEN and BRAF have been found in melanoma and shown to be cooperative events in the mouse (see below), therefore reinforcing the complexity of the interaction between components within these two pathways (PTEN-PI3K and MAPK) in cancer.

Combining *PTEN* and *BRAF* mutations to model human metastatic melanoma in mouse. Mutant activated *BRAF* (*BRAF*^{V600E}) can induce senescence in cultured melanocytes providing an explanation for the high frequency of *BRAF* mutations in benign nevi (Denoyelle et al. 2006; Dhomen et al. 2009; Michaloglou et al. 2005; Taube et al. 2009). Hence, overcoming oncogene-induced senescence may be critical for melanomagenesis. Progression to malignant melanoma is invariably accompanied by silencing of one or more tumor suppressor genes, most commonly *PTEN* or *CDKN2A* (Chin et al. 2006; Garraway et al. 2005). Additionally, the combination of mutated *BRAF* and silencing of *PTEN* expression is observed in 20% of human melanomas (Backman et al. 2004). While *Pten*-loss or *BRAF*^{V600E} activation alone did not have a dramatic consequence for melanoma onset and progression in mice, compound *BRAF*^{V600E}-*Pten*-Null mice succumbed to an aggressive form of metastatic melanoma (Dankort et al. 2009) (Fig. 2a). Of note, combinatorial inhibition of *MAPK* and *mTORC1* led to the reduction of melanoma

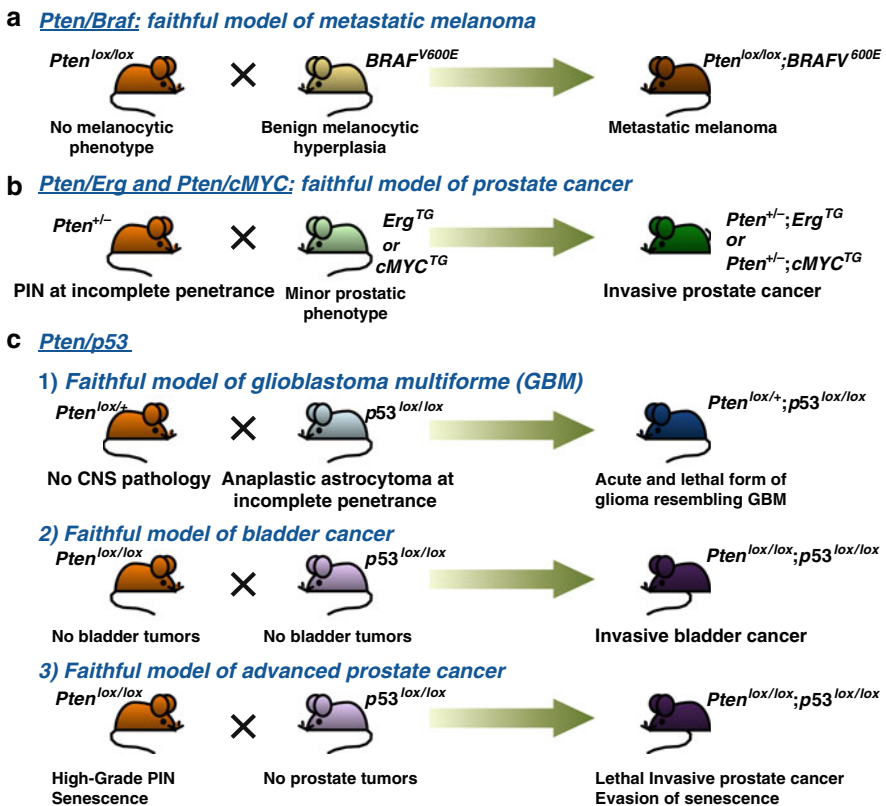


Fig. 2 Genetic interventions in the mouse that have allowed the generation of faithful mouse models resembling human disease. Faithful mouse models of human melanoma (a), prostate cancer onset and progression (b), glioblastoma multiforme, bladder cancer, and advanced prostate cancer (c)

formation, implying that this pharmacological approach may be an effective therapeutic avenue in the treatment of this type of cancer. Therefore, the *Pten*^{-/-}; BRAF^{V600E} melanoma model, together with other recently developed genetically modified melanoma mouse models (Goel et al. 2009), represent an invaluable tool for modeling of melanoma in the mouse and the evaluation of therapeutic approaches in the treatment of this deadly disease. Importantly, whether PTEN is required for the bypass and senescence induced by BRAF mutation in melanoma remains to be determined, and might add complexity to the already diverse tissue-specific outcomes of *Pten*-loss in vivo (see below).

5.2 *Pten and Transcriptional Regulators: Erg and Myc*

As mentioned above, PTEN is frequently lost or downregulated in prostate cancer (Salmena et al. 2008). Recently, the translocation of an ETS transcription factor gene (ERG or ETV1) to the TMPRSS2 gene promoter region, which contains androgen responsive elements, has been identified in prostate tumors (Tomlins et al. 2005). TMPRSS2-ERG is the first recurrent translocation event to be described in human tumors. It occurs in approximately 40% of prostate tumors and results in an aberrant androgen-regulated expression of ERG (Perner et al. 2007). In mice, transgenic ERG expression in the prostate leads to an unremarkable phenotype (Carver et al. 2009a), suggesting that ERG overexpression is not an initiating event in prostate cancer, in line with the notion that TMPRSS2-ERG translocation is rarely found in early lesions (Balmanno and Cook 2009; Carver et al. 2009b).

On the other hand, genetic lesions such as amplification and polymorphisms at 8q24, where c-MYC is located, are robustly associated with prostate cancer risk (Amundadottir et al. 2006; Bubendorf et al. 1999; El Gedaily et al. 2001; Gudmundsson et al. 2007; Haiman et al. 2007; Qian et al. 1997; Tsuchiya et al. 2002; Witte 2007; Yeager et al. 2007). Furthermore, recent studies have shown that over-expression of c-MYC is not restricted to advanced/late prostate cancer lesions, but occurs also in early lesions (Gurel et al. 2008). Additional studies are needed in order to determine the mechanisms underlying the frequent over-expression of c-MYC in prostate cancer, and to assess whether this is also a consequence of 8q24-polymorphism-relates transcriptional events or may in fact be the result of post-transcriptional mechanisms (Pomerantz et al. 2009).

Seeking a faithful model of prostate cancer in the mouse: Combinatorial mutation of PTEN with ERG and MYC. Loss of PTEN is frequently accompanied by the translocation of TMPRSS2-ERG in prostate cancer (Balmanno and Cook 2009; Carver et al. 2009a). Modeling the compound loss of *Pten* and *Erg* overexpression (Probasin-transgenic *Erg*, *Erg*^{TG}) in the mouse prostate has uncovered a strong cooperativity between these two genetic events (Balmanno and Cook 2009; Carver et al. 2009a) (Fig. 2b). Whereas *Pten* heterozygosity leads to high-grade prostate intraepithelial neoplasia (HGPIN) lesions starting at the age of 9 months, compound

Pten^{+/-} -Erg^{TG} mutants develop HGPIN by 2 months of age, which progresses to invasive cancer by the age of 6 months.

c-MYC transgenic expression in the prostate leads to hyperproliferation and PIN (Ellwood-Yen et al. 2003; Kim et al. 2009; Zhang et al. 2000). Moreover, *Pten*-heterozygous loss cooperated with c-MYC to induce high-grade prostatic intraepithelial neoplasia (HGPIN)/cancer lesions, which harbor loss of the wild type *Pten* allele (Kim et al. 2009) (Fig. 2b).

Overall, *Pten*^{+/-} -Erg^{TG} and *Pten*^{+/-} ; cMYC^{TG} prostate cancer models likely represents the most faithful models of prostate cancer initiation and progression to date, since they recapitulate the precise sequence of mutagenic events occurring in a large fraction of human prostate cancers. However, unlike human prostate cancer, which exhibits a highly metastatic tropism to the bone in later stages, mouse models have thus far failed to recapitulate these late events faithfully. Hence, additional genetic studies and novel combinatorial efforts in the mouse are required to generate better models of human prostate cancer progression to metastasis.

5.3 *Pten/p53*

In terms of overall frequency, p53 is undoubtedly the most frequently mutated tumor suppressor gene in human cancers (Levine et al. 2004; Vogelstein et al. 2000) with PTEN following in second (Cantley and Neel 1999; Simpson and Parsons 2001). The spectrum of human cancers associated with p53 and PTEN mutation are very different. (Fujisawa et al. 2000; Kato et al. 2000; Koul et al. 2002; Kurose et al. 2002). Mutations of p53 occur at high frequencies in lung, colon and breast cancers, whereas PTEN mutations are mostly found in glioblastoma, endometrial cancer, malignant melanoma, and prostate cancer. However, compound loss of *PTEN* and *p53* has been reported in glioblastoma (Han et al. 2008; Salmena et al. 2008), bladder cancer (Puzio-Kuter et al. 2009) and advanced/metastatic prostate cancer (Chen et al. 2009).

Mouse models of combined loss of Pten and p53 in glioblastoma, bladder and prostate cancer. In glioblastoma, the impact of combined *Pten* and *Trp53* loss has been recently reported in a model of concomitant deletion of *Pten* (in heterozygosity) and *Trp53* in the GFAP+ cell lineage. These mice develop an acute and lethal form of glioblastoma multiforme (Fig. 2c). Importantly, this mouse model displays features reminiscent of the pathological lesions observed in human glioblastoma multiforme (Zheng et al. 2008). Mechanistically, compound loss of *Pten* and *Trp53* in neural stem cells leads to increased cell renewal and decreased differentiation in a MYC-dependent fashion (Zheng et al. 2008).

Bladder cancer is a major cause of cancer morbidity and mortality (Jemal et al. 2005). Combined *p53* and *PTEN* losses have been identified in invasive bladder cancer and are reportedly causal factors that predict poor outcome (Puzio-Kuter et al. 2009). Indeed, combined loss of *Trp53* and *Pten* in mice results in lesions with

characteristics of human carcinoma in situ with complete penetrance at 6 months of age (Puzio-Kuter et al. 2009) (Fig. 2c).

In prostate cancer, partial loss of the *PTEN* tumor suppressor gene is a prevalent event (see above). However, complete loss of *PTEN* is infrequent in early lesions and is restricted to advanced cancers. Through the analysis of acute complete conditional loss of *Pten* in the prostatic epithelium, we found that one plausible explanation for this phenomenon is the fact that complete acute loss of *Pten* elicits a p53-dependent failsafe senescence response which opposes tumor progression, (Chen et al. 2005). In agreement with this notion and the fact that in human prostate cancer *p53* loss is a late event observed prevalently in advanced lesions, compound loss of *Pten* and *Trp53* in the mouse prostate leads to a lethal form of advanced prostate cancer where the senescence response has been evaded (Chen et al. 2005). In spite of the local aggressiveness of these tumors, *Pten/Trp53* compound mutants, surprisingly, do not develop metastatic prostate cancer (Chen et al. 2005).

Although *p53* and *PTEN* represent the most frequently lost of all tumor suppressors, further studies are required to precisely determine the frequency and the timing of their loss, and the specific tissues where it occurs. However, modeling these mutations in the mouse has already allowed the generation of faithful models of advanced prostate, bladder cancers, and glioblastoma that will prove extremely valuable to study the biology of these cancers and to test novel therapeutic modalities in preclinical studies.

6 Context-Dependent Differential Outcomes Triggered by Loss of *PTEN*

The large number of studies reporting phenotypes of *Pten* conditional knockout mice has highlighted the function of *Pten* in different cell and tissue types.

Although the PI3K pathway is ubiquitous, *PTEN*-mediated regulation of the PI3K/AKT pathway results in cell context-dependent outcomes such as cell size, proliferation, survival and senescence. Furthermore, there is a growing body of evidence suggesting that differential outcomes can be due to differential timing of *Pten* loss in specific stages of the development within the same tissue.

Cell size. Conditional knock-out mice show that loss of *Pten* may influence cell size or cell number depending on the specific context. The brain is an example of where selective deletion of *Pten* in specific cell types such as granule neurons of the cerebellum and dentate gyrus, cerebellar precursor cells and Purkinje neurons results in a cell-autonomous size increase in *Pten*-deficient cells (Backman et al. 2001; Kwon et al. 2001; Marino et al. 2002).

Cell number. In other settings, the consequences of *PTEN* loss determines changes in cell number, due to the combined effects of proliferation and cell survival, rather than aberrant cell size. Conditional deletion of *Pten* caused increased proliferation, decreased apoptosis and tumorigenesis, as exemplified in keratinocytes (Backman et al. 2004; Suzuki et al. 2004), prostatic epithelium

(Backman et al. 2004; Wang et al. 2003), mammary epithelium (Li et al. 2002), germ cells (Kimura et al. 2003) and hepatocytes (Horie et al. 2004). Overall, these examples suggest that the cellular context strongly influences the specific outcome of *PTEN* deficiency.

Cellular senescence. *PTEN* does not exert its tumor suppressive function in isolation, but cross-talks extensively with other tumor suppressors, including p53. Therefore, the status of the p19ARF/p53 network in the different tissues can also affect the differential context-dependent outcomes dictated by the loss of *Pten*.

This is nicely exemplified by the response of the prostatic epithelium upon complete inactivation of *Pten*. Surprisingly, Chen et al. showed that complete acute loss of *PTen* in the prostate did not provide a proliferative advantage as would be expected, but instead promoted a strong p53-dependent senescence response that opposed tumor progression (Chen et al. 2005). As predicted from these findings, combined inactivation of *Pten* and *Trp53* leads to unconstrained tumor growth as demonstrated by the generation of massive invasive prostate tumors. This implies that complete ablation of *PTEN* can be detrimental to tumor growth in the absence of p53 mutations and highlights the importance of haploinsufficiency or partial *PTEN* impairment in tumor progression. Clinically, these findings provide an explanation as to why complete *PTEN* loss is not frequently observed at cancer presentation.

Yilmaz et al. (2006) suggested that the hematopoietic stem cell (HSC) compartment may also be a tissue where complete loss of *Pten* triggers a senescence response (Yilmaz et al. 2006). Deletion of *Pten* in the adult HSCs results in a different outcome in normal hematopoietic stem cells versus leukemia-initiating cells (Yilmaz et al. 2006). Specifically, the authors show that deletion of *Pten* results in the generation of leukemic stem cells and concomitant depletion of normal HSCs. The mechanism responsible for the depletion of *Pten*-deficient HSCs remains to be elucidated but it has been speculated that *Pten* deficiency induces a senescence response in HSCs whereas the leukemia-initiating cells might acquire secondary mutations that inactivate the senescence response (Yilmaz et al. 2006).

Differential outcomes. In certain tissues, p53 mutations are not required for tumors to progress upon *Pten* loss because p53 is repressed through different mechanisms, and consequently cellular senescence is not observed. This is well exemplified by the deletion of *Pten* in smooth muscle, which results in the development of leiomyosarcomas with very high penetrance (80%) (Hernando et al. 2007). In response to loss of *Pten* the authors observed a substantial upregulation of p19Arf in the sarcoma cells, without concomitant induction of p53. In addition, they observed no evidence of cellular senescence either in the hyperplastic tissue or in the sarcomas. However, marked Mdm2 levels in leiomyosarcoma cells compared to normal smooth muscle of *Pten*-null mice, which kept p53 functionally repressed, thus reducing the need for p53 mutations usually required for tumor progression. In sum, Mdm2 stabilization promoted by Akt phosphorylation seems to prevail over Mdm2 inhibition by p19Arf in *Pten*-null smooth muscle cells, resulting in p53 functional inactivation and thereby tumor development (Hernando et al. 2007).

7 Conclusion

Tremendous technology advances have allowed us to gain powerful insight into the molecular and genetic determinants that drive cancer. Mouse models have been at the forefront of this revolution of information that has allowed us to faithfully recapitulate the features of tumor initiation and progression observed in human cancer. Mouse models of *Pten* loss have shed light on the critical roles of Pten in tumor suppression, specifically as a regulator of cell size, proliferation rate, and failsafe responses, such as senescence, in specific tissues. As one of the “most modeled” of all human cancer genes, Pten mouse models are exemplary of the power of genetic modeling and the success that can be achieved through such studies.

Further insight into the function of PTEN genetic mutations will rely upon the generation of specific point mutations knock-in mice models, which can inform us not only about canonical PTEN function, but the ever-increasing role of PI3K and AKT independent functions of PTEN. These models will provide further understanding of the regulatory mechanisms that affect the role of this protein in normal development and tumorigenesis.

Translation of the information acquired in mice has been and will be extremely useful for the preclinical evaluation of targeted therapeutic anti-cancer agents thereby dramatically improving our ability to cure this and other diseases.

References

- Abate Shen C, Banach Petrosky WA, Sun X, Economides KD, Desai N, Gregg JP, Borowsky AD, Cardiff RD, Shen MM (2003) Nkx3.1; Pten mutant mice develop invasive prostate adenocarcinoma and lymph node metastases. *Cancer Res* 63:3886–3890
- Abel TW, Baker SJ, Fraser MM, Tihan T, Nelson JS, Yachnis AT, Bouffard JP, Mena H, Burger PC, Eberhart CG (2005) Lhermitte Duclos disease: a report of 31 cases with immunohistochemical analysis of the PTEN/AKT/mTOR pathway. *J Neuropathol Exp Neurol* 64:341–349
- Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PR, Reese CB, Cohen P (1997) Characterization of a 3-phosphoinositide dependent protein kinase which phosphorylates and activates protein kinase B. *Curr Biol* 7:261–269
- Ali IU, Schriml LM, Dean M (1999) Mutational spectra of PTEN/MMAC1 gene: a tumor suppressor with lipid phosphatase activity. *J Natl Cancer Inst* 91:1922–1932
- Alimonti A, Carracedo A, Clohessy JG, Trotman LC, Nardella C, Egia A, Salmena L, Sampieri K, Haveman WJ, Brogi E, Richardson AL, Zhang J, Pandolfi PP (2010) Subtle variations in Pten dose determine cancer susceptibility. *Nat Genet* 42(5):454–458
- Amundadóttir LT, Sulem P, Gudmundsson J, Helgason A, Baker A, Agnarsson BA, Sigurdsson A, Benediksdóttir KR, Cazier JB, Sainz J et al (2006) A common variant associated with prostate cancer in European and African populations. *Nat Genet* 38:652–658
- Anzelon AN, Wu H, Rickert RC (2003) Pten inactivation alters peripheral B lymphocyte fate and reconstitutes CD19 function. *Nat Immunol* 4(3):287–294
- Backman SA, Stambolic V, Suzuki A, Haight J, Elia A, Pretorius J, Tsao MS, Shannon P, Bolon B, Ivy GO, Mak TW (2001) Deletion of Pten in mouse brain causes seizures, ataxia and defects in some size resembling Lhermitte Duclos disease. *Nat Genet* 29:396–403

- Backman SA, Ghazarian D, So K, Sanchez O, Wagner KU, Hennighausen L, Suzuki A, Tsao MS, Chapman WB, Stambolic V, Mak TW (2004) Early onset of neoplasia in the prostate and skin of mice with tissue specific deletion of Pten. *Proc Natl Acad Sci USA* 101:1725-1730
- Balmanno K, Cook SJ (2009) Tumour cell survival signalling by the ERK1/2 pathway. *Cell Death Differ* 16:368-377
- Bayascas JR, Leslie NR, Parsons R, Fleming S, Alessi DR (2005) Hypomorphic mutation of PDK1 suppresses tumorigenesis in *PTEN*(+/-) mice. *Curr Biol* 15:1839-1846
- Birck A, Ahrenkiel V, Zeuthen J, Hou Jensen K, Guldborg P (2000) Mutation and allelic loss of the *PTEN/MMAC1* gene in primary and metastatic melanoma biopsies. *J Invest Dermatol* 114:277-280
- Bose S, Wang SI, Terry MB, Hibshoosh H, Parsons R (1998) Allelic loss of chromosome 10q23 is associated with tumor progression in breast carcinomas. *Oncogene* 17:123-127
- Bubendorf L, Kononen J, Koivisto P, Schraml P, Moch H, Gasser TC, Willi N, Mihatsch MJ, Sauter G, Kallioniemi OP (1999) Survey of gene amplifications during prostate cancer progression by high throughout fluorescence in situ hybridization on tissue microarrays. *Cancer Res* 59:803-806
- Butler MG, Dasouki MJ, Zhou XP, Talebizadeh Z, Brown M, Takahashi TN, Miles JH, Wang CH, Stratton R, Pilarski R, Eng C (2005) Subset of individuals with autism spectrum disorders and extreme macrocephaly associated with germline *PTEN* tumour suppressor gene mutations. *J Med Genet* 42:318-321
- Buxbaum JD, Cai G, Chaste P, Nygren G, Goldsmith J, Reichert J, Anckarsater H, Rastam M, Smith CJ, Silverman JM, Hollander E, Leboyer M, Gillberg C, Verloes A, Betancur C (2007) Mutation screening of the *PTEN* gene in patients with autism spectrum disorders and macrocephaly. *Am J Med Genet B Neuropsychiatr Genet* 144B:484-491
- Cairns P, Okami K, Halachmi S, Halachmi N, Esteller M, Herman JG, Jen J, Isaacs WB, Bova GS, Sidransky D (1997) Frequent inactivation of *PTEN/MMAC1* in primary prostate cancer. *Cancer Res* 57:4997-5000
- Cairns P, Evron E, Okami K, Halachmi N, Esteller M, Herman JG, Bose S, Wang SI, Parsons R, Sidransky D (1998) Point mutation and homozygous deletion of *PTEN/MMAC1* in primary bladder cancers. *Oncogene* 16:3215-3218
- Cantley LC, Neel BG (1999) New insights into tumor suppression: *PTEN* suppresses tumor formation by restraining the phosphoinositide 3 kinase/AKT pathway. *Proc Natl Acad Sci USA* 96:4240-4245
- Carracedo A, Ma L, Teruya Feldstein J, Rojo F, Salmena L, Alimonti A, Egia A, Sasaki AT, Thomas G, Kozma SC, Papa A, Nardella C, Cantley LC, Baselga J, Pandolfi PP (2008) Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K dependent feedback loop in human cancer. *J Clin Invest* 118:3065-3074
- Carver BS, Tran J, Chen Z, Carracedo Perez A, Alimonti A, Nardella C, Gopalan A, Scardino PT, Cordon Cardo C, Gerald W, Pandolfi PP (2009a) ETS rearrangements and prostate cancer initiation. *Nature* 457:E1, discussion E2-E3
- Carver BS, Tran J, Gopalan A, Chen Z, Shaikh S, Carracedo A, Alimonti A, Nardella C, Varmeh S, Scardino PT, Cordon Cardo C, Gerald W, Pandolfi PP (2009b) Aberrant ERG expression cooperates with loss of *PTEN* to promote cancer progression in the prostate. *Nat Genet* 41:619-624
- Celebi JT, Shendrik I, Silvers DN, Peacocke M (2000) Identification of *PTEN* mutations in metastatic melanoma specimens. *J Med Genet* 37:653-657
- Chen Z, Trotman LC, Shaffer D, Lin HK, Dotan ZA, Niki M, Koutcher JA, Scher HI, Ludwig T, Gerald W, Cordon Cardo C, Pandolfi PP (2005) Crucial role of p53 dependent cellular senescence in suppression of Pten deficient tumorigenesis. *Nature* 436:725-730
- Chen ML, Xu PZ, Peng XD, Chen WS, Guzman G, Yang X, Di Cristofano A, Pandolfi PP, Hay N (2006) The deficiency of Akt1 is sufficient to suppress tumor development in *Pten*(+/-) mice. *Genes Dev* 20:1569-1574
- Chen Z, Carracedo A, Lin HK, Koutcher JA, Behrendt N, Egia A, Alimonti A, Carver BS, Gerald W, Teruya Feldstein J, Loda M, Pandolfi PP (2009) Differential p53 independent outcomes of p19(Arf) loss in oncogenesis. *Sci Signal* 2:ra44

- Chiariello E, Roz L, Albarosa R, Magnani I, Finocchiaro G (1998) PTEN/MMAC1 mutations in primary glioblastomas and short term cultures of malignant gliomas. *Oncogene* 16:541-545
- Chin L, Garraway LA, Fisher DE (2006) Malignant melanoma: genetics and therapeutics in the genomic era. *Genes Dev* 20:2149-2182
- Ciraolo E, Iezzi M, Marone R, Marengo S, Curcio C, Costa C, Azzolino O, Gonella C, Rubinetto C, Wu H et al (2008) Phosphoinositide 3 kinase p110beta activity: key role in metabolism and mammary gland cancer but not development. *Sci Signal* 1:ra3
- Crackower MA, Oudit GY, Kozieradzki I, Sarao R, Sun H, Sasaki T, Hirsch E, Suzuki A, Shioi T, Irie Sasaki J et al (2002) Regulation of myocardial contractility and cell size by distinct PI3K-PTEN signaling pathways. *Cell* 110(6):737-749
- Currie RA, Walker KS, Gray A, Deak M, Casamayor A, Downes CP, Cohen P, Alessi DR, Lucocq J (1999) Role of phosphatidylinositol 3,4,5 trisphosphate in regulating the activity and localization of 3 phosphoinositide dependent protein kinase 1. *Biochem J* 337(Pt 3):575-583
- Dahia PL (2000) PTEN, a unique tumor suppressor gene. *Endocr Relat Cancer* 7:115-129
- Dahia PL, Marsh DJ, Zheng Z, Zedenius J, Komminoth P, Frisk T, Wallin G, Parsons R, Longy M, Larsson C, Eng C (1997) Somatic deletions and mutations in the Cowden disease gene, PTEN, in sporadic thyroid tumors. *Cancer Res* 57:4710-4713
- Dankort D, Curley DP, Cartledge RA, Nelson B, Karnezis AN, Damsky WE Jr, You MJ, DePinho RA, McMahon M, Bosenberg M (2009) Braf(V600E) cooperates with Pten loss to induce metastatic melanoma. *Nat Genet* 41:544-552
- Datta K, Bellacosa A, Chan TO, Tsichlis PN (1996) Akt is a direct target of the phosphatidylinositol 3 kinase. Activation by growth factors, v-src and v-Ha-ras, in Sf9 and mammalian cells. *J Biol Chem* 271:30835-30839
- De Marzo AM, Meeker AK, Zha S, Luo J, Nakayama M, Platz EA, Isaacs WB, Nelson WG (2003) Human prostate cancer precursors and pathobiology. *Urology* 62:55-62
- Denoyelle C, Abou Rjaily G, Bezrookove V, Verhaegen M, Johnson TM, Fullen DR, Pointer JN, Gruber SB, Su LD, Nikiforov MA, Kaufman RJ, Bastian BC, Soengas MS (2006) Anti-oncogenic role of the endoplasmic reticulum differentially activated by mutations in the MAPK pathway. *Nat Cell Biol* 8:1053-1063
- Depowski PL, Rosenthal SI, Ross JS (2001) Loss of expression of the PTEN gene protein product is associated with poor outcome in breast cancer. *Mod Pathol* 14:672-676
- Dhillon AS, Hagan S, Rath O, Kolch W (2007) MAP kinase signalling pathways in cancer. *Oncogene* 26:3279-3290
- Dhomen N, Reis Filho JS, da Rocha Dias S, Hayward R, Savage K, Delmas V, Larue L, Pritchard C, Marais R (2009) Oncogenic Braf induces melanocyte senescence and melanoma in mice. *Cancer Cell* 15:294-303
- Di Cristofano A, Pesce B, Cordon Cardo C, Pandolfi PP (1998) Pten is essential for embryonic development and tumour suppression. *Nat Genet* 19:348-355
- Di Cristofano A, De Acetis M, Koff A, Cordon Cardo C, Pandolfi PP (2001) Pten and p27KIP1 cooperate in prostate cancer tumor suppression in the mouse. *Nat Genet* 27:222-224
- Duerr EM, Rollbrocker B, Hayashi Y, Peters N, Meyer Puttlitz B, Louis DN, Schramm J, Wiestler OD, Parsons R, Eng C, von Deimling A (1998) PTEN mutations in gliomas and glioneuronal tumors. *Oncogene* 16:2259-2264
- El Gedaily A, Bubendorf L, Willi N, Fu W, Richter J, Moch H, Mihatsch MJ, Sauter G, Gasser TC (2001) Discovery of new DNA amplification loci in prostate cancer by comparative genomic hybridization. *Prostate* 46:184-190
- Ellwood Yen K, Graeber TG, Wongvipat J, Iruela Arispe ML, Zhang J, Matusik R, Thomas GV, Sawyers CL (2003) Myc driven murine prostate cancer shares molecular features with human prostate tumors. *Cancer Cell* 4:223-238
- Engelman JA, Luo J, Cantley LC (2006) The evolution of phosphatidylinositol 3 kinases as regulators of growth and metabolism. *Nat Rev Genet* 7:606-619

- Fang X, Yu S, Eder A, Mao M, Bast RC Jr, Boyd D, Mills GB (1999) Regulation of BAD phosphorylation at serine 112 by the Ras mitogen activated protein kinase pathway. *Oncogene* 18:6635-6640
- Feilotter HE, Nagai MA, Boag AH, Eng C, Mulligan LM (1998) Analysis of PTEN and the 10q23 region in primary prostate carcinomas. *Oncogene* 16:1743-1748
- Fisher GH, Wellen SL, Klimstra D, Lenczowski JM, Tichelaar JW, Lizak MJ, Whitsett JA, Koretsky A, Varmus HE (2001) Induction and apoptotic regression of lung adenocarcinomas by regulation of a K Ras transgene in the presence and absence of tumor suppressor genes. *Genes Dev* 15:3249-3262
- Ford Hutchinson AF, Ali Z, Lines SE, Hallgrímsson B, Boyd SK, Jirik FR (2007) Inactivation of Pten in osteo chondroprogenitor cells leads to epiphyseal growth plate abnormalities and skeletal overgrowth. *J Bone Miner Res* 22(8):1245-1259
- Fraser MM, Zhu X, Kwon CH, Uhlmann EJ, Gutmann DH, Baker SJ (2004) Pten loss causes hypertrophy and increased proliferation of astrocytes *in vivo*. *Cancer Res* 64:7773-7779
- Fraser MM, Bayazitov IT, Zakharenko SS, Baker SJ (2008) Phosphatase and tensin homolog, deleted on chromosome 10 deficiency in brain causes defects in synaptic structure, transmission and plasticity, and myelination abnormalities. *Neuroscience* 151:476-488
- Fujisawa H, Reis RM, Nakamura M, Colella S, Yonekawa Y, Kleihues P, Ohgaki H (2000) Loss of heterozygosity on chromosome 10 is more extensive in primary (de novo) than in secondary glioblastomas. *Lab Invest* 80:65-72
- Gao X, Zhang Y, Arrazola P, Hino O, Kobayashi T, Yeung RS, Ru B, Pan D (2002) Tsc tumour suppressor proteins antagonize amino acid TOR signalling. *Nat Cell Biol* 4:699-704
- Garami A, Zwartkruis FJ, Nobukuni T, Joaquin M, Rocco M, Stocker H, Kozma SC, Hafen E, Bos JL, Thomas G (2003) Insulin activation of Rheb, a mediator of mTOR/S6K/4E BP signaling, is inhibited by TSC1 and 2. *Mol Cell* 11:1457-1466
- Garcia JM, Silva JM, Dominguez G, Gonzalez R, Navarro A, Carretero L, Provencio M, Espana P, Bonilla F (1999) Allelic loss of the PTEN region (10q23) in breast carcinomas of poor pathophenotype. *Breast Cancer Res Treat* 57:237-243
- Garraway LA, Widlund HR, Rubin MA, Getz G, Berger AJ, Ramaswamy S, Beroukhi R, Milner DA, Granter SR, Du J, Lee C, Wagner SN, Li C, Golub TR, Rimm DL, Meyerson ML, Fisher DE, Sellers WR (2005) Integrative genomic analyses identify MITF as a lineage survival oncogene amplified in malignant melanoma. *Nature* 436:117-122
- Gasser T (2007) Update on the genetics of Parkinson's disease. *Mov Disord* 22(Suppl 17):S343-S350
- Georgescu MM, Kirsch KH, Akagi T, Shishido T, Hanafusa H (1999) The tumor suppressor activity of PTEN is regulated by its carboxyl terminal region. *Proc Natl Acad Sci USA* 96:10182-10187
- Georgescu MM, Kirsch KH, Kaloudis P, Yang H, Pavletich NP, Hanafusa H (2000) Stabilization and productive positioning roles of the C2 domain of PTEN tumor suppressor. *Cancer Res* 60:7033-7038
- Goel VK, Ibrahim N, Jiang G, Singhal M, Fee S, Flotte T, Westmoreland S, Haluska FS, Hinds PW, Haluska FG (2009) Melanocytic nevus like hyperplasia and melanoma in transgenic BRAFV600E mice. *Oncogene* 28:2289-2298
- Goffin A, Hoefsloot LH, Bosgoed E, Swillen A, Fryns JP (2001) PTEN mutation in a family with Cowden syndrome and autism. *Am J Med Genet* 105:521-524
- Gray IC, Stewart LM, Phillips SM, Hamilton JA, Gray NE, Watson GJ, Spurr NK, Snary D (1998) Mutation and expression analysis of the putative prostate tumour suppressor gene PTEN. *Br J Cancer* 78:1296-1300
- Groszer M, Erickson R, Scripture Adams DD, Lesche R, Trumpp A, Zack JA, Kornblum HI, Liu X, Wu H (2001) Negative regulation of neural stem/progenitor cell proliferation by the Pten tumor suppressor gene *in vivo*. *Science* 294:2186-2189

- Groszer M, Erickson R, Scripture Adams DD, Dougherty JD, Le Belle J, Zack JA, Geschwind DH, Liu X, Kornblum HI, Wu H (2006) PTEN negatively regulates neural stem cell self renewal by modulating G0 G1 cell cycle entry. *Proc Natl Acad Sci USA* 103:111 116
- Gudmundsson J, Sulem P, Manolescu A, Amundadottir LT, Gudbjartsson D, Helgason A, Rafnar T, Bergthorsson JT, Agnarsson BA, Baker A et al (2007) Genome wide association study identifies a second prostate cancer susceptibility variant at 8q24. *Nat Genet* 39:631 637
- Guertin DA, Sabatini DM (2007) Defining the role of mTOR in cancer. *Cancer Cell* 12:9 22
- Guertin DA, Stevens DM, Saitoh M, Kinkel S, Crosby K, Sheen JH, Mullholland DJ, Magnuson MA, Wu H, Sabatini DM (2009) mTOR complex 2 is required for the development of prostate cancer induced by Pten loss in mice. *Cancer Cell* 15:148 159
- Gupta S, Ramjaun AR, Haiko P, Wang Y, Warne PH, Nicke B, Nye E, Stamp G, Alitalo K, Downward J (2007) Binding of ras to phosphoinositide 3 kinase p110alpha is required for ras driven tumorigenesis in mice. *Cell* 129:957 968
- Gurel B, Iwata T, Koh CM, Jenkins RB, Lan F, Van Dang C, Hicks JL, Morgan J, Cornish TC, Sutcliffe S, Isaacs WB, Luo J, De Marzo AM (2008) Nuclear MYC protein overexpression is an early alteration in human prostate carcinogenesis. *Mod Pathol* 21:1156 1167
- Hagenbeek TJ, Naspetti M, Malergue F, Garcon F, Nunes JA, Cleutjens KB, Trapman J, Krimpenfort P, Spits H (2004) The loss of PTEN allows TCR alphabeta lineage thymocytes to bypass IL 7 and Pre TCR mediated signaling. *J Exp Med* 200(7):883 894
- Hagenbeek TJ, Spits H (2008) T cell lymphomas in T cell specific Pten deficient mice originate in the thymus. *Leukemia* 22(3):608 619
- Haiman CA, Patterson N, Freedman ML, Myers SR, Pike MC, Waliszewska A, Neubauer J, Tandon A, Schirmer C, McDonald GJ et al (2007) Multiple regions within 8q24 independently affect risk for prostate cancer. *Nat Genet* 39:638 644
- Haillovic E, Solit DB (2008) Therapeutic strategies for inhibiting oncogenic BRAF signaling. *Curr Opin Pharmacol* 8:419 426
- Haluska FG, Tsao H, Wu H, Haluska FS, Lazar A, Goel V (2006) Genetic alterations in signaling pathways in melanoma. *Clin Cancer Res* 12:2301s 2307s
- Hamada K, Sasaki T, Koni PA, Natsui M, Kishimoto H, Sasaki J, Yajima N, Horie Y, Hasegawa G, Naito M et al (2005) The PTEN/PI3K pathway governs normal vascular development and tumor angiogenesis. *Genes Dev* 19(17):2054 2065
- Han S, Ritzenthaler JD, Zheng Y, Roman J (2008) PPAR{beta}/{delta} agonist stimulates human lung carcinoma cell growth through inhibition of PTEN expression: the involvement of PI3 K and NF {kappa}B signals. *Am J Physiol Lung Cell Mol Physiol* 294:L1238 L1249
- Herman GE, Butter E, Enrile B, Pastore M, Prior TW, Sommer A (2007) Increasing knowledge of PTEN germline mutations: Two additional patients with autism and macrocephaly. *Am J Med Genet A* 143:589 593
- Hernando E, Charytonowicz E, Dudas ME, Menendez S, Matushansky I, Mills J, Socci ND, Behrendt N, Ma L, Maki RG, Pandolfi PP, Cordon Cardo C (2007) The AKT mTOR pathway plays a critical role in the development of leiomyosarcomas. *Nat Med* 13:748 753
- Horie Y, Suzuki A, Kataoka E, Sasaki T, Hamada K, Sasaki J, Mizuno K, Hasegawa G, Kishimoto H, Iizuka M, Naito M, Enomoto K, Watanabe S, Mak TW, Nakano T (2004) Hepatocyte specific Pten deficiency results in steatohepatitis and hepatocellular carcinomas. *J Clin Invest* 113:1774 1783
- Iida S, Tanaka Y, Fujii H, Hayashi S, Kimura M, Nagareda T, Moriwaki K (1998) A heterozygous frameshift mutation of the PTEN/MMAC1 gene in a patient with Lhermitte Duclos disease only the mutated allele was expressed in the cerebellar tumor. *Int J Mol Med* 1:925 929
- Iida S, Ono A, Sayama K, Hamaguchi T, Fujii H, Nakajima H, Namba M, Hanafusa T, Matsuzawa Y, Moriwaki K (2000) Accelerated decline of blood glucose after intravenous glucose injection in a patient with Cowden disease having a heterozygous germline mutation of the PTEN/MMAC1 gene. *Anticancer Res* 20:1901 1904
- Inoki K, Li Y, Xu T, Guan KL (2003) Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes Dev* 17:1829 1834
- Inoue Narita T, Hamada K, Sasaki T, Hatakeyama S, Fujita S, Kawahara K, Sasaki M, Kishimoto H, Eguchi S, Kojima I et al (2008) Pten deficiency in melanocytes results in resistance to hair

- graying and susceptibility to carcinogen induced melanomagenesis. *Cancer Res* 68(14):5760-5768
- Jaeschke A, Hartkamp J, Saitoh M, Roworth W, Nobukuni T, Hodges A, Sampson J, Thomas G, Lamb R (2002) Tuberous sclerosis complex tumor suppressor mediated S6 kinase inhibition by phosphatidylinositol 3 OH kinase is mTOR independent. *J Cell Biol* 159:217-224
- Jemal A, Murray T, Samuels A, Tiwari RC, Ghafoor A, Feuer EJ, Thun MJ (2005) Cancer statistics, 2005. *CA Cancer J Clin* 55:10-30
- Jia S, Liu Z, Zhang S, Liu P, Zhang L, Lee SH, Zhang J, Signoretti S, Loda M, Roberts TM, Zhao JJ (2008) Essential roles of PI(3)K p110beta in cell growth, metabolism and tumorigenesis. *Nature* 454:776-779
- Johnson L, Mercer K, Greenbaum D, Bronson RT, Crowley D, Tuveson DA, Jacks T (2001) Somatic activation of the K ras oncogene causes early onset lung cancer in mice. *Nature* 410:1111-1116
- Kato H, Kato S, Kumabe T, Sonoda Y, Yoshimoto T, Han SY, Suzuki T, Shibata H, Kanamaru R, Ishioka C (2000) Functional evaluation of p53 and PTEN gene mutations in gliomas. *Clin Cancer Res* 6:3937-3943
- Kim J, Eltoum IE, Roh M, Wang J, Abdulkadir SA (2009) Interactions between cells with distinct mutations in c MYC and Pten in prostate cancer. *PLoS Genet* 5:e1000542
- Kimura T, Suzuki A, Fujita Y, Yomogida K, Lomeli H, Asada N, Ikeuchi M, Nagy A, Mak TW, Nakano T (2003) Conditional loss of PTEN leads to testicular teratoma and enhances embryonic germ cell production. *Development* 130:1691-1700
- Klinghoffer RA, Duckworth B, Valius M, Cantley L, Kazlauskas A (1996) Platelet derived growth factor dependent activation of phosphatidylinositol 3 kinase is regulated by receptor binding of SH2 domain containing proteins which influence Ras activity. *Mol Cell Biol* 16:5905-5914
- Knobbe CB, Merlo A, Reifemberger G (2002) Pten signaling in gliomas. *Neuro Oncol* 4:196-211
- Koul A, Willen R, Bendahl PO, Nilbert M, Borg A (2002) Distinct sets of gene alterations in endometrial carcinoma implicate alternate modes of tumorigenesis. *Cancer* 94:2369-2379
- Kurlawalla Martinez C, Stiles B, Wang Y, Devaskar SU, Kahn BB, Wu H (2005) Insulin hypersensitivity and resistance to streptozotocin induced diabetes in mice lacking PTEN in adipose tissue. *Mol Cell Biol* 25(6):2498-2510
- Kuroda S, Nishio M, Sasaki T, Horie Y, Kawahara K, Sasaki M, Natsui M, Matozaki T, Tezuka H, Ohteki T, Forster, I, Mak TW, Nakano T, Suzuki A (2008) Effective clearance of intracellular *Leishmania major* in vivo requires Pten in macrophages. *Eur J Immunol* 38(5):1331-1340
- Kurose K, Gilley K, Matsumoto S, Watson PH, Zhou XP, Eng C (2002) Frequent somatic mutations in PTEN and TP53 are mutually exclusive in the stroma of breast carcinomas. *Nat Genet* 32:355-357
- Kwon CH, Zhu X, Zhang J, Knoop LL, Tharp R, Smeyne RJ, Eberhart CG, Burger PC, Baker SJ (2001) Pten regulates neuronal soma size: a mouse model of Lhermitte Duclos disease. *Nat Genet* 29:404-411
- Kwon CH, Luikart BW, Powell CM, Zhou J, Matheny SA, Zhang W, Li Y, Baker SJ, Parada LF (2006) Pten regulates neuronal arborization and social interaction in mice. *Neuron* 50:377-388
- Levine AJ, Finlay CA, Hinds PW (2004) P53 is a tumor suppressor gene. *Cell* 116:S67-S69, 61 p following S69
- Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliaris C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R (1997) PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* 275:1943-1947
- Li G, Robinson GW, Lesche R, Martinez Diaz H, Jiang Z, Rozengurt N, Wagner KU, Wu DC, Lane TF, Liu X, Hennighausen L, Wu H (2002) Conditional loss of PTEN leads to precocious development and neoplasia in the mammary gland. *Development* 129:4159-4170
- Liaw D, Marsh DJ, Li J, Dahia PL, Wang SI, Zheng Z, Bose S, Call KM, Tsou HC, Peacocke M, Eng C, Parsons R (1997) Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nat Genet* 16:64-67

- Lin WM, Forgacs E, Warshal DP, Yeh IT, Martin JS, Ashfaq R, Muller CY (1998) Loss of heterozygosity and mutational analysis of the PTEN/MMAC1 gene in synchronous endometrial and ovarian carcinomas. *Clin Cancer Res* 4:2577-2583
- Liu W, James CD, Frederick L, Alderete BE, Jenkins RB (1997) PTEN/MMAC1 mutations and EGFR amplification in glioblastomas. *Cancer Res* 57:5254-5257
- Liu X, Bruxvoort KJ, Zylstra CR, Liu J, Cichowski R, Faugere MC, Bouxsein ML, Wan C, Williams BO, Clemens TL (2007) Lifelong accumulation of bone in mice lacking Pten in osteoblasts. *Proc Natl Acad Sci USA* 104(7):2259-2264
- Luchman HA, Benediktsson H, Villemaire ML, Peterson AC, Jirik FR (2008) The pace of prostatic intraepithelial neoplasia development is determined by the timing of Pten tumor suppressor gene excision. *PLoS One* 3:e3940
- Ma L, Chen Z, Erdjument-Bromage H, Tempst P, Pandolfi PP (2005a) Phosphorylation and functional inactivation of TSC2 by Erk implications for tuberous sclerosis and cancer pathogenesis. *Cell* 121:179-193
- Ma L, Teruya-Feldstein J, Behrendt N, Chen Z, Noda T, Hino O, Cordon-Cardo C, Pandolfi PP (2005b) Genetic analysis of Pten and Tsc2 functional interactions in the mouse reveals asymmetrical haploinsufficiency in tumor suppression. *Genes Dev* 19:1779-1786
- Ma X, Ziel van der Made AC, Autar B, van der Korput HA, Vermeij M, van Duijn P, Cleutjens KB, de Krijger R, Krimpenfort P, Berns A, van der Kwast TH, Trapman J (2005c) Targeted biallelic inactivation of Pten in the mouse prostate leads to prostate cancer accompanied by increased epithelial cell proliferation but not by reduced apoptosis. *Cancer Res* 65:5730-5739
- Maehama T, Dixon JE (1998) The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5 trisphosphate. *J Biol Chem* 273:13375-13378
- Manning BD, Cantley LC (2007) AKT/PKB signaling: navigating downstream. *Cell* 129:1261-1274
- Manning BD, Logsdon MN, Lipovsky AI, Abbott D, Kwiatkowski DJ, Cantley LC (2005) Feedback inhibition of Akt signaling limits the growth of tumors lacking Tsc2. *Genes Dev* 19:1773-1778
- Marandola P, Bonghi A, Jallous H, Bombardelli E, Morazzoni P, Gerardini M, Tiscione D, Albergati F (2004) Molecular biology and the staging of prostate cancer. *Ann N Y Acad Sci* 1028:294-312
- Marino S, Krimpenfort P, Leung C, van der Korput HA, Trapman J, Camenisch I, Berns A, Brandner S (2002) PTEN is essential for cell migration but not for fate determination and tumorigenesis in the cerebellum. *Development* 129:3513-3522
- Marsh DJ, Dahia PL, Zheng Z, Liaw D, Parsons R, Gorlin RJ, Eng C (1997) Germline mutations in PTEN are present in Bannayan-Zonana syndrome. *Nat Genet* 16:333-334
- Michaloglou C, Vredeveld LC, Soengas MS, Denoyelle C, Kuilman T, van der Horst CM, Majoor DM, Shay JW, Mooi WJ, Peeper DS (2005) BRAF^{V600E} associated senescence-like cell cycle arrest of human naevi. *Nature* 436:720-724
- Moodie SA, Willumsen BM, Weber MJ, Wolfman A (1993) Complexes of Ras.GTP with Raf-1 and mitogen-activated protein kinase kinase. *Science* 260:1658-1661
- Nardella C, Chen Z, Salmena L, Carracedo A, Alimonti A, Egia A, Carver B, Gerald W, Cordon-Cardo C, Pandolfi PP (2008) Aberrant Rheb-mediated mTORC1 activation and Pten haploinsufficiency are cooperative oncogenic events. *Genes Dev* 22:2172-2177
- Nardella C, Carracedo A, Alimonti A, Hobbs RM, Clohessy JG, Chen Z, Egia A, Fornari A, Fiorentino M, Loda M, Kozma SC, Thomas G, Cordon-Cardo C, Pandolfi PP (2009) Differential requirement of mTOR in postmitotic tissues and tumorigenesis. *Sci Signal* 2:ra2
- Nguyen QT, Tajmir P, Lin CH, Liadis N, Zhu XD, Eweida M, Tolasa Karaman G, Cai F, Wang R, Kitamura T et al (2006) Essential role of Pten in body size determination and pancreatic beta cell homeostasis in vivo. *Mol Cell Biol* 26(12):4511-4518
- Perner S, Mosquera JM, Demichelis F, Hofer MD, Paris PL, Simko J, Collins C, Bismar TA, Chinnaiyan AM, De Marzo AM, Rubin MA (2007) TMPRSS2-ERG fusion prostate cancer: an early molecular event associated with invasion. *Am J Surg Pathol* 31:882-888

- Perren A, Weng LP, Boag AH, Ziebold U, Thakore K, Dahia PL, Komminoth P, Lees JA, Mulligan LM, Mutter GL, Eng C (1999) Immunohistochemical evidence of loss of PTEN expression in primary ductal adenocarcinomas of the breast. *Am J Pathol* 155:1253-1260
- Plum L, Ma X, Hampel B, Balthasar N, Coppari R, Munzberg H, Shanabrough M, Burdakov D, Rother E, Janoschek R et al (2006) Enhanced PIP3 signaling in POMC neurons causes KATP channel activation and leads to diet sensitive obesity. *J Clin Invest* 116(7):1886-1901
- Podsypanina K, Ellenson LH, Nemes A, Gu J, Tamura M, Yamada KM, Cordon Cardo C, Catoretti G, Fisher PE, Parsons R (1999) Mutation of Pten/Mmac1 in mice causes neoplasia in multiple organ systems. *Proc Natl Acad Sci USA* 96:1563-1568
- Poliseno L, Salmena L, Riccardi L, Fornari A, Song MS, Hobbs RM, Sportoletti P, Varmeh S, Egia A, Fedele G, Rameh L, Loda M, Pandolfi PP (2010) Identification of the miR-106b~25 microRNA cluster as a proto oncogenic PTEN targeting intron that cooperates with its host gene MCM7 in transformation. *Sci Signal* 3(117):ra29
- Pomeroy MM, Beckwith CA, Regan MM, Wyman SK, Petrovics G, Chen Y, Hawksworth DJ, Schumacher FR, Mucci L, Penney KL et al (2009) Evaluation of the 8q24 prostate cancer risk locus and MYC expression. *Cancer Res* 69:5568-5574
- Puzio Kuter AM, Castillo Martin M, Kinkade CW, Wang X, Shen TH, Matos T, Shen MM, Cordon Cardo C, Abate Shen C (2009) Inactivation of p53 and Pten promotes invasive bladder cancer. *Genes Dev* 23:675-680
- Qian J, Jenkins RB, Bostwick DG (1997) Detection of chromosomal anomalies and c-myc gene amplification in the cribriform pattern of prostatic intraepithelial neoplasia and carcinoma by fluorescence in situ hybridization. *Mod Pathol* 10:1113-1119
- Rasheed BK, Stenzel TT, McLendon RE, Parsons R, Friedman AH, Friedman HS, Bigner DD, Bigner SH (1997) PTEN gene mutations are seen in high grade but not in low grade gliomas. *Cancer Res* 57:4187-4190
- Ratnacaram CK, Teletin M, Jiang M, Meng X, Chambon P, Metzger D (2008) Temporally controlled ablation of PTEN in adult mouse prostate epithelium generates a model of invasive prostatic adenocarcinoma. *Proc Natl Acad Sci USA* 105:2521-2526
- Reddy P, Liu L, Adhikari D, Jagarlamudi K, Rajareddy S, Shen Y, Du C, Tang W, Hamalainen T, Peng SL et al (2008) Oocyte specific deletion of Pten causes premature activation of the primordial follicle pool. *Science* 319(5863):611-613
- Rubin MA, Gerstein A, Reid K, Bostwick DG, Cheng L, Parsons R, Papadopoulos N (2000) 10q23.3 loss of heterozygosity is higher in lymph node positive (pT2-3, N+) versus lymph node negative (pT2-3, N0) prostate cancer. *Hum Pathol* 31:504-508
- Saal LH, Holm K, Maurer M, Memeo L, Su T, Wang X, Yu JS, Malmstrom PO, Mansukhani M, Enoksson J, Hibshoosh H, Borg A, Parsons R (2005) PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. *Cancer Res* 65:2554-2559
- Saal LH, Gruvberger Saal SK, Persson C, Lovgren K, Jumppanen M, Staaf J, Jonsson G, Pires MM, Maurer M, Holm K et al (2008) Recurrent gross mutations of the PTEN tumor suppressor gene in breast cancers with deficient DSB repair. *Nat Genet* 40:102-107
- Salmena L, Carracedo A, Pandolfi PP (2008) Tenets of PTEN tumor suppression. *Cell* 133:403-414
- Samuels Y, Velculescu VE (2004) Oncogenic mutations of PIK3CA in human cancers. *Cell Cycle* 3:1221-1224
- Sarbassov DD, Guertin DA, Ali SM, Sabatini DM (2005) Phosphorylation and regulation of Akt/PKB by the rictor mTOR complex. *Science* 307:1098-1101
- Schrager CA, Schneider D, Gruener AC, Tsou HC, Peacocke M (1998) Clinical and pathological features of breast disease in Cowden's syndrome: an underrecognized syndrome with an increased risk of breast cancer. *Hum Pathol* 29:47-53
- Shao X, Tandon R, Samara G, Kanki H, Yano H, Close LG, Parsons R, Sato T (1998) Mutational analysis of the PTEN gene in head and neck squamous cell carcinoma. *Int J Cancer* 77:684-688
- Simpson L, Parsons R (2001) PTEN: life as a tumor suppressor. *Exp Cell Res* 264:29-41
- Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Eystein Lonning P,

- Borresen Dale AL (2001) Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA* 98:10869-10874
- Stambolic V, Suzuki A, de la Pompa JL, Brothers GM, Mirtsos C, Sasaki T, Ruland J, Penninger JM, Siderovski DP, Mak TW (1998) Negative regulation of PKB/Akt dependent cell survival by the tumor suppressor PTEN. *Cell* 95:29-39
- Stanger BZ, Stiles B, Lauwers GY, Bardeesy N, Mendoza M, Wang Y, Greenwood A, Cheng KH, McLaughlin M, Brown D et al (2005) Pten constrains centroacinar cell expansion and malignant transformation in the pancreas. *Cancer Cell* 8(3):185-195
- Stambolic V, Tsao MS, Macpherson D, Suzuki A, Chapman WB, Mak TW (2000) High incidence of breast and endometrial neoplasia resembling human Cowden syndrome in pten+/- mice. *Cancer Res* 60:3605-3611
- Steck PA, Pershouse MA, Jasser SA, Yung WK, Lin H, Ligon AH, Langford LA, Baumgard ML, Hattier T, Davis T, Frye C, Hu R, Swedlund B, Teng DH, Tavtigian SV (1997) Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. *Nat Genet* 15:356-362
- Stiles B, Wang Y, Stahl A, Bassilian S, Lee WP, Kim YJ, Sherwin R, Devaskar S, Lesche R, Magnuson MA, Wu H (2004) Liver specific deletion of negative regulator Pten results in fatty liver and insulin hypersensitivity [corrected]. *Proc Natl Acad Sci USA* 101:2082-2087
- Stiles BL, Kuralwalla Martinez C, Guo W, Gregorian C, Wang Y, Tian J, Magnuson MA, Wu H (2006) Selective deletion of Pten in pancreatic beta cells leads to increased islet mass and resistance to STZ induced diabetes. *Mol Cell Biol* 26:2772-2781
- Subramanian KK, Jia Y, Zhu D, Simms BT, Jo H, Hattori H, You J, Mizgerd JP, Luo HR (2007) Tumor suppressor PTEN is a physiologic suppressor of chemoattractant mediated neutrophil functions. *Blood* 109(9):4028-4037
- Suzuki A, de la Pompa JL, Stambolic V, Elia AJ, Sasaki T, del Barco Barrantes I, Ho A, Wakeham A, Itie A, Khoo W, Fukumoto M, Mak TW (1998) High cancer susceptibility and embryonic lethality associated with mutation of the PTEN tumor suppressor gene in mice. *Curr Biol* 8:1169-1178
- Suzuki A, Hamada K, Sasaki T, Mak TW, Nakano T (2007) Role of PTEN/PI3K pathway in endothelial cells. *Biochem Soc Trans* 35(Pt 2):172-176
- Suzuki A, Itami S, Ohishi M, Hamada K, Inoue T, Komazawa N, Senoo H, Sasaki T, Takeda J, Manabe M, Mak TW, Nakano T (2003) Keratinocyte specific Pten deficiency results in epidermal hyperplasia, accelerated hair follicle morphogenesis and tumor formation. *Cancer Res* 63(3):674-681
- Suzuki A, Sasaki T, Mak TW, Nakano T (2004) Functional analysis of the tumour suppressor gene PTEN in murine B cells and keratinocytes. *Biochem Soc Trans* 32:362-365
- Suzuki A, Yamaguchi MT, Ohteki T, Sasaki T, Kaisho T, Kimura Y, Yoshida R, Wakeham A, Higuchi T, Fukumoto M et al (2001) T cell specific loss of Pten leads to defects in central and peripheral tolerance. *Immunity* 14(5):523-534
- Tapon N, Ito N, Dickson BJ, Treisman JE, Hariharan IK (2001) The Drosophila tuberous sclerosis complex gene homologs restrict cell growth and cell proliferation. *Cell* 105:345-355
- Tashiro H, Blazes MS, Wu R, Cho KR, Bose S, Wang SI, Li J, Parsons R, Ellenson LH (1997) Mutations in PTEN are frequent in endometrial carcinoma but rare in other common gynecological malignancies. *Cancer Res* 57:3935-3940
- Taube JM, Begum S, Shi C, Eshleman JR, Westra WH (2009) Benign nodal nevi frequently harbor the activating V600E BRAF mutation. *Am J Surg Pathol* 33:568-571
- Tee AR, Fingar DC, Manning BD, Kwiatkowski DJ, Cantley LC, Blenis J (2002) Tuberous sclerosis complex 1 and 2 gene products function together to inhibit mammalian target of rapamycin (mTOR) mediated downstream signaling. *Proc Natl Acad Sci USA* 99:13571-13576
- Teng DH, Hu R, Lin H, Davis T, Iliev D, Frye C, Swedlund B, Hansen KL, Vinson VL, Gumpfer KL et al (1997) MMAC1/PTEN mutations in primary tumor specimens and tumor cell lines. *Cancer Res* 57:5221-5225
- Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, Varambally S, Cao X, Tchinda J, Kuefer R, Lee C, Montie JE, Shah RB, Pienta KJ, Rubin MA, Chinnaiyan AM (2005) Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science* 310:644-648

- Trotman LC, Niki M, Dotan ZA, Koutcher JA, Di Cristofano A, Xiao A, Khoo AS, Roy Burman P, Greenberg NM, Van Dyke T, Cordon Cardo C, Pandolfi PP (2003) Pten dose dictates cancer progression in the prostate. *PLoS Biol* 1:E59
- Tsao H, Zhang X, Fowlkes K, Haluska FG (2000) Relative reciprocity of NRAS and PTEN/MMAC1 alterations in cutaneous melanoma cell lines. *Cancer Res* 60:1800-1804
- Tsuchiya N, Kondo Y, Takahashi A, Pawar H, Qian J, Sato K, Lieber MM, Jenkins RB (2002) Mapping and gene expression profile of the minimally overrepresented 8q24 region in prostate cancer. *Am J Pathol* 160:1799-1806
- Tsuruta H, Kishimoto H, Sasaki T, Horie Y, Natsui M, Shibata Y, Hamada K, Yajima N, Kawahara K, Sasaki M et al (2006) Hyperplasia and carcinomas in Pten deficient mice and reduced PTEN protein in human bladder cancer patients. *Cancer Res* 66(17):8389-8396
- Vasudevan KM, Burikhanov R, Goswami A, Rangnekar VM (2007) Suppression of PTEN expression is essential for antiapoptosis and cellular transformation by oncogenic Ras. *Cancer Res* 67:10343-10350
- Vogelstein B, Lane D, Levine AJ (2000) Surfing the p53 network. *Nature* 408:307-310
- Wang SI, Puc J, Li J, Bruce JN, Cairns P, Sidransky D, Parsons R (1997) Somatic mutations of PTEN in glioblastoma multiforme. *Cancer Res* 57:4183-4186
- Wang S, Gao J, Lei Q, Rozengurt N, Pritchard C, Jiao J, Thomas GV, Li G, Roy Burman P, Nelson PS, Liu X, Wu H (2003) Prostate specific deletion of the murine Pten tumor suppressor gene leads to metastatic prostate cancer. *Cancer Cell* 4:209-221
- Wiencke JK, Zheng S, Jelluma N, Tihan T, Vandenberg S, Tamguney T, Baumber R, Parsons R, Lamborn KR, Berger MS, Wrensch MR, Haas Kogan DA, Stokoe D (2007) Methylation of the PTEN promoter defines low grade gliomas and secondary glioblastoma. *Neuro Oncol* 9:271-279
- Wijesekara N, Konrad D, Eweida M, Jefferies C, Liadis N, Giacca A, Crackower M, Suzuki A, Mak TW, Kahn CR, Klip A, Woo M (2005) Muscle specific Pten deletion protects against insulin resistance and diabetes. *Mol Cell Biol* 25(3):1135-1145
- Witte JS (2007) Multiple prostate cancer risk variants on 8q24. *Nat Genet* 39:579-580
- Wu X, Wu J, Huang J, Powell WC, Zhang J, Matusik RJ, Sangiorgi FO, Maxson RE, Sucov HM, Roy Burman P (2001) Generation of a prostate epithelial cell specific Cre transgenic mouse model for tissue specific gene ablation. *Mech Dev* 101:61-69
- Wullschlegel S, Loewith R, Hall MN (2006) TOR signaling in growth and metabolism. *Cell* 124:471-484
- Yanagi S, Kishimoto H, Kawahara K, Sasaki T, Sasaki M, Nishio M, Yajima N, Hamada K, Horie Y, Kubo H, Whitsett JA, Mak TW, Nakano T, Nakazato M, Suzuki A (2007) Pten controls lung morphogenesis, bronchioalveolar stem cells, and onset of lung adenocarcinomas in mice. *J Clin Invest* 117(10):2929-2940
- Yang H, Kong W, He L, Zhao JJ, O'Donnell JD, Wang J, Wenham RM, Coppola D, Kruk PA, Nicosia SV, Cheng JQ (2008) MicroRNA expression profiling in human ovarian cancer: miR-214 induces cell survival and cisplatin resistance by targeting PTEN. *Cancer Res* 68:425-433
- Yeager M, Orr N, Hayes RB, Jacobs KB, Kraft P, Wacholder S, Minichiello MJ, Fearhead P, Yu K, Chatterjee N et al (2007) Genome wide association study of prostate cancer identifies a second risk locus at 8q24. *Nat Genet* 39:645-649
- Yilmaz OH, Valdez R, Theisen BK, Guo W, Ferguson DO, Wu H, Morrison SJ (2006) Pten dependence distinguishes haematopoietic stem cells from leukaemia initiating cells. *Nature* 441:475-482
- Yoo LI, Liu DW, Le Vu S, Bronson RT, Wu H, Yuan J (2006) Pten deficiency activates distinct downstream signaling pathways in a tissue specific manner. *Cancer Res* 66(4):1929-1939
- Yue Q, Groszer M, Gil JS, Berk AJ, Messing A, Wu H, Liu X (2005) PTEN deletion in Bergmann glia leads to premature differentiation and affects laminar organization. *Development* 132:3281-3291
- Zhang J, Grindley JC, Yin T, Jayasinghe S, He XC, Ross JT, Haug JS, Rupp D, Porter Westpfahl KS, Wiedemann LM, Wu H, Li L (2006) PTEN maintains haematopoietic stem cells and acts in lineage choice and leukaemia prevention. *Nature* 441(7092):518-522

- Zhang X, Lee C, Ng PY, Rubin M, Shabsigh A, Buttyan R (2000) Prostatic neoplasia in transgenic mice with prostate directed overexpression of the c myc oncoprotein. *Prostate* 43:278-285
- Zhang Y, Gao X, Saucedo LJ, Ru B, Edgar BA, Pan D (2003) Rheb is a direct target of the tuberous sclerosis tumour suppressor proteins. *Nat Cell Biol* 5:578-581
- Zheng H, Ying H, Yan H, Kimmelman AC, Hiller DJ, Chen AJ, Perry SR, Tonon G, Chu GC, Ding Z et al (2008) p53 and Pten control neural and glioma stem/progenitor cell renewal and differentiation. *Nature* 455:1129-1133
- Zhou XP, Marsh DJ, Hampel H, Mulliken JB, Gimm O, Eng C (2000) Germline and germline mosaic PTEN mutations associated with a Proteus like syndrome of hemihypertrophy, lower limb asymmetry, arteriovenous malformations and lipomatosis. *Hum Mol Genet* 9:765-768
- Zhou X, Hampel H, Thiele H, Gorlin RJ, Hennekam RC, Parisi M, Winter RM, Eng C (2001) Association of germline mutation in the PTEN tumour suppressor gene and Proteus and Proteus like syndromes. *Lancet* 358:210-211
- Zhou XP, Kuismanen S, Nystrom Lahti M, Peltomaki P, Eng C (2002) Distinct PTEN mutational spectra in hereditary non polyposis colon cancer syndrome related endometrial carcinomas compared to sporadic microsatellite unstable tumors. *Hum Mol Genet* 11:445-450
- Zhou XP, Marsh DJ, Morrison CD, Chaudhury AR, Maxwell M, Reifemberger G, Eng C (2003) Germline inactivation of PTEN and dysregulation of the phosphoinositol 3 kinase/Akt pathway cause human Lhermitte Duclos disease in adults. *Am J Hum Genet* 73:1191-1198
- Zhu D, Hattori H, Jo H, Jia Y, Subramanian KK, Loison F, You J, Le Y, Honczarenko M, Silberstein L, Luo HR (2006) Deactivation of phosphatidylinositol 3,4,5 trisphosphate/Akt signaling mediates neutrophil spontaneous death. *Proc Natl Acad Sci USA* 103(40):14836-14841

PI3K as a Target for Therapy in Haematological Malignancies

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Abstract Although classical mutations in genes such as PIK3CA and PTEN occur at a relatively low frequency in haematological malignancies, activation of PI3K signalling is often detected in these tumours. In some conditions, for example acute myeloid leukaemia (AML), this is due to activating mutations of upstream regulators such as the FLT3 tyrosine kinase or RAS. Primary tumour cells taken from patients with AML, acute lymphoblastic leukaemia, chronic lymphocytic leukaemia and multiple myeloma show varying levels of sensitivity to PI3K and mTOR inhibitors. The challenge now is to conduct high quality trials with novel agents that target these pathways to establish the level of clinical response and to identify those subsets of patients that are more likely to respond.

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1 Introduction

The study of the pathophysiology and pre-clinical therapeutics of haematological malignancies is made easier by ready access to primary tumour cells, as well as their normal counterparts, by blood and bone marrow sampling. A large number of studies have shown that the PI3K pathway is frequently activated in haematological tumours. In contrast to solid tumours, classical genetic aberrations leading to increased PIP3 production, such as PTEN deletion/mutation and PIK3CA activating mutation, occur with a relatively low frequency. It appears that the presence of “upstream” signalling abnormalities, such as tyrosine kinase and RAS mutations, in a high proportion of haematological tumours may reduce the evolutionary impetus to acquire such lesions. Emerging data from comprehensive genomic studies of human malignancies suggest that haematological tumours may be less complex than their epithelial counterparts. Whether this translates into increased responsiveness to agents that target PI3K remains to be seen.

Evaluating the role of PI3K in the pathophysiology and therapeutics of any tumour requires pre-clinical assays that are representative of the clinical setting. Mutational screening is readily achieved with small amounts of primary tissue and, traditionally, continuously growing cell lines originally derived from primary patient material are used to assess pathway activation and the effects of targeted therapy. However, it is apparent from a number of studies that such cell lines may not faithfully represent the broad range of tumour biology for a given malignancy. For example, tumour cell lines are more readily established with samples from patients with a more aggressive clinical disease pattern (Shimada et al. 2003). For relatively indolent haematological malignancies, such as chronic lymphocytic leukaemia (CLL), follicular non-Hodgkin’s lymphoma (NHL) and multiple myeloma (MM), tumour cell lines either cannot be established (CLL) or those that can are from the small sub-fraction of individuals with highly aggressive tumours with widespread genetic abnormalities – for example, many MM cell lines are from individuals in advanced stages of the disease who have developed plasma cell leukaemias. In acute myeloid leukaemia (AML), the two most common genetic abnormalities in the overall patient population are mutations of the FLT3 tyrosine kinase and of the nucleophosmin gene, which are present in about 25 and 40% of patients, respectively (Gale et al. 2008). However, only a small fraction of AML cell lines have been found to have either of these abnormalities (Quentmeier et al. 2003, 2005).

The ready access to primary haematological tumour samples suggests that studies of pathway activation and sensitivity to targeted therapies may be best evaluated in these cells. However, there are several caveats to this that need to be taken into account. For some tumour types, withdrawal of cells from the patient results in their rapid demise, presumably because of the loss of microenvironmental survival factors. Lymphoid cells including those from B cell CLL (Collins et al. 1989) and acute lymphoblastic leukaemia (ALL) (Savitskiy et al. 2003) are especially prone to this. Therefore, in these cells, evaluation of targeted therapies may

be taking place in the background of a stressed cell population, a large proportion of which are already committed to cell death. Primary AML, ALL, CLL and MM tumour samples usually survive and grow much better *ex vivo* in culture systems with additional stromal cell support (Grigorieva et al. 1998; Lagneaux et al. 1998; Garrido et al. 2001); in conditions of reduced oxygen tension that mimic the bone marrow microenvironment (Thompson et al. 2007) and/or with the addition of various cytokine cocktails (Delwel et al. 1987; Touw et al. 1990; Ticchioni et al. 2007). These conditions are likely to provide a more representative picture of what is going on in the patient, but this is hard to prove formally and the precise conditions for optimal primary cell culture remain unclear.

Tumour xenografts in immunodeficient mice, in particular with acute leukaemia cells, have also been widely used to understand disease pathophysiology and to evaluate therapies. The NOD-SCID model has been most widely used and was instrumental in defining leukaemia stem cell (LSC) populations in AML (Bonnet and Dick 1997). However, it is clear that there are significant deficiencies with this model. Engraftment of primary AML cells is not universal and samples from individuals with more aggressive disease are more likely to engraft (Ailles et al. 1999; Pearce et al. 2006). Clearly, human cells may not respond to certain murine microenvironmental factors or cytokines/growth factors. More recent data have shown that engraftment with human leukaemia cells is seen at a higher frequency in more immunodeficient models (Feuring-Buske et al. 2003; Agliano et al. 2008), or where tumour cells are directly injected into the bone marrow, compared with the traditional intravenous route (Mazurier et al. 2003). This indicates that residual host immunity and the failure of tumour cells to home to microenvironmental niches remain significant factors to overcome for xenografts to become established.

Due to the well-defined molecular genetic defects involved in the pathophysiology of human leukaemias, the technique of retroviral transduction of bone marrow stem and progenitor cells with oncogenes combined with transplantation assays has been widely used. In addition, transgenic models, including the use of inducible oncogenes such as BCR-ABL (Huettner et al. 2000) or deletion of regulators such as PTEN (Yilmaz et al. 2006) have been employed. All of these models have provided valuable insights into disease development and the use of targeted treatments. However, the use of such assays to conclude that the tumour cell is “addicted” to the oncogene being tested should be tempered by the fact that the order of acquisition of the oncogenic mutation may not reflect the natural order, for example a tumour that develops in the background of PTEN deletion being the initial lesion may not be representative of primary tumours, where PTEN deletion may be a late event.

Therefore, over-reaching conclusions regarding the importance of a given signalling pathway as a therapeutic target should probably not be made on the basis of results from one type of assay system, but rather rely on data from a variety of complementary approaches. Ultimately, clinical trials are the final arbiter and unexpected results may be obtained. However, the emerging availability of a wide variety of compounds that can target individual catalytic isoforms of class 1

PI3Ks inhibit all class 1 activity or act as dual inhibitors of class 1 PI3Ks and mTOR makes pre-clinical evaluation necessary, as clearly it will not be possible to test all compounds in all situations (Brachmann et al. 2009; Ihle and Powis 2009; Maira et al. 2009). The remainder of this review will concentrate on individual disease entities in haematological oncology and assess the evidence for the role of PI3K signalling as an important component of tumour biology and maintenance, and as a potential target for therapy.

2 Acute Myeloid Leukaemia

AML is the predominant acute leukaemia in adult life and usually presents with a rapidly growing tumour that results in failure of production of normal blood cells. Current treatment for AML includes combination cytotoxic chemotherapy in repeated intensive cycles and allogeneic stem cell transplant in a relatively small proportion of selected individuals. Clinical outcome is dictated by a combination of tumour-intrinsic features, patient age and the presence of co-morbidities. Specific karyotypic abnormalities are associated with low (e.g. t(8;21)) or high (e.g. monosomy 5) relapse rates. Mutations in key genes such as nucleophosmin and the FLT3 tyrosine kinase are also predictive of clinical outcome (Schiffer 2008). Overall, the cure rate ranges from approximately 60% in patients under the age of 30 years to less than 10% in those over 65. Current treatment protocols predominantly utilise drugs that have been in our formulary for over three decades.

A number of publications have shown that PI3K signalling, as judged by Akt phosphorylation, is constitutively active in a high proportion of cases of AML (Xu et al. 2003; Min et al. 2004; Grandage et al. 2005; Sujobert et al. 2005; Billotet et al. 2006). This is true for the bulk of tumour cells and also for the LSC compartment. The LSC fraction in AML is characterised by a CD34^{positive}/CD38^{low-negative} phenotype (Bonnet and Dick 1997) (although this has been recently disputed) (Taussig et al. 2008). Using a highly sensitive quantitative mass spectrometric assay, we have shown significant activation of the enzymatic activity of Akt in the LSC fraction (Cutillas et al. 2006). Similar findings have been reported by flow cytometric evaluation of Akt phosphorylation in tumour cell subsets (Bardet et al. 2006). The mechanism by which PI3K becomes activated in AML is unclear as mutations in PTEN or PIK3CA are rare (Aggerholm et al. 2000; Liu et al. 2000; Hummerdal et al. 2006). Screening for the activating Akt E17K mutation has also been negative (Kim et al. 2008; Zenz et al. 2008).

Activating mutations in FLT3, which can lead to constitutively activated PI3K/Akt signalling (Brandts et al. 2005), either by internal tandem duplication/length mutation or by point mutation are found in approximately 30% of AML cases (Mead et al. 2007; Gale et al. 2008). Mutations in KRAS and NRAS, which can directly activate PI3K, are found in about 15–20% of cases and these are largely mutually exclusive with FLT3 mutations (Bowen et al. 2005). About a further 5% of cases can be shown to have activating mutations in the KIT tyrosine kinase (Renneville et al.

2008). Therefore, half of all AML cases have mutationally activated signalling in pathways upstream of PI3K. Other possible mechanisms for PI3K activation include autocrine production of growth factors such as IGF1 and GM-CSF (Young and Griffin 1986; Tazzari et al. 2007). There are conflicting data on the potential prognostic significance of constitutive Akt phosphorylation in AML. It has been suggested that Akt S473 phosphorylation is associated with a good response to therapy (Tamburini et al. 2007) or that Akt T308 phosphorylation correlates with poor outcome (Gallay et al. 2009). Such correlations do not prove causality and do not necessarily have relevance for targeting of PI3K for therapeutic purposes.

In primary AML samples, the pattern of class 1A PI3K catalytic isoform expression has been well characterised. p110delta is ubiquitously expressed, but there is variable expression of alpha and beta isoforms (Sujobert et al. 2005; Billottet et al. 2006). In addition to the lack of mutations in p110alpha, no mutations have been found in p110delta (Cornillet-Lefebvre et al. 2006). The use of highly selective PI3K inhibitors has suggested that a significant proportion of the constitutive PI3K activity in AML is attributable to p110delta (Sujobert et al. 2005; Billottet et al. 2006). Targeting this isoform in *ex vivo* experiments reduces primary AML cell proliferation, induces a modest level of apoptosis and sensitises cells to killing by etoposide/VP16. In the acute promyelocytic leukaemia subset of AML, characterised by the t(15;17) translocation resulting in a PML-RARA fusion and retinoic acid sensitivity (Collins 2008), there is uniform high level expression of the p110beta isoform in addition to consistent p110delta expression (Billottet et al. 2009). These cells are sensitive to highly selective inhibitors of p110beta, as well as to p110delta and pan-class 1 PI3K inhibitors, suggesting that the pattern of isoform expression is a key determinant of response to isoform-selective agents in AML (Billottet et al. 2009). Vogt and colleagues have previously shown that overexpression of wild-type p110delta or p110beta isoforms is sufficient to transform fibroblasts (Kang et al. 2006).

In keeping with the findings in APL, we have found that AML samples that retain p110alpha expression are less sensitive to a p110delta inhibitor than those that lack p110alpha expression (Khwaja, unpublished data). We have also found that samples from patients with FLT3 mutations have a similar level of response to p110delta inhibitors as FLT3 WT samples, but that RAS mutant samples are more resistant to PI3K inhibition (unpublished data). It is possible that combinations with agents that target the MAPK pathway may be required in such cases (Wee et al. 2009).

Sensitisation of AML cells to chemotherapeutic agents by PI3K blockade may depend on several factors – in primary AML cells, blockade of PI3K signalling affects both p53 and NF- κ B pathways, thereby lowering the cell's apoptotic threshold (Grandage et al. 2005). Two recent papers have evaluated the effects of pan-PI3K inhibition with the class 1 PI3K/mTOR inhibitor PI103 in AML. Kojima et al. showed PI103 to have modest direct apoptotic activity but that it could enhance the effects of the MDM2 inhibitor Nutlin-3 in mediating p53-dependent apoptosis (Kojima et al. 2008). Park and colleagues showed that PI103 reduces proliferation of AML cell lines and primary cells and can induce apoptosis in the

latter (Park et al. 2008). A moderate level of cell death was induced in the CD34^{positive}/CD38^{low-negative} LSC fraction. We have also shown that PI3K inhibition with PI103 can increase the cytotoxic response to anthracyclines and arsenic trioxide, two key agents in the treatment of APL (Billottet et al. 2009).

Data from two groups have shown that PTEN is an important gene for the maintenance of normal haematopoietic stem cells – conditional deletion resulted in accelerated entry of HSC into cell-cycle, loss of self-renewal capability and consequent loss of long-term repopulating stem cell activity (Yilmaz et al. 2006; Zhang et al. 2006). PTEN-deleted animals went on to develop myeloid and lymphoid leukaemias. Treatment with the mTOR inhibitor rapamycin reduced LSC activity and restored normal HSC activity (Yilmaz et al. 2006). Extrapolation of such models to human disease should be cautious; in particular, PTEN deletion was the initiating lesion in these animals and hence the tumours may have been especially addicted to the PI3K/mTOR pathway. The genetic complexity of human tumours, and the disappointing results with mTOR inhibitors in acute leukaemias in the clinic, (Yee et al. 2006; Rizzieri et al. 2008) may also mitigate against direct comparisons. However, normal HSC are largely quiescent and also require FOXO factors, which are negatively regulated by PI3K/Akt, for their maintenance (Miyamoto et al. 2007). This suggests that PI3K targeted therapies may be less likely to cause long-term damage to the normal HSC compartment whilst retaining activity against LSCs, which show increased PI3K/Akt activity.

3 Acute Lymphoblastic Leukaemia

ALL, which can arise in B cell or T cell precursors, is the commonest malignancy of childhood and can also arise in adult life. The cure rate in children now approaches 90% (Pui et al. 2008) but is much lower in adults (Goldstone et al. 2008; Fielding et al. 2009). Treatment is with multi-agent cytotoxic chemotherapy with intensive phases and a prolonged period of lower-dose maintenance therapy, to reduce relapse rates.

Similarly to AML, mutational activation of tyrosine kinase signalling pathways is also frequently found in ALL. The t(9;22) translocation resulting in the formation of the BCR-ABL tyrosine kinase fusion protein occurs in about 25–30% of adult patients, almost exclusively with a B cell phenotype, but less frequently in childhood (Pui et al. 2008). Other abnormalities include NUP214-ABL fusions in T-ALL and mutations of JAK tyrosine kinases and RAS (Graux et al. 2004; Perentesis et al. 2004; Flex et al. 2008). PIK3CA and Akt mutations are rare (Kim et al. 2008; Mahmoud et al. 2008).

PTEN appears to play an important part in the pathophysiology of T-ALL. Palomero and colleagues initially reported the presence of PTEN mutations in 9 of 111 T-ALL patients (Palomero et al. 2007). Subsequent reports have suggested higher frequencies (Gutierrez et al. 2009), although this could be attributable to the presence of relapsed cases in some series (Larson Gedman et al. 2009). Silva et al.

found Akt phosphorylation in over 80% of T-ALL samples but detected PTEN mutations in only a small proportion (Silva et al. 2008). They showed that PTEN enzymatic activity is reduced in the majority of T-ALL samples and that this is due to a combination of CK2-mediated phosphorylation of PTEN and PTEN-oxidation due to high levels of reactive oxygen species. The genetic background resulting in these abnormalities is not clear, although high level ROS production has been demonstrated downstream of oncogenic tyrosine kinases (Sattler et al. 2000). Gutierrez and colleagues have also closely characterised a cohort of T-ALL cases for PI3K pathway genetic abnormalities (Gutierrez et al. 2009). In 47 samples, they found 16 cases with PTEN deletion/mutation, two with PIK3CA mutation, two with PIK3R1 mutation, one with Akt1 E17K mutation and four with RAS mutations. These were largely mutually exclusive indicating a genetic abnormality in a key PI3K pathway gene in approximately half the samples.

Teachey and colleagues have shown that rapamycin and analogues can inhibit proliferation and promote apoptosis of primary B-ALL cells in ex vivo culture systems and in xenograft models (Teachey et al. 2006, 2009). mTOR inhibitors can also sensitise leukaemia cells to the effects of methotrexate, most likely by reducing levels of dihydrofolate reductase (Teachey et al. 2008). In T-ALL, the majority of cases have constitutive activation of the Notch signalling pathway, predominantly due to mutations in NOTCH1 (Demarest et al. 2008). Notch signalling can be inhibited by gamma-secretase inhibitors (GSIs), which prevent processing of Notch to the active form. Previous studies have shown that GSIs can reduce proliferation of Notch mutant/PTEN WT cells, but do not induce significant apoptosis (Weng et al. 2004; De Keersmaecker et al. 2008). Early clinical trials with GSIs have been disappointing because of a lack of efficacy and gut toxicity (Aster et al. 2008). We have recently shown that combining GSI with dual PI3K/mTOR inhibitors, such as PI103 or NVP-BEZ235, converts a cytostatic to a highly cytotoxic response (Banerjee et al. 2009). The minimal requirement for this appears to be dual inhibition of class 1 PI3K and mTORC1 (in addition to Notch blockade), as it can be reproduced by combining GSI with a class 1 PI3K inhibitor, PIK90 (Knight et al. 2006), and rapamycin, but not with each agent alone. mTOR and PI3K inhibitors also show synergy with glucocorticoids, an essential component of the treatment regimen for ALL.

4 Chronic Myeloid Leukaemia and BCR-ABL Positive ALL

CML is characterised by the presence of the Philadelphia chromosome as the result of the t(9;22) translocation, which is thought to arise in a haematopoietic stem cell (Druker 2008). This generates the BCR-ABL tyrosine kinase, which is constitutively active and stimulates a number of downstream signalling pathways, including PI3K/Akt and mTOR (Kharas and Fruman 2005). The natural history of CML is of a chronic phase of variable length transforming into an acute leukaemia, most commonly to AML. Current treatment with tyrosine kinase inhibitors including

imatinib is highly effective in maintaining long-term remissions, although imatinib-resistance can arise (Druker 2008).

Ectopic expression of BCR-ABL in murine bone marrow reconstitution assays can recapitulate the clinical phenotype (Daley et al. 1990). Many groups have shown that PI3K is activated by BCR-ABL and that it plays an important part in disease pathophysiology – early studies showed that PI3K inhibition reduced clonogenic growth of BCR-ABL positive cells (Skorski et al. 1997). Deletion of both p85 alpha and beta (PIK3R1 and PIK3R2) alleles results in severe impairment of BCR-ABL-mediated transformation of B cell progenitors (Kharas et al. 2008), although this also compromises normal haematopoietic cell activity (Haneline et al. 2006). Mohi et al. showed that combining rapamycin with imatinib had a synergistic effect against BCR-ABL transformed cells and could prolong survival in a murine model of CML (Mohi et al. 2004). Combining imatinib with PI103 impairs clonogenic growth of Philadelphia-positive acute leukaemia cells (Kharas et al. 2008).

Currently, treatment with imatinib or other TKIs is effective in the majority of cases of CML. PI3K and mTOR signalling may play a part in the growth of imatinib-resistant cells (Burchert et al. 2005) and rapamycin has activity both *in vitro* and in patients in this setting (Sillaber et al. 2008). Although it has been suggested that treatment with imatinib may need to be lifelong as there is a residual pool of CML stem cells that resist therapy (Jorgensen and Holyoake 2007), recent evidence suggests that in a significant proportion of patients with molecular remission, discontinuation of IM does not result in a relapse (Rousselot et al. 2007). In the light of effective treatment for CML with imatinib and second generation Abl tyrosine kinase inhibitors, the role of PI3K inhibitors in this disease is likely to be relatively modest.

5 Chronic Lymphocytic Leukaemia

B-CLL is the most prevalent type of leukaemia worldwide and can often run a clinically indolent course, which may not require leukaemia-specific therapy. Although long thought to be a disease of low proliferative rates and cell accumulation due to reduced apoptosis, more recent data have suggested that there is a sizeable proliferating pool of CLL cells (Chiorazzi and Ferrarini 2006; Caligaris-Cappio and Ghia 2008). Cases with an absence of somatic mutations in the immunoglobulin variable region genes, which usually express the Zap-70 tyrosine kinase, have a more progressive course and may respond to chronic antigenic stimulation via the B cell receptor *in vivo* (Lanham et al. 2003). Chronic antigenic stimulation of B cells is already implicated in the development of malignancies such as gastric mucosa associated lymphoid tissue lymphomas in the context of chronic *Helicobacter pylori* infection (Caligaris-Cappio and Ghia 2008). B-CLL cells are also thought to be highly dependent on contact with supportive cells and to cytokines such as CD40L and chemokines including SDF1. Ex vivo culture of CLL

cells in the absence of cytokine or stromal support usually results in a high level of spontaneous cell death.

Some CLL samples can show constitutive activation of PI3K or may demonstrate sustained activation following cross-linking of the B cell receptor (Longo et al. 2008). A significant proportion of CLL cases, in particular those with deletion of 13q14, have reduced or absent expression of the Akt S473 phosphatase PHLPP (Gao et al. 2005; Ouillette et al. 2008). Recent data show that novel class 1 PI3K inhibitors, such as PI103 and PIK90, inhibit responses of primary CLL cells to the chemokine SDF1 and promote apoptosis, especially in combination with the cytotoxic agent fludarabine, which is highly active in CLL (Niedermeier et al. 2009).

T-prolymphocytic leukaemia (T-PLL) is a rare form of CLL. T cell leukaemia 1 (TCL1) was identified as the oncogene insertionally activated by the T cell receptor enhancer through *inv(14)* or *t(14;14)* (Virgilio et al. 1994) and is expressed at a high level in the majority of cases (Narducci et al. 1997; Herling et al. 2008). TCL1 has been shown to bind to the PH domain of Akt and to promote its activity and nuclear translocation (Laine et al. 2000; Pekarsky et al. 2000). In T-PLL cells with TCL1 dysregulation, Akt signalling is enhanced and cells are sensitive in *ex vivo* cultures to inhibitors of PI3K and Akt (Herling et al. 2008). TCL1 is also overexpressed in a subset of B-CLL cases with a more proliferative phenotype (Herling et al. 2006). In fact, when TCL1 is targeted to the B cell compartment, mice develop a B-CLL like disease (Bichi et al. 2002). TCL1 overexpression in human B-CLL may be attributable to regulation by *miR-29* and *miR-181*, two microRNAs differentially expressed in CLL (Pekarsky et al. 2006).

Several early phase clinical trials of mTOR inhibitors have included a small number of patients with CLL (Yee et al. 2006; Rizzieri et al. 2008; Decker et al. 2009). Stable disease and partial responses were seen in a significant number of cases – however, on a cautionary note, a high level of severe opportunistic infections were observed in one study (Decker et al. 2009). These patients were heavily pre-treated with other agents that compromise immune function but appropriate precautions against infection may need to be taken with PI3K/mTOR targeted agents.

6 Lymphomas

These are heterogeneous diseases with varying clinical phenotypes and growth patterns ranging from indolent, e.g. follicular lymphoma, to highly proliferative e.g. Burkitt lymphoma.

6.1 *Diffuse Large B Cell Lymphoma*

Immunohistochemistry analysis of primary tumour samples of this common and aggressive lymphoma shows a high level of positivity for phosphorylated Akt and this may correlate with worse clinical outcome (Uddin et al. 2006). Abubaker et al.

found PIK3CA mutations in 17/215 cases – these were largely mutually exclusive with samples showing reduced PTEN by immunohistochemistry (Abubaker et al. 2007). Baohua found 55/76 samples to have phosphorylated Akt but only one case had a PIK3CA mutation (Baohua et al. 2008). These variations could be due to the different ethnic populations studied and further investigation is required to establish the incidence of PIK3CA mutations in diffuse large B cell lymphoma (DLBCL).

6.2 Anaplastic Large Cell Lymphoma

A significant proportion of these lymphomas have dysregulated tyrosine kinase signalling because of the presence of the nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) fusion protein. NPM-ALK activates PI3K signalling and transformation can be inhibited by small molecule PI3K inhibitors and the expression of dominant-negative Akt (Bai et al. 2000; Slupianek et al. 2001).

6.3 Mantle Cell Lymphoma

Akt and mTOR pathways are hyperactive in primary MCL cells (Peponi et al. 2006; Rudelius et al. 2006; Dal Col et al. 2008) and this may be related to reduced PTEN expression (Rudelius et al. 2006) or enhanced PTEN phosphorylation resulting in reduced enzymatic activity (Dal Col et al. 2008). PIK3CA mutations have not been detected (Rudelius et al. 2006) but microarray analysis suggests that p110delta (PIK3CD) was overexpressed in MCL cells compared with normal mantle zone lymphocytes (Martinez et al. 2003). Primary MCL cells show variable sensitivity to PI3K and mTOR inhibition (Dal Col et al. 2008) and clinical studies have shown a significant response rate to mTOR inhibition. Ansell et al. showed a 41% response rate, predominantly partial responses, in a heavily pretreated group of relapsed MCL patients (Ansell et al. 2008). MCL is characterised by the t(11;14) translocation that results in deregulated expression of the cyclin D1 mRNA by placing this gene under control of the immunoglobulin heavy chain gene enhancer elements. The mTOR pathway is thought to regulate the translation of cyclin D1 via its effects on eIF4E-BP1 phosphorylation (Hashemolhosseini et al. 1998) but it is not clear if this is the mechanism by which rapamycin and analogues exert their effects in MCL (Yazbeck et al. 2008).

7 Multiple Myeloma

Myeloma is a malignancy of plasma cells, which is incurable in the vast majority of patients. However, a number of novel biological agents such as proteasomal inhibitors, thalidomide and its analogues show considerable activity in MM with

a resulting prolongation of survival (San-Miguel et al. 2008). Because of the relatively slow tempo of disease in the majority of patients, evaluation of targeted therapies is more readily achieved. In addition, the production of a monoclonal immunoglobulin (paraprotein) by tumour cells, which can be readily quantified by blood sampling, can give an accurate measure of treatment response.

In common with other haematological tumours, activation of the PI3K pathway in myeloma is not frequently attributable to mutations in PTEN or PIK3CA. PTEN deletions are seen in MM cell lines but are infrequent in primary samples – when found, they are associated with more advanced and aggressive disease (Chang et al. 2006). A subset of MM patients (about 15%) have the t(4;14) translocation that results in deregulated signalling from the FGFR3 receptor (Bergsagel and Kuehl 2005), which can activate PI3K (Agazie et al. 2003). In addition, MM is thought to be highly regulated by interactions with stromal elements and cytokines/growth factors (Podar et al. 2009) – both direct contact and exposure to key regulators such as IGF1 and IL-6 have been shown to activate PI3K/Akt. RAS mutations are prevalent in MM (Fonseca et al. 2004) and have been shown to directly activate PI3K, as well as MAPK, pathways in MM cells (Hu et al. 2003).

PTEN-null MM cell lines undergo cell-cycle arrest and/or apoptosis after incubation with PI3K inhibitors such as LY294002 and wortmannin (Pene et al. 2002; Zhang et al. 2003). Rapamycin can also reduce cell proliferation and co-operate positively with other agents that are active in MM (Shi et al. 2002; Raje et al. 2004). However, rapamycin has been shown to result in feedback activation of an IGF1R/IRS1/PI3K/Akt pathway (Shi et al. 2005), which may reduce its effectiveness. Zollinger and colleagues have shown that about half of primary MM samples have detectable Akt phosphorylation and that this correlates with the induction of apoptosis in response to an Akt inhibitor (Zollinger et al. 2008). Recently published data with the dual PI3K/mTOR inhibitor NVP-BEZ235 shows the induction of apoptosis in some MM cell lines and a limited number of primary samples (Baumann et al. 2009). Farag has reported the results of a phase 2 trial of the mTOR inhibitor temsirolimus in MM and this shows modest activity with one partial and five minor responses in 16 patients (Farag et al. 2009). Combination studies of mTOR inhibitors with other agents active in MM, such as dexamethasone and bortezomib, are ongoing.

8 Effects on Normal Immune Cells and Host Immunity

For many epithelial tumours, growth at the primary site and metastatic spread is influenced by a variety of stromal cells in their microenvironment. These include a number of cells of haematopoietic origin such as macrophages, myeloid derived suppressor cells, certain types of T and B lymphocytes, natural killer cells, mast cells and neutrophils (reviewed in Joyce and Pollard 2009). These can influence tumour behaviour in a variety of ways in a complex interactive network. For several tumour types, extensive macrophage infiltration correlates with poor prognosis

(Allavena et al. 2008). Experimental models suggest that loss of tumour-associated macrophages, by genetic or pharmacological approaches, can ameliorate tumour development. Because of concerns about broad depletion of cells important for the innate immune response, more targeted approaches have been tried – myeloid cell-specific deletion of vascular endothelial growth factor A in a breast cancer model was associated with decreased tumour angiogenesis but resulted in accelerated tumour progression (Stockmann et al. 2008).

PI3Ks, in particular the delta and gamma isoforms, play an important part in regulation of the immune system and of neutrophil migration. p110delta is involved in CSF1 regulated proliferation and chemotaxis in macrophages (Papakonstanti et al. 2007, 2008) but is not involved in phagocytosis, which is mediated by the beta isoform (Leverrier et al. 2003). p110gamma and delta play important roles in neutrophil trafficking and respiratory burst activation (Puri et al. 2004; Condliffe et al. 2005; Randis et al. 2008). p110delta plays a key part in mast cell activation and the allergic response. (Ali et al. 2004, 2008).

Therefore, it is possible that PI3K inhibitors could be useful in modifying the function of supportive cells. However, it is not clear if this would affect already well-established tumours and clearly this could have complex effects on host immunity. p110delta and gamma play important roles in development and function of B cells, T cells and NK cells (reviewed in (Fruman and Bismuth 2009). Certain tumours, in particular those driven by Epstein-Barr virus, can develop in immunocompromised individuals (Grulich et al. 2007) and chronic inhibition of T cell function by PI3K inhibitors could promote this. p110delta knockout mice show more rapid development of BCR-ABL driven disease and increased susceptibility to transplantable lymphomas (Saudemont et al. 2007; Zebedin et al. 2008). NK migration is compromised in p110delta null mice, which also show changes in the function of T-regulatory cells (Patton et al. 2006; Kim et al. 2007; Saudemont et al. 2009). Overall, it seems probable that more sophisticated approaches will be required to assess the effect of targeting PI3K in altering the survival and behaviour of tumour cells by modifying the functions of the immune system.

9 Conclusions

The PI3K pathway is constitutively active in a high proportion of primary patient samples from patients with haematological malignancies and constitutes an important signalling node for therapeutic targeting. In AML, the p110delta catalytic isoform appears to play an important part in mediating the activation of downstream targets and trials selectively targeting this isoform have begun. Delta-selective agents would have the potential for reduced toxicity to non-haematological tissues compared with pan-class 1 inhibitors. However, it is unlikely that single agent PI3K targeted therapy, whether isoform-selective or more broadly targeted, would be sufficient to exert long-term disease control in AML. The results of clinical trials with FLT3 tyrosine kinase inhibitors are instructive in that they show that although

patients with FLT3-mutated AML often show a response to targeted inhibitors (though this is by no means universal), this is only transient, even in the presence of effective target inhibition. Combinations of PI3K targeted agents with standard cytotoxic drugs, or with agents targeting upstream abnormalities such as FLT3, will need to be evaluated for their tolerability and efficacy.

In the more indolent haematological malignancies, such as CLL and low-grade NHLs, there is also evidence for the involvement of PI3K signalling in disease pathophysiology, although less is known about specific isoform involvement. Whereas in acute leukaemia therapy, it is likely that PI3K inhibitors will be tested in relatively short courses of treatment, more prolonged therapy may be needed in the less aggressive tumours. Because of the importance of PI3K signalling in the regulation of the immune system, extra vigilance will be required against infection, in particular as early trials are likely to be in the setting of heavily pretreated patients with already compromised immune function. The relative benefits of pan class 1 vs. isoform-selective inhibitors, with or without added activity against mTOR and PI3K-related DNA-damage kinases, adds multiple layers of complexity for the future design of clinical trials. The arrival of drugs that target PI3K signalling into the clinic should ultimately allow us to define their role in the treatment of human malignant disease.

References

- Abubaker J, Bavi PP et al (2007) PIK3CA mutations are mutually exclusive with PTEN loss in diffuse large B cell lymphoma. *Leukemia* 21(11):2368-2370
- Agazie YM, Movilla N et al (2003) The phosphotyrosine phosphatase SHP2 is a critical mediator of transformation induced by the oncogenic fibroblast growth factor receptor 3. *Oncogene* 22(44):6909-6918
- Aggerholm A, Gronbaek K et al (2000) Mutational analysis of the tumour suppressor gene MMAC1/PTEN in malignant myeloid disorders. *Eur J Haematol* 65(2):109-113
- Agliano A, Martin Padura I et al (2008) Human acute leukemia cells injected in NOD/LtSz scid/IL 2Rgamma null mice generate a faster and more efficient disease compared to other NOD/scid related strains. *Int J Cancer* 123(9):2222-2227
- Ailles LE, Gerhard B et al (1999) Growth characteristics of acute myelogenous leukemia progenitors that initiate malignant hematopoiesis in nonobese diabetic/severe combined immunodeficient mice. *Blood* 94(5):1761-1772
- Ali K, Bilancio A et al (2004) Essential role for the p110delta phosphoinositide 3 kinase in the allergic response. *Nature* 431(7011):1007-1011
- Ali K, Camps M et al (2008) Isoform specific functions of phosphoinositide 3 kinases: p110delta but not p110gamma promotes optimal allergic responses *in vivo*. *J Immunol* 180(4):2538-2544
- Allavena P, Sica A et al (2008) The inflammatory micro environment in tumor progression: the role of tumor associated macrophages. *Crit Rev Oncol Hematol* 66(1):1-9
- Ansell SM, Inwards DJ et al (2008) Low dose, single agent temsirolimus for relapsed mantle cell lymphoma: a phase 2 trial in the North Central Cancer Treatment Group. *Cancer* 113(3):508-514
- Aster JC, Pear WS et al (2008) Notch signaling in leukemia. *Annu Rev Pathol* 3:587-613

- Bai RY, Ouyang T et al (2000) Nucleophosmin anaplastic lymphoma kinase associated with anaplastic large cell lymphoma activates the phosphatidylinositol 3 kinase/Akt antiapoptotic signaling pathway. *Blood* 96(13):4319-4327
- Banerjee L, Mansour M et al (2009) Combined blockade of PI3 kinase and mTOR synergizes with gamma secretase inhibitors to induce a high level of cell death in T cell acute lymphoblastic leukaemia cells with activated Notch signalling. *Br J Haematol* 145(Suppl 1):9-10
- Baohua Y, Xiaoyan Z et al (2008) Mutations of the PIK3CA gene in diffuse large B cell lymphoma. *Diagn Mol Pathol* 17(3):159-165
- Bardet V, Tamburini J et al (2006) Single cell analysis of phosphoinositide 3 kinase/Akt and ERK activation in acute myeloid leukemia by flow cytometry. *Haematologica* 91(6):757-764
- Baumann P, Mandl Weber S et al (2009) The novel orally bioavailable inhibitor of phosphoinositide 3 kinase and mammalian target of rapamycin, NVP BEZ235, inhibits growth and proliferation in multiple myeloma. *Exp Cell Res* 315(3):485-497
- Bergsagel PL, Kuehl WM (2005) Molecular pathogenesis and a consequent classification of multiple myeloma. *J Clin Oncol* 23(26):6333-6338
- Bichi R, Shinton SA et al (2002) Human chronic lymphocytic leukemia modeled in mouse by targeted TCL1 expression. *Proc Natl Acad Sci USA* 99(10):6955-6960
- Billotet C, Grandage VL et al (2006) A selective inhibitor of the p110delta isoform of PI 3 kinase inhibits AML cell proliferation and survival and increases the cytotoxic effects of VP16. *Oncogene* 25(50):6648-6659
- Billotet C, Banerjee L et al (2009) Inhibition of class I phosphoinositide 3 kinase activity impairs proliferation and triggers apoptosis in acute promyelocytic leukemia without affecting atral induced differentiation. *Cancer Res* 69(3):1027-1036
- Bonnet D, Dick JE (1997) Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* 3(7):730-737
- Bowen DT, Frew ME et al (2005) RAS mutation in acute myeloid leukemia is associated with distinct cytogenetic subgroups but does not influence outcome in patients younger than 60 years. *Blood* 106(6):2113-2119
- Brachmann S, Fritsch C et al (2009) PI3K and mTOR inhibitors: a new generation of targeted anticancer agents. *Curr Opin Cell Biol* 21(2):194-198
- Brandts CH, Sargin B et al (2005) Constitutive activation of Akt by Flt3 internal tandem duplications is necessary for increased survival, proliferation, and myeloid transformation. *Cancer Res* 65(21):9643-9650
- Burchert A, Wang Y et al (2005) Compensatory PI3 kinase/Akt/mTOR activation regulates imatinib resistance development. *Leukemia* 19(10):1774-1782
- Caligaris Cappio F, Ghia P (2008) Novel insights in chronic lymphocytic leukemia: are we getting closer to understanding the pathogenesis of the disease? *J Clin Oncol* 26(27):4497-4503
- Chang H, Qi XY et al (2006) Analysis of PTEN deletions and mutations in multiple myeloma. *Leuk Res* 30(3):262-265
- Chiorazzi N, Ferrarini M (2006) Evolving view of the in vivo kinetics of chronic lymphocytic leukemia B cells. *Hematology Am Soc Hematol Educ Program* 273-8:512
- Collins SJ (2008) Retinoic acid receptors, hematopoiesis and leukemogenesis. *Curr Opin Hematol* 15(4):346-351
- Collins RJ, Verschuer LA et al (1989) Spontaneous programmed death (apoptosis) of B chronic lymphocytic leukaemia cells following their culture *in vitro*. *Br J Haematol* 71(3):343-350
- Condliffe AM, Davidson K et al (2005) Sequential activation of class IB and class IA PI3K is important for the primed respiratory burst of human but not murine neutrophils. *Blood* 106(4):1432-1440
- Cornillet Lefebvre P, Cuccuini W et al (2006) Constitutive phosphoinositide 3 kinase activation in acute myeloid leukemia is not due to p110delta mutations. *Leukemia* 20(2):374-376
- Cutillas PR, Khwaja A et al (2006) Ultrasensitive and absolute quantification of the phosphoinositide 3 kinase/Akt signal transduction pathway by mass spectrometry. *Proc Natl Acad Sci USA* 103(24):8959-8964

- Dal Col J, Zancai P et al (2008) Distinct functional significance of Akt and mTOR constitutive activation in mantle cell lymphoma. *Blood* 111(10):5142-5151
- Daley GQ, Van Etten RA et al (1990) Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. *Science* 247(4944):824-830
- De Keersmaecker K, Lahortiga I et al (2008) *In vitro* validation of gamma secretase inhibitors alone or in combination with other anti cancer drugs for the treatment of T cell acute lymphoblastic leukemia. *Haematologica* 93(4):533-542
- Decker T, Sandherr M et al (2009) A pilot trial of the mTOR (mammalian target of rapamycin) inhibitor RAD001 in patients with advanced B CLL. *Ann Hematol* 88(3):221-227
- Delwel R, Dorssers L et al (1987) Human recombinant multilineage colony stimulating factor (interleukin 3): stimulator of acute myelocytic leukemia progenitor cells *in vitro*. *Blood* 70(1):333-336
- Demarest RM, Ratti F et al (2008) It's T ALL about Notch. *Oncogene* 27(38):5082-5091
- Druker BJ (2008) Translation of the Philadelphia chromosome into therapy for CML. *Blood* 112(13):4808-4817
- Farag SS, Zhang S et al (2009) Phase II trial of temsirolimus in patients with relapsed or refractory multiple myeloma. *Leuk Res* 33(11):1475-1480
- Feuring Buske M, Gerhard B et al (2003) Improved engraftment of human acute myeloid leukemia progenitor cells in beta 2 microglobulin deficient NOD/SCID mice and in NOD/SCID mice transgenic for human growth factors. *Leukemia* 17(4):760-763
- Fielding AK, Rowe JM et al (2009) Prospective outcome data on 267 unselected adult patients with Philadelphia chromosome positive acute lymphoblastic leukemia confirms superiority of allogeneic transplantation over chemotherapy in the pre imatinib era: results from the International ALL Trial MRC UKALLXII/ECOG2993. *Blood* 113(19):4489-4496
- Flex E, Petrangeli V et al (2008) Somatically acquired JAK1 mutations in adult acute lymphoblastic leukemia. *J Exp Med* 205(4):751-758
- Fonseca R, Barlogie B et al (2004) Genetics and cytogenetics of multiple myeloma: a workshop report. *Cancer Res* 64(4):1546-1558
- Fruman DA, Bismuth G (2009) Fine tuning the immune response with PI3K. *Immunol Rev* 228(1):253-272
- Gale RE, Green C et al (2008) The impact of FLT3 internal tandem duplication mutant level, number, size, and interaction with NPM1 mutations in a large cohort of young adult patients with acute myeloid leukemia. *Blood* 111(5):2776-2784
- Gallay N, Dos Santos C et al (2009) The level of AKT phosphorylation on threonine 308 but not on serine 473 is associated with high risk cytogenetics and predicts poor overall survival in acute myeloid leukaemia. *Leukemia* 23(6):1029-1038
- Gao T, Furnari F et al (2005) PHLPP: a phosphatase that directly dephosphorylates Akt, promotes apoptosis, and suppresses tumor growth. *Mol Cell* 18(1):13-24
- Garrido SM, Appelbaum FR et al (2001) Acute myeloid leukemia cells are protected from spontaneous and drug induced apoptosis by direct contact with a human bone marrow stromal cell line (HS 5). *Exp Hematol* 29(4):448-457
- Goldstone AH, Richards SM et al (2008) In adults with standard risk acute lymphoblastic leukemia, the greatest benefit is achieved from a matched sibling allogeneic transplantation in first complete remission, and an autologous transplantation is less effective than conventional consolidation/maintenance chemotherapy in all patients: final results of the International ALL Trial (MRC UKALL XII/ECOG E2993). *Blood* 111(4):1827-1833
- Grandage VL, Gale RE et al (2005) PI3 kinase/Akt is constitutively active in primary acute myeloid leukaemia cells and regulates survival and chemoresistance via NF kappaB, Mapkinase and p53 pathways. *Leukemia* 19(4):586-594
- Graux C, Cools J et al (2004) Fusion of NUP214 to ABL1 on amplified episomes in T cell acute lymphoblastic leukemia. *Nat Genet* 36(10):1084-1089
- Grigorieva I, Thomas X et al (1998) The bone marrow stromal environment is a major factor in myeloma cell resistance to dexamethasone. *Exp Hematol* 26(7):597-603

- Grulich AE, van Leeuwen MT et al (2007) Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta analysis. *Lancet* 370(9581):59 67
- Gutierrez A, Sanda T et al (2009) High frequency of PTEN, PI3K and AKT abnormalities in T cell acute lymphoblastic leukemia. *Blood* 114(3):647 650
- Haneline LS, White H et al (2006) Genetic reduction of class IA PI 3 kinase activity alters fetal hematopoiesis and competitive repopulating ability of hematopoietic stem cells *in vivo*. *Blood* 107(4):1375 1382
- Hashemolhosseini S, Nagamine Y et al (1998) Rapamycin inhibition of the G1 to S transition is mediated by effects on cyclin D1 mRNA and protein stability. *J Biol Chem* 273(23): 14424 14429
- Herling M, Patel KA et al (2006) TCL1 shows a regulated expression pattern in chronic lymphocytic leukemia that correlates with molecular subtypes and proliferative state. *Leukemia* 20 (2):280 285
- Herling M, Patel KA et al (2008) High TCL1 expression and intact T cell receptor signaling define a hyperproliferative subset of T cell prolymphocytic leukemia. *Blood* 111(1):328 337
- Hu L, Shi Y et al (2003) Downstream effectors of oncogenic ras in multiple myeloma cells. *Blood* 101(8):3126 3135
- Huettner CS, Zhang P et al (2000) Reversibility of acute B cell leukaemia induced by BCR ABL1. *Nat Genet* 24(1):57 60
- Hummerdal P, Andersson P et al (2006) Absence of hot spot mutations of the PIK3CA gene in acute myeloid leukaemia. *Eur J Haematol* 77(1):86 87
- Ihle NT, Powis G (2009) Take your PIK: phosphatidylinositol 3 kinase inhibitors race through the clinic and toward cancer therapy. *Mol Cancer Ther* 8(1):1 9
- Jorgensen HG, Holyoake TL (2007) Characterization of cancer stem cells in chronic myeloid leukaemia. *Biochem Soc Trans* 35(Pt 5):1347 1351
- Joyce JA, Pollard JW (2009) Microenvironmental regulation of metastasis. *Nat Rev Cancer* 9 (4):239 252
- Kang S, Denley A et al (2006) Oncogenic transformation induced by the p110beta, gamma, and delta isoforms of class I phosphoinositide 3 kinase. *Proc Natl Acad Sci USA* 103(5):1289 1294
- Kharas MG, Fruman DA (2005) ABL oncogenes and phosphoinositide 3 kinase: mechanism of activation and downstream effectors. *Cancer Res* 65(6):2047 2053
- Kharas MG, Janes MR et al (2008) Ablation of PI3K blocks BCR ABL leukemogenesis in mice, and a dual PI3K/mTOR inhibitor prevents expansion of human BCR ABL+ leukemia cells. *J Clin Invest* 118(9):3038 3050
- Kim N, Sautemont A et al (2007) The p110delta catalytic isoform of PI3K is a key player in NK cell development and cytokine secretion. *Blood* 110(9):3202 3208
- Kim MS, Jeong EG et al (2008) Mutational analysis of oncogenic AKT E17K mutation in common solid cancers and acute leukaemias. *Br J Cancer* 98(9):1533 1535
- Knight ZA, Gonzalez B et al (2006) A pharmacological map of the PI3 K family defines a role for p110alpha in insulin signaling. *Cell* 125(4):733 747
- Kojima K, Shimanuki M et al (2008) The dual PI3 kinase/mTOR inhibitor PI 103 prevents p53 induction by Mdm2 inhibition but enhances p53 mediated mitochondrial apoptosis in p53 wild type AML. *Leukemia* 22(9):1728 1736
- Lagneaux L, Delforge A et al (1998) Chronic lymphocytic leukemic B cells but not normal B cells are rescued from apoptosis by contact with normal bone marrow stromal cells. *Blood* 91 (7):2387 2396
- Laine J, Kunstle G et al (2000) The protooncogene TCL1 is an Akt kinase coactivator. *Mol Cell* 6 (2):395 407
- Lanham S, Hamblin T et al (2003) Differential signaling via surface IgM is associated with VH gene mutational status and CD38 expression in chronic lymphocytic leukemia. *Blood* 101 (3):1087 1093
- Larson Gedman A, Chen Q et al (2009) The impact of NOTCH1, FBW7 and PTEN mutations on prognosis and downstream signaling in pediatric T cell acute lymphoblastic leukemia: a report from the Children's Oncology Group. *Leukemia* 23(8):1417 1425

- Leverrier Y, Okkenhaug K et al (2003) Class I phosphoinositide 3 kinase p110beta is required for apoptotic cell and Fcgamma receptor mediated phagocytosis by macrophages. *J Biol Chem* 278(40):38437-38442
- Liu TC, Lin PM et al (2000) Mutation analysis of PTEN/MMAC1 in acute myeloid leukemia. *Am J Hematol* 63(4):170-175
- Longo PG, Laurenti L et al (2008) The Akt/Mcl 1 pathway plays a prominent role in mediating antiapoptotic signals downstream of the B cell receptor in chronic lymphocytic leukemia B cells. *Blood* 111(2):846-855
- Mahmoud IS, Sughayer MA et al (2008) The transforming mutation E17K/AKT1 is not a major event in B cell derived lymphoid leukaemias. *Br J Cancer* 99(3):488-490
- Maira SM, Stauffer F et al (2009) PI3K inhibitors for cancer treatment: where do we stand? *Biochem Soc Trans* 37(Pt 1):265-272
- Martinez N, Camacho FI et al (2003) The molecular signature of mantle cell lymphoma reveals multiple signals favoring cell survival. *Cancer Res* 63(23):8226-8232
- Mazurier F, Doedens M et al (2003) Rapid myeloerythroid repopulation after intrafemoral transplantation of NOD SCID mice reveals a new class of human stem cells. *Nat Med* 9(7):959-963
- Mead AJ, Linch DC et al (2007) FLT3 tyrosine kinase domain mutations are biologically distinct from and have a significantly more favorable prognosis than FLT3 internal tandem duplications in patients with acute myeloid leukemia. *Blood* 110(4):1262-1270
- Min YH, Cheong JW et al (2004) Cytoplasmic mislocalization of p27Kip1 protein is associated with constitutive phosphorylation of Akt or protein kinase B and poor prognosis in acute myelogenous leukemia. *Cancer Res* 64(15):5225-5231
- Miyamoto K, Araki KY et al (2007) Foxo3a is essential for maintenance of the hematopoietic stem cell pool. *Cell Stem Cell* 1(1):101-112
- Mohi MG, Boulton C et al (2004) Combination of rapamycin and protein tyrosine kinase (PTK) inhibitors for the treatment of leukemias caused by oncogenic PTKs. *Proc Natl Acad Sci USA* 101(9):3130-3135
- Narducci MG, Stoppacciaro A et al (1997) TCL1 is overexpressed in patients affected by adult T cell leukemias. *Cancer Res* 57(24):5452-5456
- Niedermeier M, Hennessy BT et al (2009) Isoform selective phosphoinositide 3' kinase inhibitors inhibit CXCR4 signaling and overcome stromal cell mediated drug resistance in chronic lymphocytic leukemia: a novel therapeutic approach. *Blood* 113(22):5549-5557
- Ouillette P, Erba H et al (2008) Integrated genomic profiling of chronic lymphocytic leukemia identifies subtypes of deletion 13q14. *Cancer Res* 68(4):1012-1021
- Palomero T, Sulis ML et al (2007) Mutational loss of PTEN induces resistance to NOTCH1 inhibition in T cell leukemia. *Nat Med* 13(10):1203-1210
- Papakonstanti EA, Ridley AJ et al (2007) The p110delta isoform of PI 3 kinase negatively controls RhoA and PTEN. *EMBO J* 26(13):3050-3061
- Papakonstanti EA, Zwaenepoel O et al (2008) Distinct roles of class IA PI3K isoforms in primary and immortalised macrophages. *J Cell Sci* 121(pt 24):4124-4133
- Park S, Chapuis N et al (2008) PI 103, a dual inhibitor of Class IA phosphatidylinositide 3 kinase and mTOR, has antileukemic activity in AML. *Leukemia* 22(9):1698-1706
- Patton DT, Garden OA et al (2006) Cutting edge: the phosphoinositide 3 kinase p110 delta is critical for the function of CD4+CD25+Foxp3+ regulatory T cells. *J Immunol* 177(10):6598-6602
- Pearce DJ, Taussig D et al (2006) AML engraftment in the NOD/SCID assay reflects the outcome of AML: implications for our understanding of the heterogeneity of AML. *Blood* 107(3):1166-1173
- Pekarsky Y, Koval A et al (2000) Tcl1 enhances Akt kinase activity and mediates its nuclear translocation. *Proc Natl Acad Sci USA* 97(7):3028-3033
- Pekarsky Y, Santanam U et al (2006) Tcl1 expression in chronic lymphocytic leukemia is regulated by miR 29 and miR 181. *Cancer Res* 66(24):11590-11593

- Pene F, Claessens YE et al (2002) Role of the phosphatidylinositol 3 kinase/Akt and mTOR/P70S6 kinase pathways in the proliferation and apoptosis in multiple myeloma. *Oncogene* 21 (43):6587-6597
- Peponi E, Drakos E et al (2006) Activation of mammalian target of rapamycin signaling promotes cell cycle progression and protects cells from apoptosis in mantle cell lymphoma. *Am J Pathol* 169(6):2171-2180
- Perentesis JP, Bhatia S et al (2004) RAS oncogene mutations and outcome of therapy for childhood acute lymphoblastic leukemia. *Leukemia* 18(4):685-692
- Podar K, Chauhan D et al (2009) Bone marrow microenvironment and the identification of new targets for myeloma therapy. *Leukemia* 23(1):10-24
- Pui CH, Robison LL et al (2008) Acute lymphoblastic leukaemia. *Lancet* 371(9617):1030-1043
- Puri KD, Doggett TA et al (2004) Mechanisms and implications of phosphoinositide 3 kinase delta in promoting neutrophil trafficking into inflamed tissue. *Blood* 103(9):3448-3456
- Quentmeier H, Reinhardt J et al (2003) FLT3 mutations in acute myeloid leukemia cell lines. *Leukemia* 17(1):120-124
- Quentmeier H, Martelli MP et al (2005) Cell line OCI/AML3 bears exon 12 NPM gene mutation A and cytoplasmic expression of nucleophosmin. *Leukemia* 19(10):1760-1767
- Raje N, Kumar S et al (2004) Combination of the mTOR inhibitor rapamycin and CC 5013 has synergistic activity in multiple myeloma. *Blood* 104(13):4188-4193
- Randis TM, Puri KD et al (2008) Role of PI3Kdelta and PI3Kgamma in inflammatory arthritis and tissue localization of neutrophils. *Eur J Immunol* 38(5):1215-1224
- Renneville A, Roumier C et al (2008) Cooperating gene mutations in acute myeloid leukemia: a review of the literature. *Leukemia* 22(5):915-931
- Rizzieri DA, Feldman E et al (2008) A phase 2 clinical trial of deforolimus (AP23573, MK 8669), a novel mammalian target of rapamycin inhibitor, in patients with relapsed or refractory hematologic malignancies. *Clin Cancer Res* 14(9):2756-2762
- Rousselot P, Huguette F et al (2007) Imatinib mesylate discontinuation in patients with chronic myelogenous leukemia in complete molecular remission for more than 2 years. *Blood* 109(1):58-60
- Rudelius M, Pittaluga S et al (2006) Constitutive activation of Akt contributes to the pathogenesis and survival of mantle cell lymphoma. *Blood* 108(5):1668-1676
- San Miguel J, Harousseau JL et al (2008) Individualizing treatment of patients with myeloma in the era of novel agents. *J Clin Oncol* 26(16):2761-2766
- Sattler M, Verma S et al (2000) The BCR/ABL tyrosine kinase induces production of reactive oxygen species in hematopoietic cells. *J Biol Chem* 275(32):24273-24278
- Saudemont A, Okkenhaug K et al (2007) p110delta is required for innate immunity to transplantable lymphomas. *Biochem Soc Trans* 35(pt 2):183-185
- Saudemont A, Garçon F et al (2009) p110gamma and p110delta isoforms of phosphoinositide 3 kinase differentially regulate natural killer cell migration in health and disease. *Proc Natl Acad Sci USA* 106(14):5795-5800
- Savitskiy VP, Shman TV et al (2003) Comparative measurement of spontaneous apoptosis in pediatric acute leukemia by different techniques. *Cytometry B Clin Cytom* 56(1):16-22
- Schiffer CA (2008) Molecular characterization of AML: a significant advance or just another prognostic factor? *Best Pract Res Clin Haematol* 21(4):621-628
- Shi Y, Gera J et al (2002) Enhanced sensitivity of multiple myeloma cells containing PTEN mutations to CCI 779. *Cancer Res* 62(17):5027-5034
- Shi Y, Yan H et al (2005) Mammalian target of rapamycin inhibitors activate the AKT kinase in multiple myeloma cells by up regulating the insulin like growth factor receptor/insulin receptor substrate 1/phosphatidylinositol 3 kinase cascade. *Mol Cancer Ther* 4(10):1533-1540
- Shimada Y, Maeda M et al (2003) Cell culture in esophageal squamous cell carcinoma and the association with molecular markers. *Clin Cancer Res* 9(1):243-249
- Sillaber C, Mayerhofer M et al (2008) Evaluation of antileukaemic effects of rapamycin in patients with imatinib resistant chronic myeloid leukaemia. *Eur J Clin Invest* 38(1):43-52

- Silva A, Yunes JA et al (2008) PTEN posttranslational inactivation and hyperactivation of the PI3K/Akt pathway sustain primary T cell leukemia viability. *J Clin Invest* 118(11):3762-3774
- Skorski T, Bellacosa A et al (1997) Transformation of hematopoietic cells by BCR/ABL requires activation of a PI 3k/Akt dependent pathway. *EMBO J* 16(20):6151-6161
- Slupianek A, Nieborowska Skorska M et al (2001) Role of phosphatidylinositol 3 kinase Akt pathway in nucleophosmin/anaplastic lymphoma kinase mediated lymphomagenesis. *Cancer Res* 61(5):2194-2199
- Stockmann C, Doedens A et al (2008) Deletion of vascular endothelial growth factor in myeloid cells accelerates tumorigenesis. *Nature* 456(7223):814-818
- Sujobert P, Bardet V et al (2005) Essential role for the p110delta isoform in phosphoinositide 3 kinase activation and cell proliferation in acute myeloid leukemia. *Blood* 106(3):1063-1066
- Tamburini J, Elie C et al (2007) Constitutive phosphoinositide 3 kinase/Akt activation represents a favorable prognostic factor in de novo acute myelogenous leukemia patients. *Blood* 110(3):1025-1028
- Taussig DC, Miraki Moud F et al (2008) Anti CD38 antibody mediated clearance of human repopulating cells masks the heterogeneity of leukemia initiating cells. *Blood* 112(3):568-575
- Tazzari PL, Tabellini G et al (2007) The insulin like growth factor I receptor kinase inhibitor NVP AEW541 induces apoptosis in acute myeloid leukemia cells exhibiting autocrine insulin like growth factor I secretion. *Leukemia* 21(5):886-896
- Teachey DT, Obzut DA et al (2006) The mTOR inhibitor CCI 779 induces apoptosis and inhibits growth in preclinical models of primary adult human ALL. *Blood* 107(3):1149-1155
- Teachey DT, Sheen C et al (2008) mTOR inhibitors are synergistic with methotrexate: an effective combination to treat acute lymphoblastic leukemia. *Blood* 112(5):2020-2023
- Teachey DT, Grupp SA et al (2009) Mammalian target of rapamycin inhibitors and their potential role in therapy in leukaemia and other haematological malignancies. *Br J Haematol* 145(5):569-580
- Thompson JE, Conlon JP et al (2007) Enhanced growth of myelodysplastic colonies in hypoxic conditions. *Exp Hematol* 35(1):21-31
- Ticchioni M, Essafi M et al (2007) Homeostatic chemokines increase survival of B chronic lymphocytic leukemia cells through inactivation of transcription factor FOXO3a. *Oncogene* 26(50):7081-7091
- Touw I, Pouwels K et al (1990) Interleukin 7 is a growth factor of precursor B and T acute lymphoblastic leukemia. *Blood* 75(11):2097-2101
- Uddin S, Hussain AR et al (2006) Role of phosphatidylinositol 3' kinase/AKT pathway in diffuse large B cell lymphoma survival. *Blood* 108(13):4178-4186
- Virgilio L, Narducci MG et al (1994) Identification of the TCL1 gene involved in T cell malignancies. *Proc Natl Acad Sci USA* 91(26):12530-12534
- Wee S, Jagani Z et al (2009) PI3K pathway activation mediates resistance to MEK inhibitors in KRAS mutant cancers. *Cancer Res* 69(10):4286-4293
- Weng AP, Ferrando AA et al (2004) Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science* 306(5694):269-271
- Xu Q, Simpson SE et al (2003) Survival of acute myeloid leukemia cells requires PI3 kinase activation. *Blood* 102(3):972-980
- Yazbeck VY, Buglio D et al (2008) Temsirolimus downregulates p21 without altering cyclin D1 expression and induces autophagy and synergizes with vorinostat in mantle cell lymphoma. *Exp Hematol* 36(4):443-450
- Yee KW, Zeng Z et al (2006) Phase I/II study of the mammalian target of rapamycin inhibitor everolimus (RAD001) in patients with relapsed or refractory hematologic malignancies. *Clin Cancer Res* 12(17):5165-5173
- Yilmaz OH, Valdez R et al (2006) Pten dependence distinguishes haematopoietic stem cells from leukaemia initiating cells. *Nature* 441(7092):475-482
- Young DC, Griffin JD (1986) Autocrine secretion of GM-CSF in acute myeloblastic leukemia. *Blood* 68(5):1178-1181

- Zebedin E, Simma O et al (2008) Leukemic challenge unmasks a requirement for PI3Kdelta in NK cell mediated tumor surveillance. *Blood* 112(12):4655-4664
- Zenz T, Dohner K et al (2008) Chronic lymphocytic leukaemia and acute myeloid leukaemia are not associated with AKT1 pleckstrin homology domain (E17K) mutations. *Br J Haematol* 141(5):742-743
- Zhang J, Choi Y et al (2003) Preferential killing of PTEN null myelomas by PI3K inhibitors through Akt pathway. *Oncogene* 22(40):6289-6295
- Zhang J, Grindley JC et al (2006) PTEN maintains haematopoietic stem cells and acts in lineage choice and leukaemia prevention. *Nature* 441(7092):518-522
- Zollinger A, Stuhmer T et al (2008) Combined functional and molecular analysis of tumor cell signaling defines 2 distinct myeloma subgroups: Akt dependent and Akt independent multiple myeloma. *Blood* 112(8):3403-3411

Clinical Development of Phosphatidylinositol-3 Kinase Pathway Inhibitors

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Abstract The PI3K pathway is the most commonly altered in human cancer. Several recent phase I studies with therapeutic inhibitors of this pathway have shown that pharmacological inhibition of PI3K in humans is feasible and overall well tolerated. Furthermore, there has already been clinical evidence of anti-tumor activity in patients with advanced cancer. The intensity and duration of PI3K inhibition required for an antitumor effect and the optimal pharmacodynamic biomarker(s) of pathway inactivation remain to be established. Preclinical and early clinical data support focusing on trials with PI3K inhibitors that are at a minimum enriched with patients with alterations in this signaling pathway. These inhibitors are likely to be more effective in combination with established and other novel molecular therapies.

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1 Introduction

Abundant evidence indicate that the phosphatidylinositol-3 kinase (PI3K) signaling pathway is arguably the most commonly altered in human cancers (reviewed in chapters in this book). First, the p110 α catalytic subunit of PI3K is activated by mutation at a high frequency in multiple human tumors (Samuels et al. 2004). A recent review reported an overall frequency of mutations in the *PIK3CA* gene, which encodes p110 α , of 15% across all cancer types (Karakas et al. 2006). Second, the phosphatase PTEN (phosphatase and tensin homologue deleted in chromosome 10), which antagonizes PI3K signaling by dephosphorylating the second messenger phosphatidylinositol-3,4,5 trisphosphate (PIP3), is a tumor suppressor gene frequently inactivated by mutation, gene deletion, targeting by micro-RNA, and promoter methylation (Keniry and Parsons 2008; Salmena et al. 2008). Further, PI3K is potently activated by oncogenes such as mutant Ras (REF) and many tyrosine kinases that potently activate PI3K, such as Bcr-Abl, HER2 (ErbB2), MET, KIT, etc., which themselves are the target of mutational activation and/or gene amplification (Engelman et al. 2006). The serine/threonine kinase Akt is a key downstream effector of PI3K signaling output. Following growth factor-induced stimulation of PI3K, Akt is recruited to the plasma membrane where it is phosphorylated by PDK-1 in Thr308 and by TORC2 in Ser473 (Manning and Cantley 2007), respectively, resulting in its full enzymatic activation. Several human tumors, such as ovarian, pancreatic, breast, and gastric cancer, harbor Akt1 or Akt2 gene amplification. A transforming mutation in the pleckstrin homology (PH) domain of Akt1 (E17K), which results in its constitutive localization at the plasma membrane and activation, is present in a small percentage of breast, colorectal, and ovarian cancers (Carpten et al. 2007). Other components of the pathway, such as PDK-1, PIK3R1, PIK3CB, and P70S6K, are found to be amplified in human cancers (Thomas et al. 2007). All these abnormalities together identify a large repertoire of tumors with molecular alterations in the PI3K network that are potentially targetable with specific pathway inhibitors.

At this time, there is significant clinical research addressing the role of inhibition of the PI3K pathway in human cancers. In this chapter, I will review the current status of clinical investigation in this field with different types of antagonists of the PI3K network, mechanistic and preclinical considerations that are of relevance to clinical development, the rationale for combinatorial therapies that will include inhibitors of the PI3K pathway, and finally propose some clinical trial designs that may streamline the pathway to FDA approval for PI3K-targeted agents.

2 Pharmacological Approaches

Several types of compounds to block multiple levels in the PI3K signaling network have been designed and are in variable stages of clinical development. The first group comprises inhibitors of class IA PI3K isoforms. These enzymes are

heterodimeric lipid kinases that consist of a p110 catalytic subunit and a regulatory subunit, which mediates the receptor or adaptor binding, activation, and localization of the PI3K dimer. There are three genes, *PIK3CA*, *PIK3CB*, and *PIK3CD*, which encode the highly homologous p110 catalytic isoforms, p110 α , p110 β , and p110 δ , respectively (Cantley 2002; Engelman et al. 2006). The expression of p110 δ is largely restricted to immune and hematopoietic cells whereas p110 α and p110 β are expressed ubiquitously (Vanhaesebroeck et al. 1997). p110 α is essential for signaling and growth of tumors driven by *PIK3CA* mutations and/or oncogenic tyrosine kinases or mutant RAS, whereas p110 β responds to G protein-coupled receptors (GPCRs) and is the main isoform mediating tumorigenesis in PTEN-deficient cells [reviewed in (Jia et al. 2009)].

A number of pan-specific or isoform-specific PI3K antagonists have entered phase I clinical development and have the subject of several recent reviews (Garcia-Echeverria and Sellers 2008; Maira et al. 2008b). These include NVP-BEZ235, NVP-BGT226, GDC-0941, XL-765, XL-147, SF1126, CAL-101, and GSK1059615. These compounds are ATP-mimetics that bind competitively and reversibly in the ATP-binding pocket of kinase domain in p110. With the exception of CAL-101, which specifically inhibits the p110 δ kinase, the other small molecules are active against all p110 isoforms including oncogenic mutant forms of p110 α (Folkes et al. 2008; Garlich et al. 2008; Maira et al. 2008a). Some of these also have inhibitory activity against phosphatidylinositol-3 kinase-related kinases (PIKKs), such as the mTOR serine/threonine kinase (i.e., NVP-BEZ235, NVP-BGT226, XL-765, and SF1126).

Following the p110 antagonists are inhibitors of Akt isoforms. These compounds have shown antitumor activity against human xenografts and have been reviewed recently (Garcia-Echeverria and Sellers 2008). A-443654 and GSK690693 are ATP-competitive pan-Akt kinase inhibitors. They have shown antitumor activity in preclinical models and have recently entered phase I trials (Davies et al. 2007; Rhodes et al. 2008). Allosteric inhibitors of Akt that interact with its PH domain and/or hinge region thus promoting an inactive conformation of the enzyme, are also in development (Toral-Barza et al. 2007). MK-2206 is a highly selective non-ATP-competitive, allosteric inhibitor of Akt1, Akt2, and Akt3. This compound effectively inhibited the Akt kinase and its downstream effectors in vivo and caused marked suppression of growth of breast cancer xenografts with PI3K mutations and HER2 gene amplification (She et al. 2008). Early phase I clinical data in patients with advanced solid tumors have shown inhibition of P-Akt in peripheral blood mononuclear cells and good tolerability (Tolcher et al. 2009). Because of the high sequence identity among the kinase domain of Akt1, Akt2, and Akt3, it is anticipated that the development of potent isoform-selective modulators will be difficult.

A third group of compounds designed to interrupt the PI3K pathway are inhibitors of the mTOR (mammalian target of rapamycin) serine/threonine kinase (Fasolo and Sessa 2008). This kinase regulates protein translation and functions within two multiprotein complexes which share mTOR itself: TORC1 associated with RAPTOR and TORC2 associated with RICTOR (Guertin and Sabatini 2007). Rapamycin

and its analogs (see below) preferentially target TORC1. mTOR is an important component of PI3K-driven oncogenesis at different levels. TORC1 regulates protein translation and is downstream and positively modulated by Akt. On the other hand, TORC2 functions upstream where it phosphorylates and activates the Akt kinase (Sarbasov et al. 2005). The macrolide rapamycin inhibits mTOR by forming a complex with the FK506-binding protein (FKBP12), which binds to a region in the C-terminus of mTOR termed FRB (FKBP12 rapamycin-binding). The formation of this complex interferes with the kinase activity of the TORC1 but not the TORC2 complex (Sarbasov et al. 2004). The limited pharmacological properties of rapamycin prompted the development of analogs (so called “rapalogs”) such as CCI-779 (temsirolimus), RAD001 (everolimus), and AP-23573 (deferolimus). These rapalogs have already shown cytostatic activity in preclinical models and clinical trials particularly in patients with renal cell cancer and patients with mutations in TSC who harbor renal angioliopomas. Compounds that target the ATP-binding cleft of mTOR (i.e., OSI-027 and AZD8055) and are thus active against both TORC1 and TORC2 have recently entered phase I clinical trials (Fasolo and Sessa 2008).

3 Preclinical Considerations for Drug Development

The somatic DNA alterations identified above (i.e., *PIK3CA* and *AKT1* activating mutations, PTEN deletion, PI3K-activating oncogene amplification) potentially mark tumor types as well as individual cancers with aberrant activation of the PI3K pathway. This is an important consideration for the purpose of selection of patients into trials with PI3K inhibitors. In the past decade, a number of examples have shown that mutations in somatic DNA identify gene products or pathways that are critical for tumor survival and progression and that, therefore, when interrupted by pharmacological means result in a clinically important antitumor effect. Examples include the effect of imatinib and dasatinib against Philadelphia chromosome-positive chronic myelogenous leukemia (CML) harboring the *BCR-ABL* oncogene, the EGF receptor tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib against tumors with *EGFR* gene activating mutations, the anti-HER2 antibody trastuzumab and the HER2 TKI lapatinib against breast cancers with *HER2* gene amplification, and, more recently, small molecule Raf inhibitors against metastatic melanomas containing *B-RAF* activating mutations [reviewed in (Stuart and Sellers 2009)].

A number of preclinical tumor models including transgenic mice bearing cancers engineered to lack PTEN or overexpress *PIK3CA* activating mutations have already shown tumor dependence on PI3K in that administration of pharmacological inhibitors of PI3K resulted in an antitumor effect (Eichhorn et al. 2008; Engelman et al. 2008). However, in several phase I clinical trials with PI3K pathway inhibitors in progress, there have been no reports yet of major tumor reductions in patients treated with such compounds. Two previous reports using

cancer cell lines with PTEN deletions suggested that PTEN-deficient cancers would be highly sensitive to mTOR inhibitors (Neshat et al. 2001; Podsypanina et al. 2001). Again, despite the extensive clinical use of “rapalogs” and the relative frequency of PTEN loss in cancers at large, significant clinical responses to mTOR inhibitors have not been observed. Thus, although it might still be early, the dramatic clinical responses that were observed during the early clinical development of other now approved molecule-targeted inhibitors have not yet been observed with therapeutic antagonists of the PI3K pathway.

The potential dependence of some cancers over that of normal host tissues on an oncogenic pathway suggests that the possibility of a “therapeutic window” that can be exploited in the drug development process. This would allow delivery of an oncogene-directed therapy at an optimal biological dose (OBD) that would inhibit its molecular target and exert a biological effect on the tumor. This dose would be less than a maximally tolerated dose (MTD) of the inhibitor which would likely induce toxicity against normal host tissues. Imatinib and trastuzumab are examples of molecule-targeted therapies where such therapeutic window was present. Because of the role of PI3K in normal physiological processes, it is not clear whether therapy-induced toxicities will be entirely avoidable. One special concern with these therapies is the induction of insulin resistance. Under normal physiological conditions, the PI3K pathway, predominantly p110 α and less so p110 β , mediates insulin action (Foukas et al. 2006; Knight et al. 2006). Therefore, PI3K antagonists are likely to perturb glucose homeostasis and/or aggravate states of insulin resistance. Preclinical data with Akt inhibitors have already shown the induction of hyperglycemia in experimental mice (Crouthamel et al. 2009; Rhodes et al. 2008). Interestingly, mice treated with NVP-BEZ235 did not exhibit significant changes in blood glucose levels (Maira et al. 2008a). In any case, an important question in the clinical development of PI3K inhibitors is whether clinical efficacy and tolerability can be achieved without the induction of insulin resistance.

Genetically engineered mice lacking p110 α exhibit defective endothelial cell migration during vascular development (Graupera et al. 2008). Consistent with this, mice lacking PI3K regulatory subunits (p85 α , p85 β , p55 α , and p50 α) also exhibit localized vascular abnormalities (Yuan et al. 2008). Interestingly, mice expressing a p110 α mutant allele incapable of interacting with endogenous Ras display defective VEGF-C signaling to PI3K in lymphatic endothelial cells and impaired development of the lymphatic vasculature (Gupta et al. 2007). Consistent with these results, PI3K inhibitors have been shown to inhibit tumor blood vessels when administered to mice bearing human xenografts (Garlich et al. 2008; Schnell et al. 2008). These data suggest that in addition to tumor cell-autonomous effects, PI3K inhibitors could exert an additional antimetastatic effect by blocking angiogenesis and lymphangiogenesis. They also suggest that the possibility of side effects (i.e., bleeding, defective wound healing, etc.) as a result of impairment of endothelial cell function.

It has been shown that genes encoding most glycolytic enzymes are under dominant transcriptional control by Akt activation (Majumder et al. 2004). Thus, a rapid downregulation of [18F]-fluorodeoxy-D-glucose positron emission

tomography (FDG-PET) intensity might be a reliable surrogate marker of inactivation of the PI3K/Akt pathway that can be used as a noninvasive approach to predict the outcome of therapy. This also implies that tumors that are FDG-PET negative contain low glycolytic activity and, thus, are not ideal candidates for therapy with PI3K inhibitors. At this time, FDG-PET is being widely used as a pharmacodynamic biomarker of drug action in investigational trials with inhibitors of PI3K.

4 Clinical Trials

At this time, several PI3K pathway inhibitors are in phase I clinical development. This phase of the clinical development process is aimed at defining the effective dose of these compounds as well as their tolerability and toxicity profile. Preliminary results have been communicated for phase I trials with XL-147, XL-765, GDC-0941, PX-866, and CAL-101 in patients with solid tumors and hematological neoplasias (Flinn et al. 2009; Jimeno et al. 2009; LoRusso et al. 2009; Shapiro et al. 2009; Wagner et al. 2009). Overall, these compounds seem to be well tolerated with modest grade 3 and grade 4 toxicity. Main side effects have been nausea, vomiting, diarrhea, anorexia, fatigue, and rash with minimal hyperglycemia. Dose escalations are still proceeding, although pharmacodynamic evidence of drug action in skin and hair follicles has already been reported. This has been assessed by measuring levels of T308 P-Akt, S473 P-Akt, T246 P-PRAS40, T70 P-4EBP1, and S240/244 P-S6 by immunohistochemistry (IHC) using site specific antibodies in tissue sections obtained on days 21–28 after initiation of treatment.

There is significantly more clinical experience with the mTOR inhibitors temsirolimus (CCI-779), everolimus (RAD001), and deferolimus (AP23573). These drugs exhibit a comparable toxicity profile, spectrum of antitumor activity, pharmacokinetic features, and profile of biomarkers they inhibit *in situ* [recently reviewed in (Fasolo and Sessa 2008)]. Main side effects include mucositis, rash, fatigue, neutropenia, anorexia, edema, hyperglycemia, and gastrointestinal toxicities. These three compounds inhibit mainly TORC1. The TORC1 complex activates S6K which, in turn, inhibits IRS-1 through phosphorylation in Ser102 (Harrington et al. 2004). Consistent with this, in a recent paper, O'Reilly et al. demonstrated feedback activation of Akt following pharmacological inhibition of TORC1 in patients with breast cancer treated with everolimus (O'Reilly et al. 2006).

A recent phase III trial compared single-agent temsirolimus vs. interferon vs. the combination in 626 patients with poor-prognosis metastatic renal cell carcinoma. Patients receiving temsirolimus alone achieved a significantly longer overall survival (OS) and progression-free survival (PFS) than patients treated with interferon alone. In the group treated with the combination, the OS was comparable of that exhibited by patients in the single-agent interferon arm. Rash, peripheral edema, anemia, dyspnea, diarrhea, hyperglycemia, and hyperlipidemia were more common in patients treated with the mTOR inhibitor whereas asthenia was more common in the interferon group. Grade 3 and grade 4 toxicities were more common in the

combination group, resulting in more delays and reductions in the dose of temsirolimus potentially explaining the lack of advantage of the combination over interferon alone. Median OS in the interferon, temsirolimus, and combination therapy groups was 7.3, 10.9, and 8.4 months, respectively (Hudes et al. 2007). Based on these results, temsirolimus was approved by the FDA for the initial treatment of patients with advanced poor-prognosis renal cell cancer.

A double-blind, multicenter phase III trial in patients with renal cell cancer who have progressed on primary therapy for metastatic disease was recently completed (Motzer et al. 2008). In this study, 400 patients were randomized to everolimus 10 mg/day vs. placebo, both with the best supportive care. Everolimus produced a significant extension in PFS of 4 vs. 1.9 months, with an overall favorable safety profile. Stomatitis, anemia, and asthenia were the most common grade 3 and grade 4 toxicities (Motzer et al. 2008). Finally, Baselga et al. (2009) just reported the results of a neoadjuvant randomized phase II study of the aromatase inhibitor letrozole vs. letrozole plus everolimus in postmenopausal patients with newly diagnosed ER-positive breast cancer. Clinical response rate and inhibition of tumor cell proliferation as measured by Ki67 IHC were higher in the combination arm compared to the group treated with single-agent letrozole (Baselga et al. 2009).

Promising clinical activity in single-arm phase II studies with temsirolimus and everolimus has been reported in endometrial cancer and relapsed mantle cell lymphoma (Oza et al. 2005; Slomovitz et al. 2008; Witzig et al. 2005). Because of their ability to inhibit TORC1 and TORC2 and thus, potentially bypass feedback activation of Akt, higher single-agent clinical activity compared to everolimus, temsirolimus, and deferolimus is anticipated for AZD8055 and OSI-027. Up to now, however, the original concept that dysregulation of PI3K signaling predicts sensitivity to mTOR inhibitors has not been verified in clinical practice. In fairness though, most of these therapeutic studies have not actively explored a correlation between clinical benefit and detectable genetic alterations in the PI3K pathway by profiling a meaningful number of tumors from patients enrolled in these trials. At the time of this writing, combination studies of mTOR inhibitors with EGFR, VEGF, PI3K, and IGF-IR inhibitors are in development.

5 Patient Selection and Role of Presurgical Trials

As with other targeted therapies, it is likely that only a fraction of patients treated with PI3K inhibitors will benefit from these drugs. Because of this, there is an expectation that the clinical development of a molecule-targeted therapy will also include the deployment of a diagnostic test(s) that will identify patients that are likely to respond to and thus be offered such therapy. Examples include fluorescent in situ hybridization (FISH) and IHC for HER2 which identify patients with breast cancer for whom trastuzumab and lapatinib are approved (Press et al. 2008); and *EGFR* activating mutations which identify patients with nonsmall-cell lung cancer (NSCLC) with a high likelihood of response to EGFR TKIs (Lynch et al. 2004;

Paez et al. 2004), among others. An example of a negative predictor of response is the presence of mutant K-RAS, which identifies patients with colon cancer that do not benefit from therapy with the neutralizing EGFR antibodies panitumumab or cetuximab (Amado et al. 2008; Benvenuti et al. 2007).

There is an agreement that early therapeutic studies should be enriched with patients harboring known detectable abnormalities in the PI3K pathway. However, it is not clear whether clinical responses will be limited to these patients. Testing the possible selectivity of PI3K inhibitors against cancers with PI3K pathway alterations and/or another molecular signature in single-arm phase II trials in patients with metastatic disease is intrinsically problematic because of (1) the difficulty in obtaining biopsies from metastatic sites and (2) the limitations of assessment of tumor response as a meaningful clinical endpoint in the absence of a placebo control arm.

There are, however, examples of short-term, tissue-based pharmacodynamic novel trial designs which could provide information that can be later used for patient selection or exclusion into early trials with novel targeted therapies such as PI3K antagonists. For example, administration of antiestrogens for a period of 1–3 weeks has been shown to induce a significant antiproliferative effect, as measured by Ki67 IHC (Assersohn et al. 2003), in ER-positive but not ER-negative breast cancers (DeFriend et al. 1994; Dowsett et al. 2000, 2001). Treatment-induced tumor cell apoptosis, as measured by cleaved caspase-3 IHC 1 week after administration of single-agent trastuzumab correlated with clinical response of HER2-overexpressing breast cancers to trastuzumab plus chemotherapy (Mohsin et al. 2005). The neoadjuvant IMPACT trial compared the aromatase inhibitor anastrozole vs. tamoxifen vs. the combination of both drugs. Drug-induced inhibition of cancer cell proliferation *in situ* as measured by Ki67 IHC in a tumor biopsy obtained after 2 weeks of therapy was better in anastrozole-treated patients compared to patients in the other two arms (Dowsett et al. 2005). Interestingly, this change in proliferation (Ki67) after only 2 weeks of therapy mirrors the results of the adjuvant ATAC trial where >9,000 patients with ER + tumors were randomized to the same three arms as in the IMPACT study following surgical resection of the primary tumor. In this large study, relapse-free survival was also better in patients treated with anastrozole compared to the other two treatment arms (Howell et al. 2005). In terms of PI3K pathway-targeted drugs, Cloughesy and colleagues demonstrated a dramatic effect of rapamycin on the Ki67 index in a group of patients with recurrent glioblastoma. Tumors were surgically-resected after 7 days of therapy with the mTOR inhibitor. Interestingly, the reduction in Ki67 after short-term rapamycin was limited to PTEN-deficient tumors and correlated with an improved PFS in patients treated with the mTOR inhibitor following surgery (Cloughesy et al. 2008).

The above mentioned examples suggest that the use of presurgical nontherapeutic trials with PI3K pathway inhibitors to ensure that critical endpoints in their clinical development are met. For example, after a safe dose of the inhibitor has been defined in a conventional phase I study, patients with *operable* breast cancer (or another tumor known to exhibit PI3K alterations where this approach is ethical and feasible) that are not candidates for neoadjuvant therapy can be treated with the inhibitor for

2 weeks, which is likely a period of time adequate for the drug to achieve steady-state levels in plasma. Effects on cell proliferation (Ki67), apoptosis (TUNEL, cleaved caspase-3 IHC), and inhibition of the drug target in situ (i.e., with P-Akt, P-PRAS40, P-S6, etc. antibodies) can be easily assessed in formalin-fixed tumor cores from the surgical specimen. A gene expression signature indicative to kinase inactivation can be generated from fixed or frozen tumor material that is not further required for clinical purposes. Evidence of inhibition of the molecular target of the inhibitor will validate the therapeutic dose selected by the early drug development (phase I) process. Lack of inhibition of the target in situ would suggest that the drug is not reaching its target despite adequate drug levels or another pharmacological limitation. This possibility can then be studied by measuring drug levels in tumor homogenates. Addressing these questions would be critically important before engaging in larger and (potentially) uninformative efficacy trials. Evidence of inhibition of cell proliferation (Ki67) and/or induction of apoptosis (TUNEL, etc.) can be correlated with *PIK3CA* or *AKT1* mutations, *PTEN* deletion, etc. as well as other routine clinical markers, such as ER, PR, and HER2 levels in the case of breast cancer, to determine if the drug has or has not activity against an obvious cancer subtype. In turn, this can potentially identify cancer subtypes in which the clinical development should be focused and/or subtypes that can be enriched for in early phase II studies. A flow diagram of this presurgical approach using Ki67, pathway activation markers, and FDG-PET for the testing of novel PI3K inhibitors during the preapproval process of clinical development is shown below in Fig. 1.

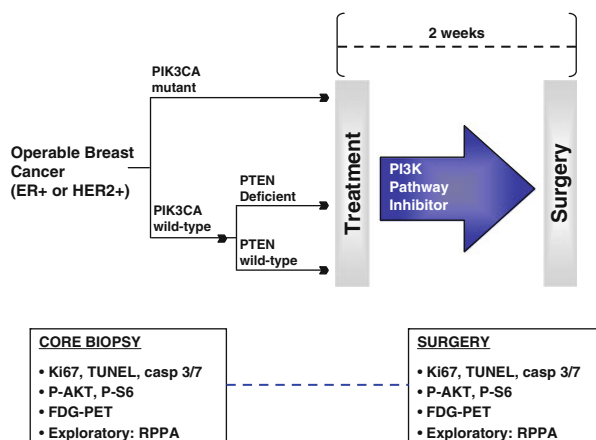


Fig. 1 Diagram of presurgical clinical trial with PI3K pathway inhibitor(s). Each of these three groups of patients with newly diagnosed operable breast cancer (*PIK3CA* mutant, *PIK3CA* wild type/*PTEN* mutant, and *PIK3CA* wild type/*PTEN* wild type) will be treated with the PI3K pathway inhibitor for 2 weeks until the day before surgical resection of the primary tumor. All patients/tumors will be evaluated with the indicated immunohistochemical markers and FDG PET at the start of the study and at the completion of therapy (in tumor sections from the surgical specimen and the day of surgery, respectively). An estimate of 90 patients, 30 per arm, will be required to achieve the study endpoints

6 Rationale for Combination Therapies

The PI3K pathway is highly interconnected with multiple negative feedback loops and with complex cross-talk with other signaling networks. The redundancy with the MAPK pathway and with the LKB1/AMPK energy-sensing pathway has been reviewed in chapters in this book. Much of this network is conserved back to flies and worms and this cross-talk and negative autoregulation has apparently evolved to ensure homeostatic control of cell growth in response to mitogenic factors, and to prevent inappropriate growth under conditions of energy stress. The mutations that involve the PI3K network in human cancers invariably circumvent one or more of the negative feedback pathways that provide homeostatic control to the network (Shaw and Cantley 2006). Nonetheless, interruption of single nodes within the PI3K network can suppress this negative feedback autoregulation and endow tumor cells with compensatory molecular signals that counteract drug action. Moreover, the prior experience with other molecule-targeted drugs (Arteaga 2007) strongly suggest that, even in patients who initially respond to these drugs, single-agent PI3K inhibitors will be insufficient to cure patients with advanced disease.

The existence of a TORC1-PI3K/Akt negative feedback loop has been well documented in studies with cells in culture (reviewed in chapter). Recently, however, two clinical studies elegantly documented that pharmacological inhibition of TORC1 (with rapamycin or everolimus) led to Akt activation as measured by tumor levels of Ser473 P-Akt in patients with breast cancer and glioblastoma (Cloughesy et al. 2008; O'Reilly et al. 2006). These findings have important therapeutic implications as they imply that the limited efficacy of TORC1 inhibitors might be due to their intrinsic capacity to abrogate this negative feedback to Akt. Indeed, in the study by O'Reilly et al., inhibition of TORC1 with everolimus led to insulin-like growth factor (IGF)-I receptor/IRS-1-dependent activation of Akt. IGF-IR inhibition with small molecule TKIs prevented RAD001-induced Akt phosphorylation and sensitized tumor cells to the TORC1 inhibitor (O'Reilly et al. 2006). Based, in part, on these data, at this time, clinical trials testing combinations of mTOR inhibitors with neutralizing IGF-IR monoclonal antibodies are in progress.

In another relevant example, inhibition of TORC1 with rapalogs in primary breast tumors and in xenografts induced a dose-dependent increase in MAPK activation which was dependent on an S6K-PI3K-RAS pathway (Carracedo et al. 2008). Supporting the notion that this compensation limits the therapeutic inhibition of a single pathway, the combined inhibition of mTOR and MEK has shown synergistic activity against several cancer xenografts (Carracedo et al. 2008; Kinkade et al. 2008; Legrier et al. 2007). Therefore, although PI3K inhibitors have not yet been shown to induce upregulation of MEK (or an upstream activator of MEK), it is not unreasonable to expect they will do so in cells where PI3K inhibitors downregulate TORC1 activity downstream. Based in part on these data, combinations of TORC1/TORC2 inhibitors with MEK inhibitors and Akt inhibitors

with MEK inhibitors are under early planning. Furthermore, since activation of mTOR downregulates PDGF receptor signaling (Zhang et al. 2007), it is likely that inhibition of mTOR will also lead to PDGFR activation in some cancers. In tumors where this receptor is overexpressed, this response would limit the action of mTOR inhibitors and potentially inform the use of novel therapeutic combinations aimed at blocking such compensatory response.

Two papers have recently shown that inhibition of MEK with a small molecule inhibitor, although partially effective, leads to feedback upregulation of PI3K/Akt in human breast cancer cells with a basal-like gene expression signature (Hoefflich et al. 2009; Mirzoeva et al. 2009). This compensatory response upon therapeutic inhibition of MEK was enhanced in cells lacking PTEN (Hoefflich et al. 2009). Further, studies with human cancer cell lines and transgenic tumors that harbor both PI3K pathway and Ras mutations do not respond to PI3K inhibitors (Engelman et al. 2008; Ihle et al. 2009). One example of therapeutic synergy conferred by the addition of a PI3K pathway inhibitor to a MEK inhibitor was recently reported by Engelman et al. Transgenic mice harboring lung cancers driven by mutant K-RAS did not respond to the MEK inhibitor ARRY-142886 or to the PI3K/mTORC inhibitor NVP-BEZ235 when given alone. However, the combination was markedly synergistic in inducing tumor shrinkage (Engelman et al. 2008). This combined approach may be applicable to other tumors if we consider recent studies showing that cancers with mutant p110 α often possess mutations or alterations in other components of the PI3K pathway, such as Ras, HER2 (ErbB2), and PTEN (Oda et al. 2008; Perez-Tenorio et al. 2007; Stemke-Hale et al. 2008). In any case, these data suggest that basal-like breast cancers and NSCLC with K-Ras mutations are tumor types where combinations of PI3K and MEK inhibitors are worthy of clinical testing.

Aberrant PI3K activity has also been associated with resistance to multiple drugs, thus suggesting a role for PI3K pathway inhibitors with other established primary therapies. For example, presence of *PIK3CA* mutations and loss of PTEN in HER2-overexpressing cancers correlates with a lower response to the HER2 antibody trastuzumab (Berns et al. 2007; Nagata et al. 2004) and the HER2 TKI lapatinib (Eichhorn et al. 2008). Overexpression of constitutively active Akt renders HER2-overexpressing breast cancer cells insensitive to trastuzumab (Yakes et al. 2002). Treatment with the p110/TORC1 inhibitors NVP-BEZ235 or GDC-0941 has been shown to restore the action of trastuzumab and lapatinib against HER2-overexpressing cells and xenografts that also harbor PTEN loss or *PIK3CA* activating mutations (Eichhorn et al. 2008; Junttila et al. 2009; Serra et al. 2008). EGFR TKIs are ineffective in high-grade gliomas that lack PTEN expression (Mellinghoff et al. 2005). Restoration of PTEN expression into *PTEN* mutant cancer cells sensitizes them to EGFR inhibitors (Bianco et al. 2003; She et al. 2003) and downregulation of PTEN using shRNAs dampens the apoptotic effect of EGFR TKIs against receptor-dependent tumor cells (She et al. 2003; Wang et al. 2006). Recently, *MET* gene amplification was shown to engage HER3 in order to activate PI3K/Akt and induce acquired resistance to gefitinib in lung cancer cells and primary NSCLC (Bean et al. 2007; Engelman et al.

2007). These data suggest that inhibitors of the PI3K pathway, currently in clinical development, can be used to potentially reverse acquired and de novo drug resistance.

7 Neoadjuvant Clinical Trials

Amplification of PI3K signaling has also been associated with resistance to endocrine therapy in breast cancer (Perez-Tenorio and Stal 2002; Tokunaga et al. 2006). Breast cancer cells with upregulated Akt signaling exhibit resistance to antiestrogens which can be abrogated by cotreatment with everolimus and other mTOR inhibitors (Beeram et al. 2007; Boulay et al. 2005; deGraffenried et al. 2004). Based on these data, Baselga et al. conducted an exploratory randomized phase II study of the aromatase inhibitor letrozole vs. letrozole plus everolimus administered over a 4-month period to 270 postmenopausal women with operable ER-positive breast cancer (Baselga et al. 2009). The primary endpoint was clinical response by palpation. Mandatory biopsies were obtained at baseline and after 2 weeks (day 15) of treatment. Specimens were assessed for presence of exon 9 (E545K, E542K) and exon 20 (H1047R) PIK3CA mutations, and for pharmacodynamic changes in Ki67, P-S6, P-Akt, cyclin D1, and progesterone receptor (PgR) by IHC. Response rate as assessed by clinical palpation was statistically higher in the everolimus-containing arm vs. single-agent letrozole. Consistent with target inhibition, a marked downregulation of P-S6 levels occurred only in the day 15 biopsy in patients receiving everolimus. A significant reduction in tumor cell proliferation as measured by Ki67 IHC was observed in 57% of patients in the everolimus arm vs. 30% of patients in the letrozole alone arm ($p < 0.01$) (Baselga et al. 2009). The results of this trial have important implications that could not have been arrived to in the absence of this elegant design. First, because of the better response rate to the combination, this result provides a signal that the combination should be explored further. Second, they suggest that early pharmacodynamic biomarkers (Ki67, noninvasive imaging) might identify tumors that benefit from the combination vs. not. Finally, this approach ensures the access to abundant tumor tissue in a large proportion of patients (since all are operated) where unbiased molecular profiling aimed at identifying a signature of response or lack thereof can be investigated.

The neoadjuvant trial described above illustrates a clinical platform that can be utilized in breast and other cancers for testing of feasibility and identifying early signals for “go-no go” decisions to pursue combinations of PI3K inhibitors with the current standards of care (i.e., chemotherapy, endocrine therapy, other targeted agents). Obviously, these would have to be done after safety of the combinations has been documented in traditional phase I studies. A diagram of such generic approach in breast cancer is shown in Fig. 2 but can be modified to other tumor types where neoadjuvant therapy is used. Patients are randomized to the “standard” therapy with or without the PI3K pathway inhibitor. A “research” biopsy can be obtained after 2 weeks in order to document effects on tumor cell proliferation/

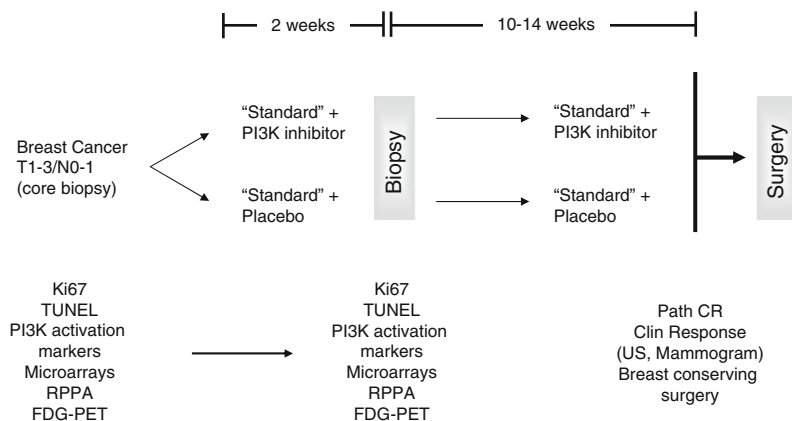


Fig. 2 Schema of neoadjuvant clinical trial with PI3K pathway inhibitor. Patients with breast cancers requiring neoadjuvant therapy prior to breast conserving surgery are randomized to the “standard” therapy \pm the PI3K pathway inhibitor. Formalin fixed and flash frozen core biopsies are obtained after 2 weeks of therapy in order to document effects on tumor cell proliferation and/or apoptosis as well as pathway inactivation (i.e., downregulation of P Akt, P S6, P PRAS40, etc., by IHC). Incorporation of noninvasive FDG PET at 2 weeks could identify early metabolic changes (or lack thereof). Clinical response can be evaluated after approximately 4 months of therapy by measuring the tumor with calipers, ultrasound, and/or mammography. Absence of tumor in the surgical specimen would be scored as a path CR. Rate of breast conserving surgery is another endpoint that can be compared between both arms. No difference in terms of clinical and/or pathological response in favor of the “standard” therapy plus PI3K inhibitor arm would indicate the clinical development of the combination is not a priority

apoptosis as well as pathway inactivation. Incorporation of noninvasive FDG-PET could identify early metabolic changes as a function of PI3K/Akt inhibition (or lack thereof). Clinical and pathological complete response can be evaluated after approximately 4 months of therapy. As designed, this approach asks three questions: (1) is there a difference in the cellular and molecular response between the two treatment arms during the first 2 weeks? (2) is clinical and/or pathological complete response statistically better in the arm containing the PI3K pathway inhibitor, and (3) is there a tissue and/or noninvasive imaging pharmacodynamic biomarker in the pretherapy, the 2-week, and/or the surgical specimen that correlates with response or lack of response to the combination? A difference in favor of the combination of the “standard” therapy plus the PI3K inhibitor would support the further development of the combination.

8 Conclusions

The introduction of antagonists of the PI3K signaling pathway as a therapeutic anticancer strategy is still at a relatively early stage of development. Early clinical data, however, suggest that this strategy is clinically feasible and that these drugs, at

least as single agents, will be well tolerated. Temsirolimus, an inhibitor of one element of this pathway, TORC1, has already been approved for treatment of high risk, metastatic renal cell cancer. A significant number of unknowns that apply to the wide clinical use of these inhibitors still remain. These include pharmacodynamic tissue and/or imaging biomarkers of drug action against its target(s), mid-term and long-term toxicities associated with their use, the need or not to develop isoform-specific p110 and Akt inhibitors, the combined inhibition of TORC1 and TORC2 with single agents, novel mechanisms of compensation (i.e., feedback) deployed upon therapeutic inhibition of this pathway, the development of rational combinations that will include PI3K pathway inhibitors, and perhaps more importantly, the use of an unbiased approach to determine the patients that will likely benefit from these drugs as well as the better combinatorial therapies to pursue. With the plethora of PI3K pathway inhibitors in development and the increased perception of the need to assess the effect of these drugs in tumor tissues in real time and link such assessment to clinical benefit, it is likely we will have answers to most of these questions in the next few years.

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References

- Amado RG, Wolf M, Peeters M, Van Cutsem E, Siena S, Freeman DJ, Juan T, Sikorski R, Suggs S, Radinsky R, Patterson SD, Chang DD (2008) Wild type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol* 26:1626-1634
- Arteaga CL (2007) HER3 and mutant EGFR meet MET. *Nat Med* 13:675-677
- Assersohn L, Salter J, Powles TJ, A'Hern R, Makris A, Gregory RK, Chang J, Dowsett M (2003) Studies of the potential utility of Ki67 as a predictive molecular marker of clinical response in primary breast cancer. *Breast Cancer Res Treat* 82:113-123
- Baselga J, Semiglazov V, van Dam P, Manikhas A, Bellet M, Mayordomo J, Campone M, Kubista E, Greil R, Bianchi G, Steinseifer J, Molloy B, Tokaji E, Gardner H, Phillips P, Stumm M, Lane HA, Dixon JM, Jonat W, Rugo HS (2009) Phase II randomized study of neoadjuvant everolimus plus letrozole compared with placebo plus letrozole in patients with estrogen receptor positive breast cancer. *J Clin Oncol* 27:2630-2637
- Bean J, Brennan C, Shih JY, Riely G, Viale A, Wang L, Chitale D, Motoi N, Szoke J, Broderick S, Balak M, Chang WC, Yu CJ, Gazdar A, Pass H, Rusch V, Gerald W, Huang SF, Yang PC, Miller V, Ladanyi M, Yang CH, Pao W (2007) MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. *Proc Natl Acad Sci USA* 104:20932-20937
- Beeram M, Tan QT, Tekmal RR, Russell D, Middleton A, DeGraffenried LA (2007) Akt induced endocrine therapy resistance is reversed by inhibition of mTOR signaling. *Ann Oncol* 18:1323-1328
- Benvenuti S, Sartore Bianchi A, Di Nicolantonio F, Zanon C, Moroni M, Veronese S, Siena S, Bardelli A (2007) Oncogenic activation of the RAS/RAF signaling pathway impairs the response of metastatic colorectal cancers to anti epidermal growth factor receptor antibody therapies. *Cancer Res* 67:2643-2648

- Berns K, Horlings HM, Hennessy BT, Madiredjo M, Hijmans EM, Beelen K, Linn SC, Gonzalez Angulo AM, Stemke Hale K, Hauptmann M, Beijersbergen RL, Mills GB, van de Vijver MJ, Bernards R (2007) A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. *Cancer Cell* 12:395-402
- Bianco R, Shin I, Ritter CA, Yakes FM, Basso A, Rosen N, Tsurutani J, Dennis PA, Mills GB, Arteaga CL (2003) Loss of PTEN/MMAC1/TEP in EGF receptor expressing tumor cells counteracts the antitumor action of EGFR tyrosine kinase inhibitors. *Oncogene* 22:2812-2822
- Boulay A, Rudloff J, Ye J, Zumstein Mecker S, O'Reilly T, Evans DB, Chen S, Lane HA (2005) Dual inhibition of mTOR and estrogen receptor signaling in vitro induces cell death in models of breast cancer. *Clin Cancer Res* 11:5319-5328
- Cantley LC (2002) The phosphoinositide 3 kinase pathway. *Science* 296:1655-1657
- Carpten JD, Faber AL, Horn C, Donoho GP, Briggs SL, Robbins CM, Hostetter G, Boguslawski S, Moses TY, Savage S, Uhlik M, Lin A, Du J, Qian YW, Zeckner DJ, Tucker Kellogg G, Touchman J, Patel K, Mousses S, Bittner M, Schevitz R, Lai MH, Blanchard KL, Thomas JE (2007) A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. *Nature* 448:439-444
- Carracedo A, Ma L, Teruya Feldstein J, Rojo F, Salmena L, Alimonti A, Egia A, Sasaki AT, Thomas G, Kozma SC, Papa A, Nardella C, Cantley LC, Baselga J, Pandolfi PP (2008) Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K dependent feedback loop in human cancer. *J Clin Invest* 118:3065-3074
- Cloughesy TF, Yoshimoto K, Nghiemphu P, Brown K, Dang J, Zhu S, Hsueh T, Chen Y, Wang W, Youngkin D, Liao L, Martin N, Becker D, Bergsneider M, Lai A, Green R, Oglesby T, Koleto M, Trent J, Horvath S, Mischel PS, Mellinghoff IK, Sawyers CL (2008) Antitumor activity of rapamycin in a Phase I trial for patients with recurrent PTEN deficient glioblastoma. *PLoS Med* 5:e8
- Crouthamel MC, Kahana JA, Korenchuk S, Zhang SY, Sundaresan G, Eberwein DJ, Brown KK, Kumar R (2009) Mechanism and management of AKT inhibitor induced hyperglycemia. *Clin Cancer Res* 15:217-225
- Davies TG, Verdonk ML, Graham B, Saalau Bethell S, Hamlett CC, McHardy T, Collins I, Garrett MD, Workman P, Woodhead SJ, Jhota H, Barford D (2007) A structural comparison of inhibitor binding to PKB, PKA and PKA/PKB chimera. *J Mol Biol* 367:882-894
- DeFriend DJ, Howell A, Nicholson RI, Anderson E, Dowsett M, Mansel RE, Blamey RW, Bundred NJ, Robertson JF, Saunders C et al (1994) Investigation of a new pure antiestrogen (ICI 182780) in women with primary breast cancer. *Cancer Res* 54:408-414
- deGraffenried LA, Friedrichs WE, Russell DH, Donzis EJ, Middleton AK, Silva JM, Roth RA, Hidalgo M (2004) Inhibition of mTOR activity restores tamoxifen response in breast cancer cells with aberrant Akt Activity. *Clin Cancer Res* 10:8059-8067
- Dowsett M, Dixon JM, Horgan K, Salter J, Hills M, Harvey E (2000) Antiproliferative effects of idoxifene in a placebo controlled trial in primary human breast cancer. *Clin Cancer Res* 6:2260-2267
- Dowsett M, Bundred NJ, Decensi A, Sainsbury RC, Lu Y, Hills MJ, Cohen FJ, Veronesi P, O'Brien ME, Scott T, Muchmore DB (2001) Effect of raloxifene on breast cancer cell Ki67 and apoptosis: a double blind, placebo controlled, randomized clinical trial in postmenopausal patients. *Cancer Epidemiol Biomarkers Prev* 10:961-966
- Dowsett M, Ebbs SR, Dixon JM, Skene A, Griffith C, Boeddinghaus I, Salter J, Detre S, Hills M, Ashley S, Francis S, Walsh G, Smith IE (2005) Biomarker changes during neoadjuvant anastrozole, tamoxifen, or the combination: influence of hormonal status and HER 2 in breast cancer - a study from the IMPACT trialists. *J Clin Oncol* 23:2477-2492
- Eichhorn PJ, Gili M, Scaltriti M, Serra V, Guzman M, Nijkamp W, Beijersbergen RL, Valero V, Seoane J, Bernards R, Baselga J (2008) Phosphatidylinositol 3 kinase hyperactivation results in lapatinib resistance that is reversed by the mTOR/phosphatidylinositol 3 kinase inhibitor NVP BEZ235. *Cancer Res* 68:9221-9230

- Engelman JA, Luo J, Cantley LC (2006) The evolution of phosphatidylinositol 3 kinases as regulators of growth and metabolism. *Nat Rev Genet* 7:606–619
- Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, Lindeman N, Gale CM, Zhao X, Christensen J, Kosaka T, Holmes AJ, Rogers AM, Cappuzzo F, Mok T, Lee C, Johnson BE, Cantley LC, Janne PA (2007) MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* 316:1039–1043
- Engelman JA, Chen L, Tan X, Crosby K, Guimaraes AR, Upadhyay R, Maira M, McNamara K, Perera SA, Song Y, Chirieac LR, Kaur R, Lightbown A, Simendinger J, Li T, Padera RF, Garcia Echeverria C, Weissleder R, Mahmood U, Cantley LC, Wong KK (2008) Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. *Nat Med* 14:1351–1356
- Fasolo A, Sessa C (2008) mTOR inhibitors in the treatment of cancer. *Expert Opin Investig Drugs* 17:1717–1734
- Flinn IW, Byrd JC, Furman RR et al (2009) Preliminary evidence of clinical activity in a phase I study of CAL 101, a selective inhibitor of the p110delta isoform of phosphatidylinositol 3 kinase (PI3K) in patients with selective hematological malignancies. *Proc Am Soc Clin Oncol* 27 (15S):156s
- Folkes AJ, Ahmadi K, Alderton WK, Alix S, Baker SJ, Box G, Chuckowree IS, Clarke PA, Depledge P, Eccles SA, Friedman LS, Hayes A, Hancox TC, Kugendradas A, Lensun L, Moore P, Olivero AG, Pang J, Patel S, Pergl Wilson GH, Raynaud FI, Robson A, Saghir N, Salphati L, Sohal S, Ultsch MH, Valenti M, Wallweber HJ, Wan NC, Wiesmann C, Workman P, Zhyvoloup A, Zvelebil MJ, Shuttleworth SJ (2008) The identification of 2 (1H indazol 4 yl) 6 (4 methanesulfonyl piperazin 1 ylmethyl) 4 morpholin 4 yl thieno[3,2 d] pyrimidine (GDC 0941) as a potent, selective, orally bioavailable inhibitor of class I PI3 kinase for the treatment of cancer. *J Med Chem* 51:5522–5532
- Foukas LC, Claret M, Pearce W, Okkenhaug K, Meek S, Peskett E, Sancho S, Smith AJ, Withers DJ, Vanhaesebroeck B (2006) Critical role for the p110alpha phosphoinositide 3 OH kinase in growth and metabolic regulation. *Nature* 441:366–370
- Garcia Echeverria C, Sellers WR (2008) Drug discovery approaches targeting the PI3K/Akt pathway in cancer. *Oncogene* 27:5511–5526
- Garlich JR, De P, Dey N, Su JD, Peng X, Miller A, Murali R, Lu Y, Mills GB, Kundra V, Shu HK, Peng Q, Durden DL (2008) A vascular targeted pan phosphoinositide 3 kinase inhibitor prodrug, SF1126, with antitumor and antiangiogenic activity. *Cancer Res* 68:206–215
- Graupera M, Guillermet Guibert J, Foukas LC, Phng LK, Cain RJ, Salpekar A, Pearce W, Meek S, Millan J, Cutillas PR, Smith AJ, Ridley AJ, Ruhrberg C, Gerhardt H, Vanhaesebroeck B (2008) Angiogenesis selectively requires the p110alpha isoform of PI3K to control endothelial cell migration. *Nature* 453:662–666
- Guertin DA, Sabatini DM (2007) Defining the role of mTOR in cancer. *Cancer Cell* 12:9–22
- Gupta S, Ramjaun AR, Haiko P, Wang Y, Warne PH, Nicke B, Nye E, Stamp G, Alitalo K, Downward J (2007) Binding of ras to phosphoinositide 3 kinase p110alpha is required for ras driven tumorigenesis in mice. *Cell* 129:957–968
- Harrington LS, Findlay GM, Gray A, Tolkacheva T, Wigfield S, Rebholz H, Barnett J, Leslie NR, Cheng S, Shepherd PR, Gout I, Downes CP, Lamb RF (2004) The TSC1/2 tumor suppressor controls insulin PI3K signaling via regulation of IRS proteins. *J Cell Biol* 166:213–223
- Hoefflich KP et al (2009) *In vivo* anti tumor activity of MEK and PI3K inhibitors in basal like breast cancer models: implications for rational combination therapy. *Clin Cancer Res* 15:4649–4664
- Howell A, Cuzick J, Baum M, Buzdar A, Dowsett M, Forbes JF, Hoctin Boes G, Houghton J, Locker GY, Tobias JS (2005) Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. *Lancet* 365:60–62
- Hudes G, Carducci M, Tomczak P, Dutcher J, Figlin R, Kapoor A, Staroslawska E, Sosman J, McDermott D, Bodrogi I, Kovacevic Z, Lesovoy V, Schmidt Wolf IG, Barbarash O,

- Gokmen E, O'Toole T, Lustgarten S, Moore L, Motzer RJ (2007) Temsirolimus, interferon alfa, or both for advanced renal cell carcinoma. *N Engl J Med* 356:2271-2281
- Ihle NT, Lemos R Jr, Wipf P, Yacoub A, Mitchell C, Siwak D, Mills GB, Dent P, Kirkpatrick DL, Powis G (2009) Mutations in the phosphatidylinositol 3 kinase pathway predict for antitumor activity of the inhibitor PX 866 whereas oncogenic Ras is a dominant predictor for resistance. *Cancer Res* 69:143-150
- Jia S, Roberts TM, Zhao JJ (2009) Should individual PI3 kinase isoforms be targeted in cancer? *Curr Opin Cell Biol* 21:199-208
- Jimeno A, Hong DS, Hecker S et al (2009) Phase I trial of PX 866, a novel phosphoinositide 3 kinase inhibitor. *Proc Am Soc Clin Oncol* 27 (15S):156s
- Junttila TT, Akita RW, Parsons K, Fields C, Lewis Phillips GD, Friedman LS, Sampath D, Sliwkowski MX (2009) Ligand independent HER2/HER3/PI3K complex is disrupted by trastuzumab and is effectively inhibited by the PI3K inhibitor GDC 0941. *Cancer Cell* 15:429-440
- Karakas B, Bachman KE, Park BH (2006) Mutation of the PIK3CA oncogene in human cancers. *Br J Cancer* 94:455-459
- Keniry M, Parsons R (2008) The role of PTEN signaling perturbations in cancer and in targeted therapy. *Oncogene* 27:5477-5485
- Kinkade CW, Castillo Martin M, Puzio Kuter A, Yan J, Foster TH, Gao H, Sun Y, Ouyang X, Gerald WL, Cordon Cardo C, Abate Shen C (2008) Targeting AKT/mTOR and ERK MAPK signaling inhibits hormone refractory prostate cancer in a preclinical mouse model. *J Clin Invest* 118:3051-3064
- Knight ZA, Gonzalez B, Feldman ME, Zunder ER, Goldenberg DD, Williams O, Loewith R, Stokoe D, Balla A, Toth B, Balla T, Weiss WA, Williams RL, Shokat KM (2006) A pharmacological map of the PI3 K family defines a role for p110alpha in insulin signaling. *Cell* 125:733-747
- Legrier ME, Yang CP, Yan HG, Lopez Barcons L, Keller SM, Perez Soler R, Horwitz SB, McDaid HM (2007) Targeting protein translation in human non small cell lung cancer via combined MEK and mammalian target of rapamycin suppression. *Cancer Res* 67:11300-11308
- LoRusso P, Taberero J, Shazer R et al (2009) A phase I dose escalation study of the safety, pharmacokinetics, and pharmacodynamics of XL765, a PI3K/TORC1/TORC2 inhibitor administered orally to patients with advanced solid tumors. *Proc Am Soc Clin Oncol* 27 (15S):146s
- Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J, Haber DA (2004) Activating mutations in the epidermal growth factor receptor underlying responsiveness of non small cell lung cancer to gefitinib. *N Engl J Med* 350:2129-2139
- Maira SM, Stauffer F, Brueggen J, Furet P, Schnell C, Fritsch C, Brachmann S, Chene P, De Pover A, Schoemaker K, Fabbro D, Gabriel D, Simonen M, Murphy L, Finan P, Sellers W, Garcia Echeverria C (2008a) Identification and characterization of NVP BEZ235, a new orally available dual phosphatidylinositol 3 kinase/mammalian target of rapamycin inhibitor with potent in vivo antitumor activity. *Mol Cancer Ther* 7:1851-1863
- Maira SM, Voliva C, Garcia Echeverria C (2008b) Class IA phosphatidylinositol 3 kinase: from their biologic implication in human cancers to drug discovery. *Expert Opin Ther Targets* 12:223-238
- Majumder PK, Febbo PG, Bikoff R, Berger R, Xue Q, McMahon LM, Manola J, Brugarolas J, McDonnell TJ, Golub TR, Loda M, Lane HA, Sellers WR (2004) mTOR inhibition reverses Akt dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1 dependent pathways. *Nat Med* 10:594-601
- Manning BD, Cantley LC (2007) AKT/PKB signaling: navigating downstream. *Cell* 129:1261-1274
- Mellinghoff IK, Wang MY, Vivanco I, Haas Kogan DA, Zhu S, Dia EQ, Lu KV, Yoshimoto K, Huang JH, Chute DJ, Riggs BL, Horvath S, Liau LM, Cavenee WK, Rao PN, Beroukhim R, Peck TC, Lee JC, Sellers WR, Stokoe D, Prados M, Cloughesy TF, Sawyers CL, Mischel PS

- (2005) Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. *N Engl J Med* 353:2012-2024
- Mirzoeva OK, Das D, Heiser LM, Bhattacharya S, Siwak D, Gendelman R, Bayani N, Wang NJ, Neve RM, Guan Y, Hu Z, Knight Z, Feiler HS, Gascard P, Parvin B, Spellman PT, Shokat KM, Wyrobek AJ, Bissell MJ, McCormick F, Kuo WL, Mills GB, Gray JW, Korn WM (2009) Basal subtype and MAPK/ERK kinase (MEK) phosphoinositide 3 kinase feedback signaling determine susceptibility of breast cancer cells to MEK inhibition. *Cancer Res* 69:565-572
- Mohsin SK, Weiss HL, Gutierrez MC, Chamness GC, Schiff R, Digiovanna MP, Wang CX, Hilsenbeck SG, Osborne CK, Allred DC, Elledge R, Chang JC (2005) Neoadjuvant trastuzumab induces apoptosis in primary breast cancers. *J Clin Oncol* 23:2460-2468
- Motzer RJ, Escudier B, Oudard S, Hutson TE, Porta C, Bracarda S, Grunwald V, Thompson JA, Figlin RA, Hollaender N, Urbanowitz G, Berg WJ, Kay A, Lebowitz D, Ravaud A (2008) Efficacy of everolimus in advanced renal cell carcinoma: a double blind, randomised, placebo controlled phase III trial. *Lancet* 372:449-456
- Nagata Y, Lan KH, Zhou X, Tan M, Esteva FJ, Sahin AA, Klos KS, Li P, Monia BP, Nguyen NT, Hortobagyi GN, Hung MC, Yu D (2004) PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. *Cancer Cell* 6:117-127
- Neshat MS, Mellinghoff IK, Tran C, Stiles B, Thomas G, Petersen R, Frost P, Gibbons JJ, Wu H, Sawyers CL (2001) Enhanced sensitivity of PTEN deficient tumors to inhibition of FRAP/mTOR. *Proc Natl Acad Sci USA* 98:10314-10319
- Oda K, Okada J, Timmerman L, Rodriguez Viciano P, Stokoe D, Shoji K, Taketani Y, Kuramoto H, Knight ZA, Shokat KM, McCormick F (2008) PIK3CA cooperates with other phosphatidylinositol 3' kinase pathway mutations to effect oncogenic transformation. *Cancer Res* 68:8127-8136
- O'Reilly KE, Rojo F, She QB, Solit D, Mills GB, Smith D, Lane H, Hofmann F, Hicklin DJ, Ludwig DL, Baselga J, Rosen N (2006) mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. *Cancer Res* 66:1500-1508
- Oza A, Elit L, Biagi J et al (2005) A phase II study of temsirolimus (CCI 779) in patients with metastatic and/or recurrent endometrial cancer. *Clin Cancer Res* 11:9099s
- Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel H, Herman P, Kaye FJ, Lindeman N, Boggon TJ, Naoki K, Sasaki H, Fujii Y, Eck MJ, Sellers WR, Johnson BE, Meyerson M (2004) EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 304:1497-1500
- Perez Tenorio G, Stal O (2002) Activation of AKT/PKB in breast cancer predicts a worse outcome among endocrine treated patients. *Br J Cancer* 86:540-545
- Perez Tenorio G, Alkhori L, Olsson B, Waltersson MA, Nordenskjold B, Rutqvist LE, Skoog L, Stal O (2007) PIK3CA mutations and PTEN loss correlate with similar prognostic factors and are not mutually exclusive in breast cancer. *Clin Cancer Res* 13:3577-3584
- Podsypanina K, Lee RT, Politis C, Hennessy I, Crane A, Puc J, Neshat M, Wang H, Yang L, Gibbons J, Frost P, Dreisbach V, Blenis J, Giaciong Z, Fisher P, Sawyers C, Hedrick Ellenson L, Parsons R (2001) An inhibitor of mTOR reduces neoplasia and normalizes p70/S6 kinase activity in Pten^{+/−} mice. *Proc Natl Acad Sci USA* 98:10320-10325
- Press MF, Finn RS, Cameron D, Di Leo A, Geyer CE, Villalobos IE, Santiago A, Guzman R, Gasparyan A, Ma Y, Danenberg K, Martin AM, Williams L, Oliva C, Stein S, Gagnon R, Arbushites M, Koehler MT (2008) HER 2 gene amplification, HER 2 and epidermal growth factor receptor mRNA and protein expression, and lapatinib efficacy in women with metastatic breast cancer. *Clin Cancer Res* 14:7861-7870
- Rhodes N, Heerding DA, Duckett DR, Eberwein DJ, Knick VB, Lansing TJ, McConnell RT, Gilmer TM, Zhang SY, Robell K, Kahana JA, Geske RS, Kleymenova EV, Choudhry AE, Lai Z, Leber JD, Minthorn EA, Strum SL, Wood ER, Huang PS, Copeland RA, Kumar R (2008) Characterization of an Akt kinase inhibitor with potent pharmacodynamic and anti-tumor activity. *Cancer Res* 68:2366-2374

- Salmena L, Carracedo A, Pandolfi PP (2008) Tenets of PTEN tumor suppression. *Cell* 133:403-414
- Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell SM, Riggins GJ, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Velculescu VE (2004) High frequency of mutations of the PIK3CA gene in human cancers. *Science* 304:554
- Sarbassov DD, Ali SM, Kim DH, Guertin DA, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM (2004) Rictor, a novel binding partner of mTOR, defines a rapamycin insensitive and raptor independent pathway that regulates the cytoskeleton. *Curr Biol* 14:1296-1302
- Sarbassov DD, Guertin DA, Ali SM, Sabatini DM (2005) Phosphorylation and regulation of Akt/PKB by the rictor mTOR complex. *Science* 307:1098-1101
- Schnell CR, Stauffer F, Allegrini PR, O'Reilly T, McSheehy PM, Dartois C, Stumm M, Cozens R, Littlewood-Evans A, Garcia-Echeverria C, Maira SM (2008) Effects of the dual phosphatidylinositol 3 kinase/mammalian target of rapamycin inhibitor NVP-BEZ235 on the tumor vasculature: implications for clinical imaging. *Cancer Res* 68:6598-6607
- Serra V, Markman B, Scaltriti M, Eichhorn PJ, Valero V, Guzman M, Botero ML, Llonch E, Atzori F, Di Cosimo S, Maira M, Garcia-Echeverria C, Parra JL, Arribas J, Baselga J (2008) NVP-BEZ235, a dual PI3K/mTOR inhibitor, prevents PI3K signaling and inhibits the growth of cancer cells with activating PI3K mutations. *Cancer Res* 68:8022-8030
- Shapiro G, Kwak E, Baselga J et al (2009) Phase I dose escalation study of XL147, a PI3K inhibitor administered orally to patients with solid tumors. *Proc Am Soc Clin Oncol* 27 (15S):146s
- Shaw RJ, Cantley LC (2006) Ras, PI(3)K and mTOR signalling controls tumour cell growth. *Nature* 441:424-430
- She QB, Solit D, Basso A, Moasser MM (2003) Resistance to gefitinib in PTEN null HER overexpressing tumor cells can be overcome through restoration of PTEN function or pharmacologic modulation of constitutive phosphatidylinositol 3' kinase/Akt pathway signaling. *Clin Cancer Res* 9:4340-4346
- She QB, Chandrapaty S, Ye Q, Lobo J, Haskell KM, Leander KR, DeFeo Jones D, Huber HE, Rosen N (2008) Breast tumor cells with PI3K mutation or HER2 amplification are selectively addicted to Akt signaling. *PLoS ONE* 3:e3065
- Slomovitz B et al (2008) A phase II study of oral mammalian target of rapamycin (mTOR) inhibitor, RAD001 (everolimus) in patients with recurrent endometrial carcinoma. *Proc Am Soc Clin Oncol* 26 (15S):293s
- Stemke-Hale K, Gonzalez-Angulo AM, Lluch A, Neve RM, Kuo WL, Davies M, Carey M, Hu Z, Guan Y, Sahin A, Symmans WF, Pusztai L, Nolden LK, Horlings H, Berns K, Hung MC, van de Vijver MJ, Valero V, Gray JW, Bernard R, Mills GB, Hennessy BT (2008) An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. *Cancer Res* 68:6084-6091
- Stuart D, Sellers WR (2009) Linking somatic genetic alterations in cancer to therapeutics. *Curr Opin Cell Biol* 21:304-310
- Thomas RK, Baker AC, DeBiasi RM, Winckler W, Laframboise T, Lin WM, Wang M, Feng W, Zander T, MacConaill L, Lee JC, Nicoletti R, Hatton C, Goyette M, Girard L, Majumdar K, Ziaugra L, Wong KK, Gabriel S, Beroukhim R, Peyton M, Barretina J, Dutt A, Emery C, Greulich H, Shah K, Sasaki H, Gazdar A, Minna J, Armstrong SA, Mellinghoff IK, Hodi FS, Dranoff G, Mischel PS, Cloughesy TF, Nelson SF, Liao LM, Mertz K, Rubin MA, Moch H, Loda M, Catalona W, Fletcher J, Signoretti S, Kaye F, Anderson KC, Demetri GD, Dummer R, Wagner S, Herlyn M, Sellers WR, Meyerson M, Garraway LA (2007) High throughput oncogene mutation profiling in human cancer. *Nat Genet* 39:347-351
- Tokunaga E, Kataoka A, Kimura Y, Oki E, Mashino K, Nishida K, Koga T, Morita M, Kakeji Y, Baba H, Ohno S, Maehara Y (2006) The association between Akt activation and resistance to hormone therapy in metastatic breast cancer. *Eur J Cancer* 42:629-635
- Tolcher AW, Yap TA, Fearon I et al (2009) A phase I study of MK-2206, an oral potent allosteric Akt inhibitor, in patients with advanced solid tumors. *Proc Am Soc Clin Oncol* 27 (15S):146s

- Toral Barza L, Zhang WG, Huang X, McDonald LA, Salaski EJ, Barbieri LR, Ding WD, Krishnamurthy G, Hu YB, Lucas J, Bernan VS, Cai P, Levin JI, Mansour TS, Gibbons JJ, Abraham RT, Yu K (2007) Discovery of lactoquinomycin and related pyranonaphthoquinones as potent and allosteric inhibitors of AKT/PKB: mechanistic involvement of AKT catalytic activation loop cysteines. *Mol Cancer Ther* 6:3028-3038
- Vanhaesebroeck B, Leevers SJ, Panayotou G, Waterfield MD (1997) Phosphoinositide 3-kinases: a conserved family of signal transducers. *Trends Biochem Sci* 22:267-272
- Wagner AJ, Von Hoff DH, LoRusso, PM et al (2009) A first in human phase I study to evaluate the pan PI3K inhibitor GDC 0941 administered QD or BID in patients with advanced solid tumors. *Proc Am Soc Clin Oncol* 27 (15S):146s
- Wang MY, Lu KV, Zhu S, Dia EQ, Vivanco I, Shackleford GM, Cavenee WK, Mellinghoff IK, Cloughesy TF, Sawyers CL, Mischel PS (2006) Mammalian target of rapamycin inhibition promotes response to epidermal growth factor receptor kinase inhibitors in PTEN deficient and PTEN intact glioblastoma cells. *Cancer Res* 66:7864-7869
- Witzig TE, Geyer SM, Ghobrial I, Inwards DJ, Fonseca R, Kurtin P, Ansell SM, Luyun R, Flynn PJ, Morton RF, Dakhil SR, Gross H, Kaufmann SH (2005) Phase II trial of single agent temsirolimus (CCI 779) for relapsed mantle cell lymphoma. *J Clin Oncol* 23:5347-5356
- Yakes FM, Chinratanalab W, Ritter CA, King W, Seelig S, Arteaga CL (2002) Herceptin induced inhibition of phosphatidylinositol 3-kinase and Akt is required for antibody mediated effects on p27, cyclin D1, and antitumor action. *Cancer Res* 62:4132-4141
- Yuan TL, Choi HS, Matsui A, Benes C, Lifshits E, Luo J, Frangioni JV, Cantley LC (2008) Class IA PI3K regulates vessel integrity during development and tumorigenesis. *Proc Natl Acad Sci USA* 105:9739-9744
- Zhang H, Bajraszewski N, Wu E, Wang H, Moseman AP, Dabora SL, Griffin JD, Kwiatkowski DJ (2007) PDGFRs are critical for PI3K/Akt activation and negatively regulated by mTOR. *J Clin Invest* 117:730-738

From the Bench to the Bed Side: PI3K Pathway Inhibitors in Clinical Development

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Abstract A number of intracellular kinase components of the PI3K/Akt/mTOR pathway have been targeted over the past few years, leading to a new generation of anticancer agents that effectively and specifically disrupt this pathway in tumor cells. Here, progress in the identification and clinical evaluation of compounds designed to modulate the enzymatic activity of PI3K, Akt, mTOR, and Hsp90 is reviewed.

1 Introduction

Due to their crucial role in signal transduction, the dysregulated metabolism of phosphoinositides (PI) represents a key step in many disease settings. Multiple enzymes participate in the phosphorylation and dephosphorylation of the inositol

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head group of PI lipids (Hawkins et al. 2006). Among them, Phosphoinositide 3-Kinases (PI3Ks) have been the focus of extensive research and drug discovery activities for the last 20 years. To date, eight PI3K catalytic proteins encoded by eight separate genes have been identified and are classified based on their primary and secondary structures as originally proposed by Domin and Waterfield (Vanhaesebroeck et al. 1997; Domin and Waterfield 1997). The members of the PI3K catalytic protein family have been grouped into three sub-classes. Class I enzymes are heterodimers of catalytic subunits (Class 1A: p110 α , β , δ and Class 1B: p110 γ) with adaptor subunits which regulate activity of the complex and the conversion of PtdIns (4,5)P₂ to PtdIns (3,4,5)P₃. Class IA enzymes are integral components of many growth factor pathways, and activation often occurs through recruitment of the enzymes to cell membranes via phosphotyrosine binding SH2 domains present in the adaptor subunits. P110 α and β are widely expressed whilst the expression of p110 δ is more restricted to blood cells. P110 γ is regulated by G-protein coupled receptors and is expressed in multiple tissues. PI3KC2 α , β and γ belong to Class II and their mode of regulation is less clear. Vps34 is the only member of Class III and it controls vesicular trafficking through the production of PtdIns (3)P. Class I, II, and III proteins contain highly conserved catalytic domain, and homology searches have revealed the presence of similar kinase domains in a sub-group of serine/threonine protein kinases hence referred as *Phosphoinositide-3-Kinase-related Kinases* (PIKK) or Class IV PI3Ks, encompassing the growth control protein mTOR and the DNA damage induced proteins DNA-PK, ATM, and ATR (Maira et al. 2008b; Ihle and Powis 2009).

The first part of this chapter will focus on the identification and development of PI3K pathway inhibitors in the context of cancer and, to less extent, non-cancer treatment. Genetic alterations at multiple nodes in the PI3K pathway have been implicated in oncogenesis and cancer (Zhao and Vogt 2008; Yuan and Cantley 2008). PI3K activation occurs in response to (1) constitutively active mutants or amplification of growth factor receptor tyrosine kinases, (2) amplification of PI3K, (3) presence of activating mutations in the PIK3CA gene encoding the p110 α catalytic subunit, (4) overexpression of the downstream effector kinase Akt, (5) loss or inactivating mutations of PTEN, the phosphatase that breaks down PtdIns (3,4,5)P₃, or (6) constitutive recruitment and activation by mutant forms of the Ras oncogene. The signaling cascades downstream of PI3K activation are well understood and have facilitated the design of pharmacodynamic biomarkers for assessing pathway inhibition in animal models and clinical trials (e.g., antibodies recognizing phosphorylation sites in Akt or ribosomal protein S6). PI3K is essential for many physiological processes such as insulin signaling and glucose homeostasis. Hence, some on-target toxicities are expected during clinical development, but they can be easily monitored.

In addition to PI3K, other intracellular components of the PI3K pathway have been targeted as anticancer drug discovery activities. The second part of this chapter summarizes current knowledge of other modulators of the PI3K/Akt/mTOR pathway in which drug discovery and early clinical activities have been advanced focusing on inhibitors of mTOR, Akt, and Hsp90. As shown in this

chapter, intervention at different levels within PI3K/Akt/mTOR pathway is rapidly emerging as a promising approach for the treatment of cancer although patient stratification and the selection of the right inhibitor profile will be further refined, as more preclinical data are generated and clinical trials are initiated.

2 PI3K Inhibitors: Path to the Clinic

2.1 *PI3K Inhibitors in Oncology Drug Discovery and Development*

Prototypical PI3K inhibitors such as the natural product wortmannin (Powis et al. 1994) (compound 1, Fig. 1) or the quercetin derivative LY294002 (Vlahos et al. 1994) (compound 2, Fig. 1), are generally considered as pan Class I PI3K inhibitors. They retain significant activities toward other PI3K super-family members and do not possess drug like properties suitable for clinical development. Follow-up medicinal chemistry activities on both molecules, have led to the identification of PX-866 (compound 3, Fig. 1) and SF-1126 (compound 4, Fig. 1), respectively.

PX-866 is a pan Class I PI3K inhibitor that has the particularity (such as wortmannin) to bind PI3K in an irreversible manner (Ihle et al. 2004). Pre-clinically, PX-866 has demonstrated *in vivo* antitumor activity against various tumor models in mice (Ihle et al. 2004, 2005). PX-866 was shown to induce hyperglycemia in mice, but this effect could be overcome by co-treatment with the PPAR γ inhibitor pioglitazone (Ihle et al. 2009a). PX-866 was shown to be more efficacious against tumor cell lines containing PIK3CA mutations whereas KRAS mutant tumor cells were essentially insensitive (Ihle et al. 2009b). PX-866 was also used to validate the use of hair follicles as surrogate tissue to assess PI3K inhibition (Williams et al. 2006). This assay is now used to demonstrate target inhibition in clinical trials. PX-866 is developed by Oncothyreon, and has entered Phase I clinical trial in June 2008. Patients with advanced metastatic cancers received PX-866 orally, for 5 out 7 days per week for 2 weeks (each cycle encompassing 28 days). The starting dose was 0.5 mg, and the actual dose escalation reached a dose of 4.5 mg. No Dose-Limiting Toxicities (DLTs) were encountered till date, and the Maximum Tolerated Dose (MTD) was not established yet. However, three patients had stable disease and were treated (or are still on treatment) for more than 100 days. Target modulation could be demonstrated in PBMCs as reflected by the reduction of pRPS6 and phospho-mTOR (pmTOR) levels in this surrogate tissue, in a dose-dependent manner (Jimeno et al. 2009).

SF-1126 is composed of a vasculature-targeting RGDS tag linked to LY294002, providing solubility and acid stability. The peptidic tag is naturally cleaved at neutral pH. The full profile of this pan-PI3K inhibitor has been disclosed. The compound, given either subcutaneously or i.v. produces significant antitumor activity in tumor xenografts in mice, and could reduce glucose avidity as measured

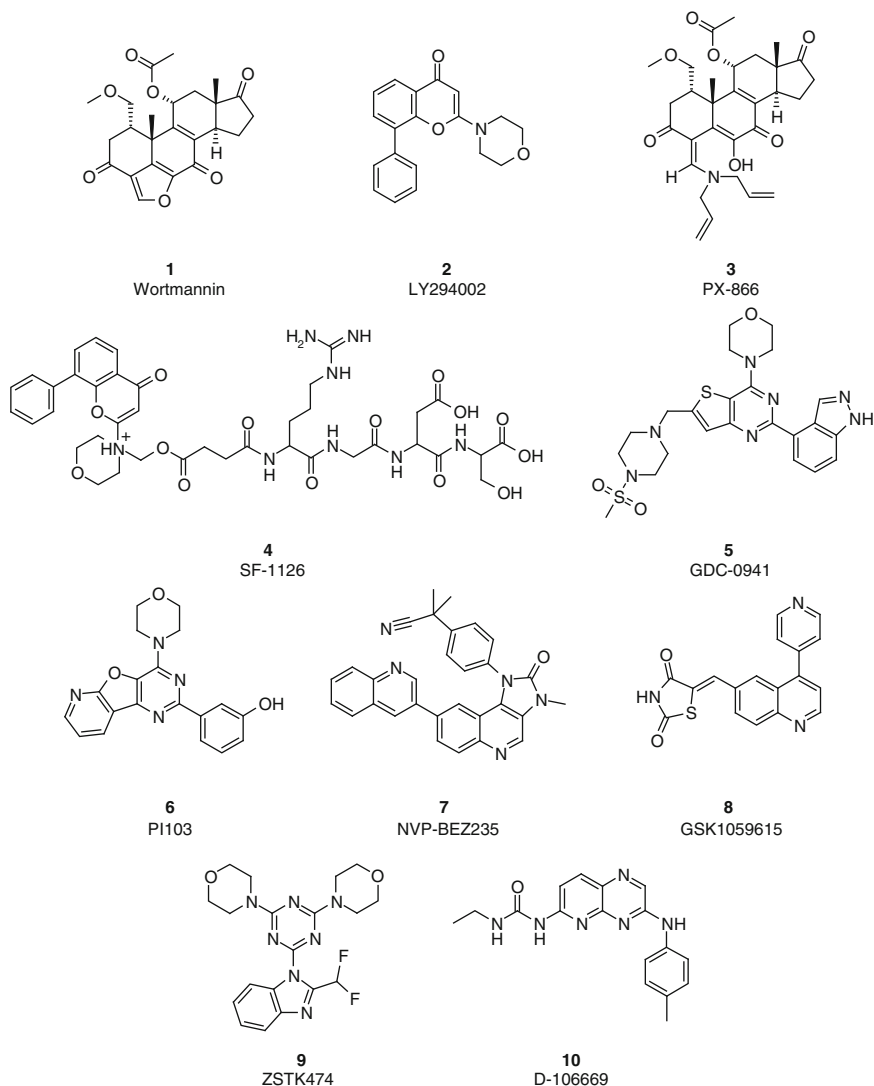


Fig. 1 Selected examples of PI3K inhibitors in oncology drug discovery and development

by FDG-PET of MDA-MB468 tumors (Garlich et al. 2008). SF-1126 is developed by Semafore, and entered Phase I clinical trial in March 2007. It is administered by i.v. infusion (90 min) on day 1 every 4 weeks. This first dose level was 90 mg/m². Although one DLT was observed in one patient in the third dose cohort (180 mg/m²), the compound was well tolerated up to 630 mg/m² and the MTD has not been established yet. Pharmacokinetic measurements of the active hydrolysis product (LY294002, referred also as SF1101) showed a $t_{1/2}$ of 2 h, and a dose proportional increase in C_{max} (21 μ M at 630 mg/m²). Target modulation in tumor biopsies could

be demonstrated through pRPS6 levels reduction by immuno-histochemistry (Chiorean et al. 2009).

The elucidation of PI3K γ X-ray crystal structure alone (Walker et al. 1999) or complexed to various PI3K inhibitors (Walker et al. 2000; Knight et al. 2006), has provided the first key elements to better understanding the molecular determinants for ATP competitive inhibition within the catalytic ATP cleft. More recently, a similar effort has shed light on the structure of the PI3K α isoform (Huang et al. 2007, 2008; Miled et al. 2007; Amzel et al. 2008; Williams et al. 2009). The increasing amount of detailed molecular structures available has allowed the design of more selective and potent synthetic PI3K inhibitors. Representative examples of this new generation of structure-based design PI3K modulators are reviewed below.

The clinical candidate GDC-0941 (compound 5, Fig. 1) is a pan-Class I inhibitor (IC₅₀ for PI3K α , β , δ and γ of 3, 33, 3, and 75 nM, respectively) based on PI-103 (compound 6, Fig. 1), which is a prototypic molecule from the pyridofuopyrimidine scaffold (Folkes et al. 2008). Most notably, GDC-0941 is less potent on mTOR and DNA-PK than PI-103 (30 and 23 nM vs. 580 and 1,230 nM, respectively). GDC-0941 shows high oral bioavailability in contrast to PI-103 and related molecules PI-540 and PI-620 (structures not shown). GDC-0941 displays strong anti-tumor activity as a single agent against PTEN null U87MG or PC3 and breast cancer HER2 amplified HER2 tumors when given once a day at doses from 75 to 150 mg/kg daily (Folkes et al. 2008; Friedman 2008). Interestingly, the potential of this compound in combination with other targeted agents has been demonstrated. In that respect, the combination of GDC-0941 with the HER2 antagonist agent Trastuzumab was found to produce profound effects in the treatment of HER2 positive breast cancers, *in vitro* and *in vivo* (Junttila et al. 2009; Yao et al. 2009). Engagingly, it was shown that in basal-like breast cancer cell lines (generally, HER2, ER, and PR negative), which are dependent on the ERK/MEK signaling pathway, MEK inhibitor treatment increases PI3K pathway activity. Hence, in this setting, the combination of MEK inhibitors with GDC-0941 results in synergistic inhibition of cell proliferation (Hoeflich et al. 2009).

GDC-0941 is being developed by Genentech/Roche (Piramed was purchased by Roche in April 2008) and entered Phase I dose escalation trial in patients with advanced solid tumors in April 2008. Five cohorts with dose levels of 15, 30, 45 mg (given once a day), 60 and 80 mg (given as a b.i.d regimen), for a period of 3 weeks followed by 1 week without treatment (composing one cycle) have been enrolled so far. Grade 3 headache (80 mg b.i.d) and pleural effusion (60 mg b.i.d) DLTs were observed but the MTD has not been reached so far. The best response observed to date is stable disease and lymph node shrinkage associated with normalization of plasma CA-125 levels in a patient with ovarian cancer (bearing a PIK3CA mutation), treated at dose of 30 mg (Wagner et al. 2009). The PK/PD relationship of GDC-0941 was investigated in a separate Phase I study (Sarker et al. 2009). The compound is rapidly absorbed (C_{\max} 1–3 h), has moderate to long half life (8.4–28.2 h), and produces dose proportional exposures (352–1,230, 516–3,590, and 1,030–2,370 h*ng/mL for 15, 30, and 45 mg dose levels, respectively).

Sanofi-Aventis is currently developing two different molecules, XL-147 and XL-765, originally identified by Exelixis that differ in their target profile. Although both molecules are potent pan Class I PI3K inhibitors, they differ in their activity on Class IV protein kinase mTOR (for PI3K α , β , δ , γ and mTOR, the IC₅₀ are respectively 39, 383, 36, 32, and >1,500 nM for XL-147; 39, 113, 43, 9, and 157 nM for XL-765). The chemical structures of both compounds have not yet been disclosed and all available profiling data are from either posters or oral communications. Both drugs were recently licensed in by Sanofi-Aventis (April 2009).

XL-147 and XL-765 are both currently in dose escalation Phase-I trials in patients with solid tumors, since June 2007. For XL-147, 48 patients have been enrolled so far, treated at dose levels from 30 to 900 mg, orally, once a day for 3 weeks followed by 1 week without treatment (composing one cycle). Several DLTs (including grade 3 rash and arterial thrombosis) have been observed, identifying the MTD as 600 mg. XL-147 has a very long half life (3.7–6.3 days), and a long lasting absorption (2–24 h) in man. The steady state levels are achieved after 15–20 days of treatment, and accumulation is observed (5- to 13-fold). From the efficacy perspective, 43 patients stayed on trial for more than 12 weeks (three cycles). Interestingly, this included 13 patients with NSCLC, and one of them (EGFR wt, PI3K wt, no data on KRAS and LKB1) encountered partial response (PR) by RECIST Criteria. In parallel, pathway modulation was measured by IHC measurement on hair follicles of p4EBP1, pRPS6, and pAkt. Moreover, 40–80% and 40–70% decrease in pAkt and p4EBP1, respectively, could be measured in a paired tumor biopsy from a breast cancer patient (Shapiro et al. 2009).

For XL-765, 34 patients have enrolled in a dose-escalation Phase I study. The compound is given orally, either once or twice a day. The MTD levels were either 120 mg (60 mg b.i.d), or 100 mg (once a day), respectively. The DLTs included elevated hepatic transaminases, anorexia/hypophosphatemia, rash and nausea/vomiting. In man, XL-765 is characterized by rapid absorption (T_{max} 1–3 h), long half life (3–11 h), and steady state levels after repeated dosing is achieved by day 8. Also, the exposure increased proportionally with the dose levels, and no accumulation was observed. No PR could be evidenced for XL-765 but efficacy could be demonstrated as stable disease for five patients. As for XL-147, pathway modulation could be monitored in a similar fashion (LoRusso et al. 2009).

NVP-BEZ235 (compound 7, Fig. 1) developed by Novartis Pharma AG is an imidazoquinoline derivative that has been optimized for potent PI3K (IC₅₀ of 4, 75, 7 and 5 for PI3K α , β , δ and γ , respectively) and mTOR inhibitory properties (Maira et al. 2008a; Stauffer et al. 2008). NVP-BEZ235 is orally available and was the first compound with this dual class I PI3K/mTOR inhibition profile to enter Phase I clinical trials at the end of 2006 for dose escalation in patients with advanced solid tumors, particularly enriched for patients with advanced breast cancer. The compound has been extensively profiled in a variety of preclinical models reflecting different diseases and lineages. Hence, NVP-BEZ235 has been shown to be efficacious against melanoma (Marone et al. 2009), multiple myeloma (McMillin et al. 2009; Baumann et al. 2009), prostate cancer (Cao et al. 2009; Dubrovskaja et al. 2009), and breast cancer (Serra et al. 2008). Moreover, NVP-BEZ235 was shown to

act synergistically in combination with MEK inhibitors in models of KRAS mutant lung tumors (Engelman et al. 2008), or with anti-estrogen therapies and anti-EGFR therapies in breast cancer (Crowder et al. 2009; Eichhorn et al. 2008). The data of the Phase I trials for this compound has not yet been released. A second inhibitor from the same company (NVP-BGT226, structure not disclosed) has entered a Phase I clinical trial for patients with advanced solid tumors, including advanced breast cancer.

The structure and biological profile of the pyridinylquinoline derivative GSK615 (also known as GSK1059615; compound 8, Fig. 1) have been disclosed recently (Auger et al. 2008; Bachman 2008; Greshock 2008). GSK615 is a pan-class I (K_i of 0.42, 0.6, 1.7, and 0.47 nM against PI3K α , β , δ and γ respectively) and mTOR (the IC_{50} has not been reported yet) dual inhibitor. In *in vivo* studies, oral treatment with GSK615 led to stasis or tumor regression of BT474 or HCC1954 breast cancer tumors, when given at a dose of 25 mg/kg, b.i.d. GSK615, which is developed by GlaxoSmithKline, has entered Phase I dose escalation trials in patients with solid tumors and lymphoma in September 2008. No data have yet been reported from this trial.

ZSTK474 (compound 9, Fig. 1) is a pan class I PI3K inhibitor (IC_{50} of 16, 44, 4.6 and 49 nM for PI3K α , β , δ and γ , respectively) that was discovered by Zenakyo Kogyo, through a bio-informatic method aiming at identifying compounds with anti-proliferative activity similar to LY294002, when tested in the NCI60 panel (Yaguchi et al. 2006; Kong and Yamori 2007). ZSTK474 is not an mTOR inhibitor and has limited activity against DNA-PK (Kong et al. 2009). Although extensively profiled in pre-clinical settings, ZSTK474 has not reached clinical development. The compound is orally available in mice, and showed significant antitumor activity against various xenograft models, at dose levels ranging from 100 to 400 mg/kg, given once a day. This compound possesses antitumor angiogenic activities as reflected by its anti-proliferative activities against HUVEC cells stimulated with VEGF or bFGF, and ability to reduce expressed HIF1 α and VEGF levels. ZSTK474 also potently blocks migratory and microtubule-like formation properties of HUVEC cells (Kong et al. 2009).

UCB1320437 is an optimized molecule whose structure and complete biochemical profile has not yet been disclosed. UCB1320437 was shown to possess anti-proliferative activities as a single agent against a range of human tumor lines and synergizes with Erlotinib, Rapamycin, and MEK inhibitors, most notably resulting in apoptosis induction (Kavorkian et al. 2008). No information has been reported about Phase I clinical studies with this PI3K inhibitor. This compound class has been identified by UCB.

Although the structure of AEZS-126 has not yet been disclosed, the compound is probably an optimized derivative of the precursor molecule D-106669 (compound 10, Fig. 1). Remarkably, both molecules are pan-class I PI3K and ERK2 inhibitors (Seipelt et al. 2007, 2009). AEZS-126 was shown to possess strong anti-proliferative activities in a range of tumor lines (A549, HCT116, U87MG, PC3 and MDA-MB468), and that is correlated with concomitant diminution of S473P-Akt levels in cells. This compound possesses good pharmacokinetic properties in mice with oral

bioavailability of 60%, translating in good antitumor activities achieved at 30 mg/kg against HCT116 and A549 tumor models (Seipelt et al. 2009). No information has been released so far regarding entry into Phase I clinical trials. The compound is being developed by Eterna Zentaris Inc.

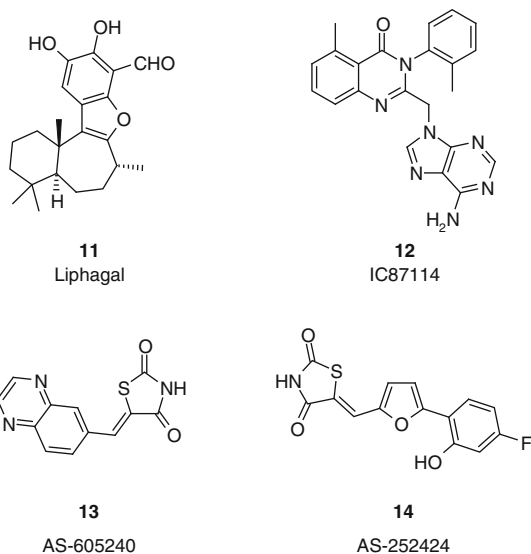
2.2 *Identification of Isoform Specific PI3K Inhibitors for Oncology*

The elucidation of the roles of different class I isoforms in normal nonpathological tissues has been studied using genetically manipulated mice. Limited information was gained from the constitutive knockout of the p110 α and p110 β isoforms as the ablation was found to be embryonic lethal (Bi et al. 1999, 2002). The conditional knock-out of the PIK3CA and PIK3CB gene or even better, replacement of the endogenous gene by a kinase dead version was recently published. Not surprisingly, p110 α has been found to be critical for regulation of cell growth, cell signaling through growth factors, and cell transformation (Foukas et al. 2006; Zhao et al. 2006). The unexpected finding came with the results that p110 β seems to also have a strong involvement in cell growth and metabolism and that this is in part due to its activation by GPCRs, a feature that was believed to be the hallmark of p110 γ (Ciraolo et al. 2008). Indeed, functional redundancies between these two isoforms were identified (Guillermet-Guibert et al. 2008). Even more striking is the fact that a PTEN null induced prostate intra-epithelial neoplasia phenotype could be reverted by loss of p110 β expression (Jia et al. 2008). Confirming this, the ablation of p110 β in the PTEN null cell line PC3 was found to reduce its tumorigenicity (Wee et al. 2008). All these data suggest that isoform specific inhibition might achieve similar efficacy depending on tumor type, and as a consequence of the higher specificity might present higher therapeutic windows than pan-Class I inhibitors (Jia et al. 2009).

Efforts have been undertaken to identify Class I PI3K isoform specific inhibitors (Knight et al. 2006), including the identification of Liphagal (compound 11, Fig. 2), a natural product claimed to selectively inhibit PI3K α (Marion et al. 2006). In addition, a major breakthrough has been the identification of p110 δ inhibitors suitable for clinical development.

Based on the fact that p110 δ expression is generally restricted to hematopoietic lineages, and that p110 δ expression was increased in hematological malignancies, there is a good rationale for specific p110 δ inhibitors for cancer treatment. The original chemical series described by the former ICOS and Lilly, exemplified by the IC-87114 example (compound 12, Fig. 2; covered in next section), was further optimized and led to the identification of CAL-101 (structure not disclosed) (Giese 2008; Lannutti et al. 2009). This molecule is being developed for treatment of haematological malignancies. CAL-101 inhibits PI3K δ with an IC₅₀ of 65 nM, and achieves 40- to 300-fold selectivity toward the other class I isoforms. The compound has been shown to potently block pAkt phosphorylation in CLL and AML

Fig. 2 Selected examples of isoform selective PI3K inhibitors in cancer and non cancer indications



cell lines. Moreover, CAL-101, which is developed by Calistoga Pharmaceuticals, potently blocked the proliferation and induced apoptosis of freshly derived primary AML, CML, ALL, and MM tumor lines. To date, CAL-101 has been given to a total of six patients with either relapse CLL or B Cell Non Hodgkin lymphoma at dose level of 50 and 100 mg. No DLTs have been reported but interestingly out the 12 patients, 6 of them showed a PR. This very promising study is currently ongoing and enrolling at a dose level of 200 mg (Flinn et al. 2009).

2.3 *Development of PI3K Pathway Inhibitors in Non-Cancer Indications*

The prospects for the use of PI3K inhibitors in the treatment of various inflammatory disorders and cardiovascular disease look promising. Although the clinical development of PI3K inhibitors in these disease settings has lagged behind the oncology field, there is a vast array of supporting preclinical data for these diseases (Marone et al. 2008; Ward and Finan 2003). While in cancer, the role of PI3K dysregulation in the disease is supported by genetic alteration (mutation, gene amplification, loss of PTEN, or aberrant activation of receptor tyrosine kinases), the rationale for modulation of PI3K in other disease settings is based on the pivotal role of PI3K isoform signaling in response to various growth factors, inflammatory cytokines, chemokines, and adhesion molecules. Many of the studies supporting these findings have utilized both genetically modified mice and

pharmacological tools. In the former case, kinase-dead targeted mice which retain close to endogenous levels of the targeted isoform have proven to be particular useful as the direct removal of PI3K isoforms can disturb the stoichiometry of other PI3K complexes (Vanhaesebroeck et al. 2005). In addition, it has been recently demonstrated that some of the effect of p110 γ loss in models of cardiac function are attributable to a scaffolding function rather than catalytic activity (Patrucco et al. 2004). A wide array of pharmacological tool agents has been utilized for validation studies and these molecules have extensively profiled in PI3K assays *in vitro* (Knight et al. 2006). In particular, the function of p110 δ has been probed with IC87114 (compound 12, Fig. 2) which inhibits p110 δ with an IC₅₀ of 500 nM and p110 γ , p110 α and p110 β with IC₅₀s in excess of 20 μ M (Sadhu et al. 2003). AS-605240 (compound 13, Fig. 2) have been used extensively to assess the function of p110 γ . This thiazolidinedione inhibits p110 γ with an IC₅₀ of 8 nM and has greater than sevenfold selectivity against p110 α , p110 β and p110 δ (Camps et al. 2005b). These inhibitors are a vast improvement over LY294002 and wortmannin as pharmacological tools but they would still be expected to have overlapping inhibition curves for different p110 isoforms. Coupled with the absence of detailed pharmacokinetic and tissue exposure data for these inhibitors, the interpretation of *in vivo* studies using these inhibitors needs to be treated with some caution.

2.3.1 PI3K Inhibition for the Treatment of Respiratory Diseases

Many respiratory diseases are the outcome of underlying inflammatory conditions which are manifest in the aberrant recruitment and activation of different leukocyte populations. Chronic obstructive pulmonary disease (COPD) is caused by tobacco smoke leading to damage to the lung epithelium and recruitment of neutrophils by chemo-attractants such as IL-8, GRO α , and leukotrienes. Neutrophil influx is then accompanied by the release of reactive oxygen species and proteases which cause alveolar destruction and further release of inflammatory mediators. Asthma is a chronic inflammatory disorder associated with airway hyperresponsiveness. The underlying inflammation involves multiple cell types and includes dysregulation of T helper cell function, aberrant mast cell activation, and recruitment and activation of eosinophils. All of these cell types have been shown to utilize PI 3-kinase in the control of various cellular functions. In many cases, there is interplay between p110 γ and p110 δ , and it is still unclear whether the best efficacy and safety profile will be observed with a p110 γ inhibitor, a p110 δ inhibitor, or a dual inhibitor. In mouse models of asthma, intratracheal administration of the p110 δ inhibitor, IC87114 has been shown to inhibit OVA-induced influx of leucocytes and led to a reduction of Th2 cytokines. These findings are further supported by studies in genetically modified animals which demonstrate a role for p110 δ in mast cell activation (Ali et al. 2004). The role of p110 γ in mast cell function has also been demonstrated with genetically modified mice. Mast cell activation is potentiated by various GPCR ligands such as

adenosine and these have been shown to utilize p110 γ (Laffargue et al. 2002). These studies have not been fully recapitulated with the pharmacological tool AS-252424 (compound 14; Fig. 2) which inhibits p110 γ (Ali et al. 2008). A further confounding observation has been made with p110 δ genetically modified mice. In these studies p110 δ inactivation was shown to enhance IgE production (Zhang et al. 2008). Clearly this adds an extra layer of complexity that needs to be considered before progressing PI3K inhibitors for the treatment of allergic diseases such as asthma. Although there are plenty of data supporting the development of p110 γ and p110 δ inhibitors, there has been limited transition into the clinic. By the inhaled route, such molecules would have to demonstrate superior efficacy to other anti-inflammatories delivered locally such as inhaled steroids. By the oral route, the inhibitors would have to be well tolerated for chronic treatment and insights into the safety profile associated with the inhibition of these enzymes, is likely to be gained indirectly through the development of pan-PI3K inhibitors for cancer. CAL-263 (structure not disclosed) is an oral, p110 δ inhibitor which is reported to have favorable pharmacokinetics and safety profile in Phase I trials. CAL-263 (structure not disclosed), when dosed as a racemic mixture is reported to demonstrate efficacy in ovalbumin-induced allergic inflammation in rodents and allergic rhinitis models in ragweed sensitized dogs although this data is unpublished (Calistoga Pharmaceuticals Website).

2.3.2 PI3K Inhibition for the Treatment of Arthritis and Systemic Lupus Erythematosus

Rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are chronic autoimmune inflammatory diseases caused by an inappropriate balance of B and T cell function (Marone et al. 2008). In RA, this leads to inflammatory synovitis that often progresses to destruction of the articular cartilage and ankylosis of the joints caused in part by an accumulation of neutrophils, macrophages, and fibroblasts in the synovium. In mouse models of RA, deletion of p110 γ leads to a reduction in paw swelling, leukocyte infiltration, and joint erosion (Camps et al. 2005a). This reduction in RA pathology is also observed with AS-605240 when dosed therapeutically. No clinical development of p110 γ inhibitors for RA is reported although unpublished data has supported the development of the p110 δ inhibitor CAL-263 (Calistoga Pharmaceuticals Website).

SLE mostly affects women of African or Asian descent and is caused by B cell expansion, hyper-gamma-globulinemia, and production of antibodies to DNA. Mouse models of this disease use the MRL-*lpr* mice which are highly prone to the development of SLE. When dosed at 30mg/kg i.p., AS-605240 increased the number of mice which were alive at 5 months in comparison to animals treated with vehicle alone (Barber et al. 2005). The treated mice had lower numbers of CD4+ T cells, reduced levels of auto-antibodies, and reduced kidney malfunction.

2.3.3 PI3K Inhibition for the Treatment of Atherosclerosis

Atherosclerosis is a chronic inflammatory disease caused in a large part by the accumulation of macrophages and the deposition of oxidized low-density lipoproteins in arterial blood vessels. In macrophages derived from p110 γ knockout mice, oxidized LDL no longer activates the PI3K signaling pathway. Furthermore, when these mice are crossed with mice lacking the apolipoprotein E gene, a reduction in the severity of atherosclerosis was observed (Fougerat et al. 2008). No clinical development of PI3K inhibitors for this disease has been reported.

3 mTOR Inhibitors: Allosteric and ATP Competitive Inhibitors

Amongst other downstream effectors, the PI3K/Akt pathway has been shown to exert tumorigenic potential by activating the mTORC1 complex (composed of mTOR and raptor). The best example to illustrate this is the fact that a rapamycin derivative, which is an allosteric mTORC1 inhibitor, is able to suppress Akt induced PIN lesions in mice (Majumder et al. 2004). Recent studies have identified the other mTOR containing complex, mTORC2 (composed of mTOR and rictor) as one of the direct Akt upstream activator (Sarbasov et al. 2005). Rapamycin (sirolimus, Wyeth; compound 15, Fig. 3), a macrolide isolated from *Streptomyces hygroscopicus* is an allosteric inhibitor of the mTORC1 activity displaying antifungal and immunosuppressive effects. The unfavorable physicochemical properties of rapamycin have triggered the development of rapamycin derivatives suitable for clinical development, such as Everolimus (RAD001, Novartis Pharma AG; compound 16, Fig. 3) Temsirolimus (CCI-779, Wyeth; compound 17, Fig. 3), and Deforolimus (AP23573, Merck/Ariad; compound 18, Fig. 3). The identification and clinical development of these compounds have been extensively reviewed in numerous publications (Abraham and Eng 2008) and is not covered herein. Recent evidence has shown that ATP-competitive inhibition produces a more profound anti-proliferative effect than rapamycin analogs. In addition to inhibition of the mTORC2 complex, this increased efficacy is thought to be due to a more complete inhibition of mTORC1 and to the blockade of a rapamycin insensitive pool of mTORC1 pool (Feldman et al. 2009). These findings have catalyzed an interest in developing mTOR active-site inhibitors.

KU-0063794 is a pyridopyrimidine derivative (compound 19, Fig. 4) lacking PI3K inhibition (IC_{50} of 8,867, >30,000, 5,251, and >30,000 nM for PI3K α , β , δ , and γ , respectively) but inhibiting the mTOR kinase with an IC_{50} of 2.5 nM. As expected for an mTOR inhibitor, KU-0063794 is able to reduce S473P-Akt (mTORC2 inhibition, IC_{50} of 100 and 43 nM in U87MG and MCF7, respectively) and pRPS6 (mTORC1 inhibition, IC_{50} of 150 and 230 nM in U87MG and MCF7) levels in cells. When administered at dose levels above 50 mg/kg to mice tumor bearing animals, the compound resulted in stable disease in MCF7 and U87MG models (Davies et al. 2008).

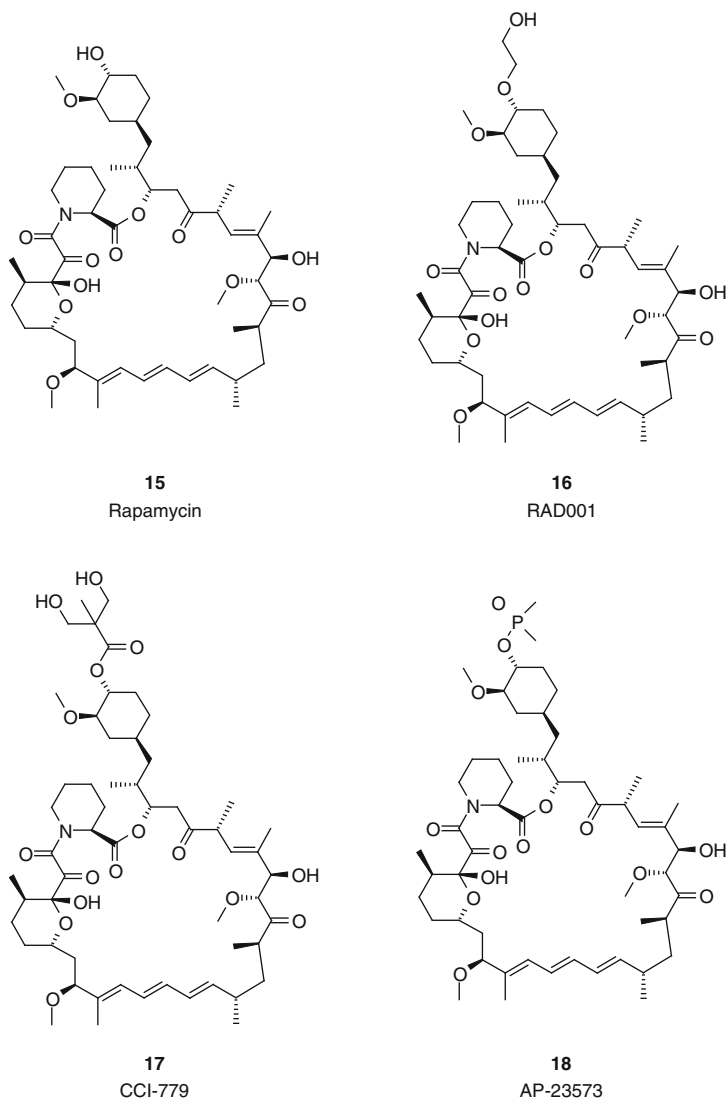


Fig. 3 Allosteric inhibitors of mTOR

AZD8055 is an mTOR catalytic that is currently being developed by Astra Zeneca. The compound entered Phase I trials in patients with solid tumors, in July 2008. The structure has not yet been disclosed but it is likely that this molecule belongs to the same chemical series as KU-0063794. The profile of this molecule is very similar to KU-0063794 (IC_{50} of 3,590, 18,900, 3,200, and $>14,780$ nM for PI3K α , β , δ , and γ , respectively, and 0.8 nM for mTOR). Differences are seen *in vivo*, as efficacy (tumor regression) is achieved for dose levels of 10 mg given

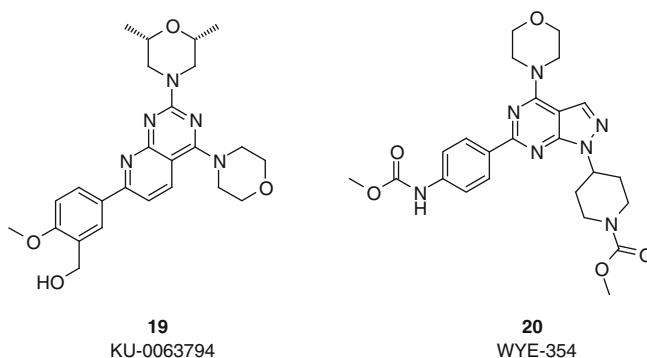


Fig. 4 ATP competitive inhibitors of mTOR

b.i.d or 20 mg once a day, orally given to U87MG tumor bearing animals (Chresta et al. 2009).

WYE-354 (compound 20, Fig. 4) is an optimized pyrazolopyrimidine (Zask et al. 2009a), originating from a high-throughput screen performed by Wyeth, and is highly selective for mTOR (IC_{50} of 5 nM) over Class I PI3K (>100- to 500-fold versus p110 α and p110 γ , respectively). Similar to KU-063794, WYE-354 potently shuts off mTORC1 and mTORC2 signaling in cells, resulting in profound protein translation and subsequent cell cycle arrest in G1. These effects were superior to those observed with mTORC1 allosteric inhibitors such as rapamycin, in agreement with other reports (Feldman et al. 2009; Thoreen et al. 2009). Long term exposure to the compound also induced cell death in some of the cell lines tested such as the HER2 positive breast cancer line MDA-MB361 cells. Finally, when given by the i.p. route, b.i.d, to PC3 tumor bearing animals, WYE-354 caused tumor stasis at a dose of 50 mg/kg (Yu et al. 2009). WYE-132 (structure not disclosed) is the clinical development candidate probably from the same chemical class, and with a similar profile to WYE-354 (subnanomolar activity against mTOR, 6,000 selectivity over the class I PI3K's). WYE-132 was shown to shrink MB-361 tumors in mice at a dose of 20 mg/kg, given orally, once a day, showing its superiority with regard to biopharmaceutical and pharmacokinetic properties compared to WYE-354 (Zask et al. 2009b).

The structure of OXA-01 has not been disclosed, but the generic scaffold is a pyrrolo[2,3-d]-pyrimidine, reminiscent of the one used for the identification of the IGF1-R inhibitor OSI-906. The compound is a potent inhibitor of mTOR (IC_{50} : 29 nM), and very selective over p110 β and the class IV DNAPK (IC_{50} > 30,000 and 3,200 nM, respectively). The compound inhibits mTORC1 and mTORC2 effectors in cells but the activity is modest with respect to downstream markers of pathway activity (IC_{50} of 1.1, 1.7 and 1.7 μ M against p4EBP1, pRPS6, and pAkt, respectively). Not surprisingly, OXA-01 displays modest anti-proliferative activities (IC_{50} s ranging from 0.5 to 4.5 μ M). In *in vivo* studies, OXA-01 achieved a T/C of 40% when administered to BT474 tumor bearing mice, at a dose of

100 mg/kg once a day, and regression is observed at a dose of 75 mg/kg, twice a day (Bhagwat et al. 2008). It is not clear if this compound has yet reached Phase I clinical trials, and if the compound claimed to be in development OSI-027 is actually OXA-01. These compounds are being developed by OSI Pharmaceuticals.

Exelixis is currently working on the selection of mTOR catalytic inhibitors for further clinical development. No information is available yet either in terms of chemical series and structure. Preclinical profile has been presented for a selection of four molecules (Miller 2008) but no information regarding potential Phase I trials with these molecules have been presented up to now.

4 Akt Kinase Inhibitors and Perifosine

After the discovery of Akt, non-selective Akt inhibitors based on staurosporine (compound 21, Fig. 5; $IC_{50} = 48$ to 11 nM for Akt-1) and derivatives thereof were extensively used as tool compounds to elucidate the role of this kinase in the biology of human cancers (Li and Zhu 2002; Kumar and Madison 2001). As for other serine/threonine kinases, the identification of potent and selective Akt kinase inhibitors has proven particularly difficult and only recently the first compounds with some level of selectivity for Akt have entered clinical trials. As shown in this section, the acute hyperglycemia observed in animal models has raised concerns

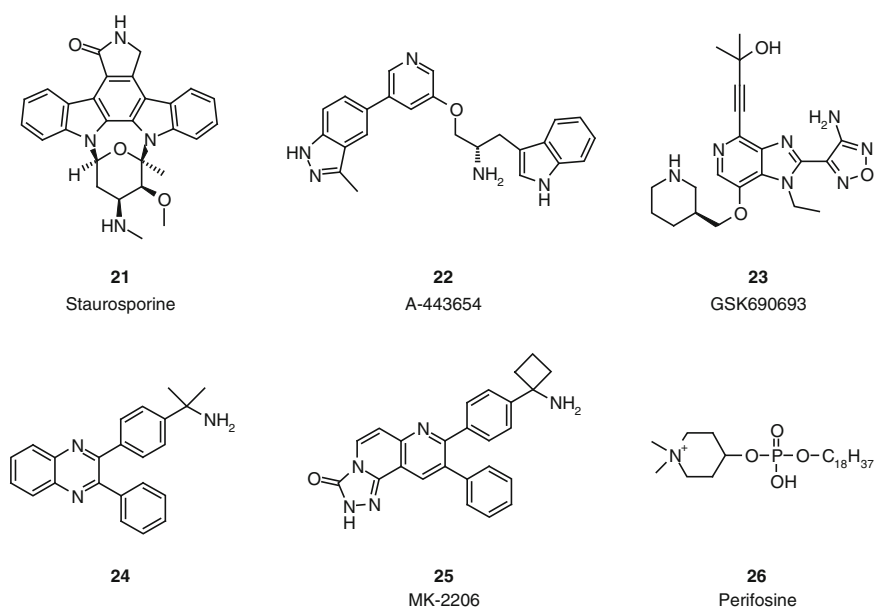


Fig. 5 Selected examples of Akt inhibitors and structure of perifosine

that the therapeutic application of Akt inhibitors will be limited by mechanism-based metabolic toxicities.

A-443654 (compound 22, Fig. 5) is a potent Akt modulator ($K_i = 160$ pM for Akt1) that shows some level of selectivity against other members of the AGC family: PKA, 40-fold; PKC γ , 150-fold; PKC δ , 200-fold, and PDK1, >120,000-fold (Luo et al. 2005). As determined by X-ray crystallography, the reported differences in potency of A-443654 for Akt compared to PKA seem to arise as a result of one of the three key residue differences between the ATP-binding clefts: namely the presence of methionine-282 in place of leucine at the base of the Akt cleft (Davies et al. 2007). The change in the bulkiness of the side-chain allows the inhibitor to adopt a folded, energetically favorable conformation where it can form increased lipophilic contacts with the enzyme.

The compound decreases the phosphorylation of Akt downstream targets in cells (e.g., GSK3 α/β , FOXO3, TSC2, and mTOR) and *in vivo* in a dose-dependent manner. Interestingly, these biological effects appear to induce significant Akt Ser-473 phosphorylation in human cancer cell lines, including PTEN- and TSC2-deficient cell lines (Han et al. 2007). Studies performed with catalytically inactive mutants of Akt showed that binding of a low molecular mass compound to the ATP-binding cleft is sufficient to cause the observed phosphorylation of the regulatory amino acids. Additional experiments to determine the mechanism(s) involved in this experimental observation and the biological consequences of this occurrence show that Akt ATP-competitive inhibitors impart regulatory phosphorylation of their target kinase (Okuzumi et al. 2009). Although not fully proven this can be due to a change in the conformation of the protein that makes it (1) more susceptible to kinase phosphorylation and/or (2) less prone to dephosphorylation by phosphatases. The impact of this finding on the development of Akt-modulators is unclear at this point in time.

In preclinical *in vivo* efficacy experiments, the compound showed significant anti-tumor activity as a single agent and in combination regimens in a number of human tumor xenografts, but the dosing period was limited due to malaise and weight loss (Shi et al. 2005). Recently, compounds with improved potency, selectivity, and cardiovascular safety as compared with A-443654 and derivatives thereof have been reported by Abbott (Zhu et al. 2007), but none of these indazole-pyridine based compounds have entered clinical trials yet.

GSK690693 (compound 23, Fig. 5) is an ATP-competitive, pan-AKT kinase inhibitor ($IC_{50} = 2, 13$ and 9 nM for Akt-1, -2 and -3, respectively) that recently entered Phase I clinical trials (Rhodes et al. 2008). This aminofurazan derivative is also active ($IC_{50} < 80$ nM) against members of the AGC, CAMK, and group II PAK kinase families that can contribute to its reported pre-clinical anti-tumor activity. Thus, the compound suppresses the proliferation of multiple human tumor cell lines, including breast carcinoma BT-474 and SK-BR-3, and prostate cancer LNCaP ($IC_{50} = 0.021$ – 0.298 μ M). A single intraperitoneal dose treatment of SCID mice bearing BT474 tumors with GSK690693 (20 mg/kg) resulted in acute hyperglycemia with blood glucose levels returning to baseline as the circulating drug concentration decreases. A rapid elevation of plasma insulin levels was also

observed, reaching an 880-fold increase in relation to the baseline level at 4 h post-dosing. Preclinical studies in mice to determine the mechanism of GSK690693 induced hyperglycemia have shown that this adverse effect is related to peripheral insulin resistance, increased gluconeogenesis, and/or glycogenesis. Specific dietary modifications (e.g., a low carbohydrate diet) or pre-dose fasting could reduce the magnitude of the increase in glucose and insulin. In spite of these observations, the compound was well tolerated in *in vivo* efficacy experiments with i.p. administration once daily and showed significant anti-tumor activity in human cancer xenografts (e.g., SKOV-3, LNCap, BT474 and HCC-1954).

A first in human, Phase I study to investigate the safety, tolerability, PK, and pharmacodynamics of GSK690693 given weekly or twice weekly (25, 50, 75 and 115 mg) as an i.v. infusion over 1–4 h is ongoing. Assessment of Akt inhibition in peripheral blood mononuclear cells and glucose levels in plasma indicated target modulation with doses of 50 mg and higher. In concordance with the pre-clinical AE findings, drug-related increases in blood glucose levels above 250 mg/dL have already been reported at the 50 and 75 mg doses (LoRusso et al. 2008). The compound is being developed by GlaxoSmithKline.

Other pockets besides the ATP-binding cleft have been exploited for the identification and development of selective Akt kinase modulators. It is notable that allosteric inhibitors of Akt containing the 2,3-diphenylquinoxaline or 5,6-diphenylpyrazin-2(1H)-one scaffolds have been described (Barnett et al. 2005; Zhao et al. 2005; Lindsley et al. 2005). The original hits were identified by screening around 270,000 compounds using a homogenous time-resolved fluorescence kinase assay. The Akt inhibitors exhibited a linear mixed-type inhibition against ATP and peptide substrate, showed isozyme selectivity, and were only active against the full length protein (e.g., compound 24, Fig. 5; IC_{50} 2.7 μ M for Akt1 versus $IC_{50} > 250$ μ M for Δ PH-Akt1). Although the mechanism of inhibition by these allosteric kinase inhibitors has not been fully elucidated and there is no structural data yet, it seems that these molecules may bind outside the ATP-binding pocket, interacting with the PH domain and/or hinge region likely promoting the formation of an inactive kinase conformation.

Several follow-up papers have illustrated medicinal chemistry efforts directed to improving the potency and drug-like properties of this compound class (Zhao et al. 2008; Hartnett et al. 2008). This optimization work resulted in the identification of MK-2206 (compound 25, Fig. 5), which has recently entered Phase I clinical trials. This compound, which is developed by Merck & Co, inhibits the kinase activity of Akt isozymes 1, 2, and 3 with IC_{50} values of 5, 12, and 65 nM, respectively. As for the previous precursors, MK-2206 is dependent on the Pleckstrin homology domain for activity, and was highly selective for Akt, with no inhibitory activity observed against a panel of 250 protein kinases when profiled at 1 μ M. When tested against a panel of human cancer cell lines, additive or synergistic interactions were found with MK-2206 and cytotoxic agents with different mechanisms of action (e.g., doxorubicin, camptothecin, gemcitabine, 5-FU, docetaxel, and carboplatin) and targeted anticancer agents (e.g., erlotinib or lapatinib). Oral administration at 240 mg/kg was associated with significant inhibition of Akt in A2780 ovarian

s.c. xenografts in mice. The same oral dose when administered three times a week inhibited tumor growth by 60%.

Single doses of MK-2206 in the range of 0.25–100 mg were generally well tolerated in healthy volunteers enrolled in a randomized, double-blind, placebo-controlled study. The most common adverse events were headache, common cold, and diarrhea, but no serious clinical adverse events were reported. Pharmacokinetic evaluation yielded a T_{\max} of 6–8 h, median half-life of 55–78 h, and dose-proportional exposure for doses between 2 and 100 mg. Single doses of 40, 80, and 100 mg inhibited Akt in whole blood, with maximum inhibition obtained at 6 h post-dose for the 80 and 100 mg doses. Akt inhibition lasted as long as 24 h. The compound has also been tested in a safety and tolerability Phase-I clinical trial in cancer patients. In this study, the compound was administered q.o.d in 28-days cycles at 30, 60, 75, and 90 mg. Dose escalation is complete and the 60 mg q.o.d has been identified as the MTD since CTCAE G3/4 skin rash and CTCAE G3 mucositis were reported at higher doses. The PK parameters are similar to the ones identified in the healthy volunteer study and evidence of target modulation was observed in whole blood and included reversible CTCAE G1/2 hyperglycemia and CTCAE G1 insulin c-peptide elevation. The compound is currently undergoing Phase I studies to compare safety and tolerability at two different doses in combination with chemotherapy and targeted therapy agents (e.g., carboplatin + paclitaxel, docetaxel, or erlotinib) in patients with locally advanced or metastatic solid tumors.

It is important to note that these allosteric inhibitors are slightly less potent than the classical ATP-competitive inhibitors in blocking the kinase activity of a recently reported Akt1 mutant found in breast cancer samples. The mutated protein contains a lysine amino acid instead of glutamic acid at position 17 in the lipid-binding pocket of Akt1 (Carpten et al. 2007). This single amino acid mutation leads to constitutive association of Akt1 with the plasma membrane, constitutive activation of the pathway, and induces leukemia in mice. In spite of relatively low frequency, the sensitivity of this mutant to the currently reported Akt modulators, including the allosteric inhibitors reported in the preceding paragraphs, may serve as a clinically relevant predictive marker for directing Akt drug development.

In addition to directly targeting the kinase activity of Akt, alternative therapeutic modes have been pursued to control the enzymatic activity of this protein. Thus, phospholipid containing molecules have also been used to modulate membrane function and signaling targets that use naturally occurring lipid moieties as substrates or co-factors. Perifosine (KRX-0401/NSC 639966/D-21266 compound 26, Fig. 5), which is an alkylphosphocholine structurally related to miltefosine, appears to block the activation and phosphorylation of Akt in cellular settings (Kondapaka et al. 2003; Ruitter et al. 2003). Although the mechanism of action of perifosine, which is currently developed by Keryx Biopharmaceuticals, is not fully understood, one hypothesis is that, following insertion into the intracellular membrane, perifosine interferes with Akt membrane localization by inhibiting the association of its PH domain with $\text{PtdIns}(3,4,5)\text{P}_3$. In keeping with this notion, perifosine blocks Akt plasma membrane localization as assessed by immuno-fluorescence imaging (Kondapaka et al. 2003).

Perifosine has marked cytotoxic effects on human tumor cell lines when used alone or in combination with targeted anticancer agents (Dasmahapatra et al. 2004; Li et al. 2006; Huston et al. 2008). Moreover, immuno-modulation may partly contribute to the anticancer activity of this compound (Safa et al. 1998). Despite these data, the specific anti-tumor mechanisms of perifosine and its relationship to Akt inhibition remain unclear.

An extensive Phase II clinical program is currently under way with perifosine, including trials in breast, head and neck, kidney, lung, prostate, renal cell carcinoma, glioma, leukemia, sarcoma, and multiple myeloma. Nausea, vomiting, and diarrhea (grade 1-3) have been described as the most common side effects. Although encouraging evidence of anti-tumor activity was reported in Phase I clinical trials, no major clinical breakthrough has been reported yet.

5 ATPase Inhibitors of Hsp90

As an alternative to targeting the enzymatic function of components of the PI3K pathway directly, inhibitors of heat-shock protein 90 (Hsp90) have been pursued to block the abnormal activation of the PI3K pathway in cancer cells. Heat-shock proteins are ATP-dependent molecular chaperones involved in the conformational maturation, stability, and function of a selected range of substrates, the so-called “client proteins” (Whitesell and Lindquist 2005). The Hsp90 family of chaperones is composed of four isoforms: Hsp90 α , Hsp90 β , GRP94, and TRAP-1. The 90KDa chaperone binds to “client proteins” in the presence of other partner proteins to produce a multi-protein complex that folds the target substrate into its biologically active conformation(s). Binding and release of Hsp90 “client proteins” is regulated by the activity of the *N*-terminal ATPase domain and is driven by the hydrolysis of ATP to ADP and by ATP/ADP exchange.

Many of the proteins that interact with Hsp90 are key players in signal transduction pathways that are essential to mediate and sustain tumor cell growth and survival. For example, a functional Hsp90 multi-chaperone complex is required for the correct folding and stability of Akt and PDK1 (Basso et al. 2002; Fujita et al. 2002; Solit et al. 2003), which are key components of the PI3K pathway. Loss of chaperone function causes client proteins to be degraded by the ubiquitin-proteasome pathways. Given the prevalence of a high-affinity form of Hsp90 in tumor cells and the oncogenic addiction of cancer cells to certain client proteins of hsp90, inhibition of Hsp90 has emerged as a possible strategy for the treatment of human tumors. This section is focused on the identification and clinical development of several structurally diverse inhibitors that target the *N*-terminal ATP binding cleft of Hsp90 (for a recent review on this topic, see Bishop et al. 2007).

As for other therapeutic targets covered in this chapter, the initial discovery of the potential use of Hsp90 inhibitors as novel anticancer agents was based on two natural products, geldanamycin (compound 27, Fig. 6) and radicicol (also called monorden, compound 28, Fig. 6). The successful preclinical target validation

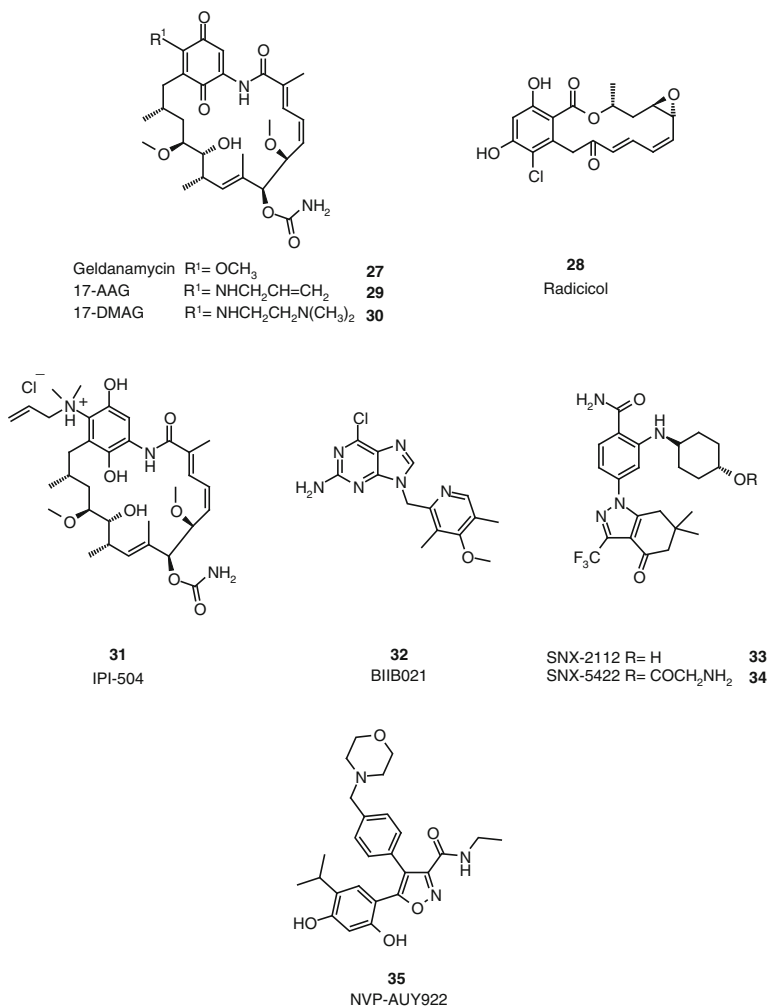


Fig. 6 Selected examples of Hsp90 inhibitors

achieved with these tool compounds has catalyzed the identification of derivatives with improved pharmaceutical properties and better toxicological profile. In the case of geldanamycin, extensive medicinal chemistry efforts have been made to replace the 17-methoxy substituent and one of these derivatives, 17-allylamino-17-demethoxygeldanamycin (17-AAG, compound 29, Fig. 6) (Jia et al. 2003) has been the first Hsp90 modulator to enter Phase I clinical trials (Phase I studies are reviewed in Egorin et al. 2002). In spite of its relative low affinity to Hsp90 (Chiosis et al. 2003), a considerable amount of understanding has been obtained from the clinical experience of this compound using several intravenous formulations. Thus, KOS953 (tanespimycin; Kosan Biosciences), which contains a proprietary form of

17-AAG in a novel, optimized formulation, has achieved clinical proof-of-concept with trastuzumab. This combination was well tolerated and tumor regressions were observed exclusively in patients with human epidermal growth factor receptor 2 (erbB2)-positive metastatic breast cancer whose disease progressed following treatment with trastuzumab (clinical benefit rate was 57%) (Modi et al. 2007). Phase III monotherapy and combination trials with bortezomib are currently undergoing in relapse and refractory multiple myeloma cancer patients. 17-AAG has also been formulated in an oil-in-water nanoemulsion (CNF1010; Conforma Therapeutics), but this alternative delivery system is not currently pursued.

Additional geldanamycin analogs with increased potency, chemical/metabolic stability, and formulation options have been reported. Included among these are, 17-(2-dimethylaminoethyl)-amino-17-demethoxygeldanamycin (17-DMAG, KOS1022/Kosan Biosciences; alvespimycin; compound 30, Fig. 6) (Tian et al. 2004) and IPI-504 (compound 31, Fig. 6) each having entered clinical trials. The clinical development of this last compound is still ongoing, while the progression of alvespimycin was halted in March 2008 due to its unfavorable overall toxicity profile.

IPI-504, which is a hydroquinone hydrochloride derivative of 17-AAG, interconverts with 17-AAG *in vitro* and *in vivo* via an oxidation/reduction equilibrium (Ge et al. 2008). It shows a comparable binding affinity to Hsp90 ($EC_{50} = 63$ nM) and Grp94 ($EC_{50} = 119$ nM), and potently inhibits the growth of tumor cell lines that overexpress Hsp90 client protein Her2 (e.g., SKBR3, $GI_{50} = 22$ nM and SKOV3, $GI_{50} = 52$ nM). It is preferentially retained in tumor tissues relative to plasma, which seems to be a general feature of all Hsp90 modulators. The compound is efficacious alone (e.g., 100 mg/kg 2xw) and in combination with the 20S proteasome inhibitor bortezomib when administered i.v. to mice bearing the RPMI-8226 multiple myeloma model (Sydor et al. 2006). On the basis of its significant improvement in water solubility (>250 mg/ml vs. 50 μ g/ml) and pharmacological properties in relation to 17-AAG, IPI-504 entered Phase I clinical trials in 2005, and its i.v. formulation is currently in advanced clinical trials. Clinical activity (as defined by PET response) has been reported for IPI504 in advanced non-small cell lung cancer tumors and in Gastro-intestinal stromal tumors (GIST). Orphan drug designation was assigned to the compound by the FDA for the treatment of GIST, but, in spite of early signs of therapeutic activity (as defined by PET response), the development of IPI-504 was discontinued for this indication due to a higher than anticipated mortality rate in the Phase III trial that was initiated in August 2008. Phase II trial to evaluate IPI-504 with trastuzumab in patients with pretreated, locally advanced, or metastatic erbB positive breast cancer is ongoing. This compound is being developed by Infinity Pharmaceuticals.

Despite the clinical activity and improvements in pharmacological properties, several drawbacks of the current geldanamycin derivatives, including instability in solution, drug resistance, hepatotoxicity, and variable pharmacokinetics, may limit their optimal clinical development. To address the preceding limitations, novel Hsp90 inhibitor chemotypes based on structure-based design and high-throughput screening approaches have been identified (Bishop et al. 2007; Janin 2005).

Representative examples of low-molecular mass Hsp90 modulators that have entered clinical trials are described in the following paragraphs.

BIIB021 (originally called CNF2024; compound 32, Fig. 6) is a synthetic orally-available Hsp90 modulator ($IC_{50} = 30$ nM) that entered clinical trials in October 2005 (Lundgren et al. 2007, 2009). BIIB021 promotes the degradation of the Hsp90 client protein HER-2 with an EC_{50} of 38 nM in MCF-7 cells and inhibits tumor growth at tolerated doses (e.g., 125 mg/kg p.o., qdx5) in human tumor xenograft models expressing high levels of HER2 (e.g., N87 and BT474 87% and 94% tumor growth inhibition; respectively). CNF2024 is in Phase II clinical trials for the treatment of GIST, Phase I trials in advanced solid tumors and B-cell chronic lymphocytic leukemia, and in Phase Ib/II in combination with trastuzumab in patients with advanced breast cancer. Preliminary results indicate generally good tolerance and evidence of biological activity. The compound is being developed by Biogen Idec.

The SNX-2112 scaffold was identified using a protein affinity-displacement assay. A compound library was tested and molecules that displaced Hsp90 family members from the purine-based affinity resin were then identified by mass spectrometry. SNX-2112 (compound 33, Fig. 6) binds to Hsp90 α and β with low nanomolar affinity ($IC_{50} = 30$ nM), with moderate selectivity over the other hsp90 family members ($IC_{50} = 862$ and 4,275 nM for Trap-1 and Grp94, respectively) (Chandarlapaty et al. 2008; Okawa et al. 2009). As in the case of geldanamycin and derivatives thereof, SNX-2112 significantly inhibits the proliferation of tumor cell lines by inducing the degradation of relevant client proteins. SNX-5422 (compound 34, Fig. 6), which is a water soluble prodrug of SNX-2112, can be administered orally to tumor bearing mice and is rapidly converted to SNX-2112 where it preferentially accumulates in tumor tissues. Anti-tumor activity as a single agent (50 mg/kg, p.o., qdx5) was reported in BT474 and H1650 xenografts (partial regression and 40% inhibition of tumor growth, respectively). SNX-5422 has entered Phase I clinical trials, but no additional information is available for this compound. The compound was originally discovered by Serenex and is being developed by Pfizer.

NVP-AUY922 (compound 35, Fig. 6) is one of the most potent synthetic small-molecular mass hsp90 inhibitor yet described ($K_i = 9.0$ and 8.2 nM for Hsp90 α and β , respectively) (Brough et al. 2008; Eccles et al. 2008; Stümer et al. 2008). This isoxazole derivative resulted from the structure-based elaboration of a hit 4, 5-diarylpyrazole scaffold identified by a medium high-throughput screening campaign. NVP-AUY922 inhibits the proliferation of a wide range of human tumor cell lines with an average GI_{50} value of 9 nM, and induces marked accumulation in either G_1 or G_1 plus G_2 -M phases in most cell lines (Eccles et al. 2008). Daily dosing of NVP-AUY922 (50 mg/kg i.p. or i.v.) to mice bearing human tumor xenografts with diverse oncogenic profiles produced statistically significant growth inhibition and/or regression, and significant target inhibition evidenced by depletion of client proteins and induction of hsp72. Significant antitumor activity was also observed when the compound was administered on a weekly i.v. schedule (Jensen et al. 2008). NVP-AUY922 is currently in Phase I/II studies in patients with

a range of cancer indications. The compound is administered i.v. on a once a week schedule. FDG-PET scans have demonstrated partial metabolic responses at well tolerated doses. The compound was originally identified by Vernalis and is currently being developed by Novartis Pharma AG.

STA-9090 (structure not disclosed, Synta Pharmaceuticals) is a novel Hsp90 inhibitor based on the triazolone scaffold. As the other compounds covered in this section, it induces the proteasome degradation of multiple Hsp90 client protein and significantly inhibits the *in vitro* and *in vivo* growth of tumor cell lines (Lin et al. 2008). The compound is undergoing Phase I clinical trials.

Other compound that are currently undergoing Phase I clinical trials are KW2578 (Kyowa Hakko), AT13387 (Astex Therapeutics), and HSP990 (Novartis Pharma AG); structures not disclosed.

6 Outlook

The development of modulators of the PI3k/Akt/mTOR pathway either as single agents or in combination with inhibitors of other oncogenic pathways is progressing rapidly. Based on known roles of the components of this pathway in diverse biological functions including glucose homeostasis, cellular proliferation, and immune cell function, there are obvious safety signals that will need to be monitored in the clinic. In the case of PI3K, the first inhibitors to enter the clinic inhibit multiple isoforms although they have exquisite selectivity over protein kinases and, to less extent, over lipid kinases. This broad spectrum activity will guide our understanding of the liabilities associated with the inhibition of PI3K isoforms and may facilitate the development of inhibitors for non-cancer indications such as chronic inflammatory conditions. In addition to PI3K, other intracellular components of this pathway have been successfully targeted leading to current panoply of clinical trials of inhibitors of Akt and mTOR in man, including Hsp90, which is an indirect way to block the preceding targets. The clinical efficacy and tolerability of this new generation of anticancer agents are eagerly awaited.

References

- Abraham RT, Eng CH (2008) Mammalian target of rapamycin as a therapeutic target in oncology. *Expert Opin Ther Targets* 12:209–222
- Ali K, Bilancio A, Thomas M, Pearce W, Gilfillan AM, Tkaczyk C, Kuehn N, Gray A, Giddings J, Peskett E, Fox R, Bruce I, Walker C, Sawyer C, Okkenhaug K, Finan P, Vanhaesebroeck B (2004) Essential role for the p110 δ phosphoinositide 3 kinase in the allergic response. *Nature* 431:1007–1011
- Ali K, Camps M, Pearce WP, Ji H, Ruckle T, Kuehn N, Pasquali C, Chabert C, Rommel C, Vanhaesebroeck B (2008) Isoform specific functions of phosphoinositide 3 kinases: p110 δ but not p110 γ promotes optimal allergic responses *in vivo*. *J Immunol* 180:2538–2544

- Amzel LM, Huang CH, Mandelker D, Lengauer C, Gabelli SB, Vogelstein B (2008) Structural comparisons of class I phosphoinositide 3 kinases. *Nat Rev Cancer* 8:665–669
- Auger KR, Luo L, Knight S, Van Aller G, Tummino PJ, Copeland RA, Diamond M, Sutton D, Jackson JR, Dhanak D (2008) A novel inhibitor of phosphoinositide 3 kinase for the treatment of cancer (poster). Presented at 20th EORTC NCI AACR symposium on molecular targets and cancer therapeutics, Geneva, Switzerland, 21–24 October 2008
- Bachman KE (2008) GSK615: A novel inhibitor of phosphoinositide 3 kinase for the treatment of cancer. Presented at AACR special conference in cancer research Targeting the PI3 Kinase pathway in cancer, Cambridge, USA, 11–14 November 2008
- Barber DF, Bartolome A, Hernandez C, Flores JM, Redondo C, Fernandez Arias C, Camps M, Ruckle T, Schwarz MK, Rodriguez S, Martinez A, Balomenos D, Rommel C, Carrera AC (2005) PI3K γ inhibition blocks glomerulonephritis and extends lifespan in a mouse model of systemic lupus. *Nat Med* 11:933–935
- Barnett SF, Defeo Jones D, Fu S, Hancok PJ, Haskell K, Jones RE, Kahana JA, Kral AM, Leander K, Lee LL, Malinowski J, McAvoy EM, Nahas DD, Robinson RG, Huber HE (2005) Identification and characterization of pleckstrin homology domain dependent and isozyme specific Akt inhibitors. *Biochem J* 385:399–408
- Basso AD, Solit DB, Munster PN, Rosen N (2002) Ansamycin antibiotics inhibit Akt activation and cyclin D expression in breast cancer cells that overexpresses HER2. *Oncogene* 21:1159–1166
- Baumann P, Mandl Weber S, Oduncu F, Schmidmaier R (2009) The novel orally bioavailable inhibitor of phosphoinositid 3 kinase and mammalian target of rapamycin, NVP BEZ235, inhibits growth and proliferation in multiple myeloma. *Exp Cell Res* 315:485–497
- Bhagwat SV, Crew AP, Gokhale PC, Cooke A, Kahler J, Yao Y, Chan A, Arnold LD, Wild R, Pachter JA (2008) Cellular characterization of OXA 0, a potent and selective dual mTORC1 and mTORC2 kinase inhibitor. Presented at 20th EORTC NCI AACR symposium on molecular targets and cancer therapeutics, Geneva, Switzerland, 21–24 October 2008
- Bi L, Okabe I, Bernard DJ, Wynshaw Boris A, Nussbaum RL (1999) Proliferative defect and embryonic lethality in mice homozygous for a deletion in the p110 α subunit of phosphoinositide 3 kinase. *J Biol Chem* 274:10963–10968
- Bi L, Okabe I, Bernard DJ, Nussbaum RL (2002) Early embryonic lethality in mice deficient in the p110 β catalytic subunit of PI 3 kinase. *Mamm Genome* 13:169–172
- Bishop SC, Burlison JA, Blagg BJS (2007) Hsp90: a novel target for the disruption of multiple signaling cascades. *Curr Med Chem* 7:369–388
- Brough PS, Aherne W, Barril X et al (2008) 4, 5 Diaryl isoxazole hsp90 chaperone inhibitors: potential therapeutic agents for the treatment of cancer. *J Med Chem* 51:196–218
- Camps M, Ruckle T, Ji H, Ardisson V, Rintelen F, Shaw J, Ferrandi C, Chabert C, Gillieron C et al (2005a) Blockade of PI3K γ suppresses joint inflammation and damage in mouse models of rheumatoid arthritis. *Nat Med* 11:936–943
- Camps M, Ruckle T, Ji H, Ardisson V, Rintelen F, Shaw J, Ferrandi C, Chabert C, Gillieron C, Francon B, Martin T, Gretener D, Perrin D, Leroy D, Vitte PA, Hirsch E, Wymann MP, Cirillo R, Schwarz MK, Rommel C (2005b) Blockade of PI3K γ suppresses joint inflammation and damage in mouse models of rheumatoid arthritis. *Nat Med* 11:936–943
- Cao P, Maira SM, Garcia Echeverria C, Hedley DW (2009) Activity of a novel, dual PI3 kinase/mTor inhibitor NVP BEZ235 against primary human pancreatic cancers grown as orthotopic xenografts. *Br J Cancer* 100:1267–1276
- Carpten JD, Faber AL, Horn C, Donoho GP, Briggs SL, Robbins CM, Hostetter G, Boguslawski S et al (2007) A transforming mutation in the pleckstrin homology domain of Akt1 in cancer. *Nature* 448:439–444
- Chandarlapaty S, Sawai A, Ye Q, Scott A, Silinski M, Huang K, Fadden P, Partridge J, Hall S, Steed P, Norton L, Rosen N, Solit DB (2008) SNX2112, a synthetic heat shock protein 90 inhibitor, has potent antitumor activity against HER kinase dependent cancers. *Clin Cancer Res* 14:240–248

- Chiorean EG, Mahadevan D, Harris WB, Von Hoff DD, Younger AE, Rensvold DM, Shelton CF, Hennessy BT, Garlich JR, Ramanathan RK (2009) Phase I evaluation of SF1126, a vascular targeted PI3K inhibitor, administered twice weekly IV in patients with refractory solid tumors. *J Clin Oncol (Meeting Abstracts)* 27:2558
- Chiosis G, Huezio H, Rosen N, Mimnaugh E, Whitesell L, Neckers L (2003) 17AAG: Low Target Binding Affinity and Potent Cell Activity—Finding an Explanation. *Mol Cancer Ther* 2:123–129
- Chresta CM, Davies BR, Harding T, Hickson I, Cosulich S, Crichtlow S, Sini P, James D, Malagu K, Hummerson M, Smith GCM, Pass M, Guichard M (2009) Characterization of AZD8055, a potent selective inhibitor of mTORC1 and mTORC2 kinase (poster). Presented at 100th AACR annual meeting, Denver, USA, 18–22 April 2009
- Ciraolo E, Iezzi M, Marone R, Marengo S, Curcio C, Costa C, Azzolino O, Gonella C, Rubinetto C, Wu H, Dastru W, Martin EL, Silengo L, Altruda F, Turco E, Lanzetti L, Musiani P, Ruckle T, Rommel C, Backer JM, Forni G, Wymann MP, Hirsch E (2008) Phosphoinositide 3 kinase p110beta activity: key role in metabolism and mammary gland cancer but not development. *Sci Signal* 1:ra3
- Crowder RJ, Phommaly C, Tao Y, Hoog J, Luo J, Perou CM, Parker JS, Miller MA, Huntsman DG, Lin L, Snider J, Davies SR, Olson JA Jr, Watson MA, Saporita A, Weber JD, Ellis MJ (2009) PIK3CA and PIK3CB inhibition produce synthetic lethality when combined with estrogen deprivation in estrogen receptor positive breast cancer. *Cancer Res* 69:3955–3962
- Dasmahapatra GP, Didolkar P, Alley MC, Ghosh S, Sausville EA, Roy KK (2004) *In vitro* combination treatment with perifosine and UCN 01 demonstrates synergism against prostate (PC 3) and lung (A549) epithelial adenocarcinoma cell lines. *Clin Cancer Res* 10:5242–5252
- Davies TD, Verdonk ML, Graham B, Saalau Bethell S, Hamlett CCF, McHardy T, Collins I, Garrett MD, Workman P, Woodhead SJ, Jhota H, Barford D (2007) A structural comparison of inhibitor binding to PKB, PKA and PKA/PKB chimera. *J Mol Biol* 367:882–894
- Davies BR, Chresta CM, Dudley P, Hewitt N, Brady M, Jacobsen M, Hickson I, Martin NMB, Smith GCM, Pass M (2008) Pharmacodynamics and anti tumor activity of KU 0063794, a potent and specific inhibitor of mTOR kinase. Presented at 20th EORTC NCI AACR symposium on molecular targets and cancer therapeutics, Geneva, Switzerland, 21–24 October 2008
- Domin J, Waterfield MD (1997) Using structure to define the function of phosphoinositide 3 kinase family members. *FEBS Lett* 410:91–95
- Dubrovskaya A, Kim S, Salamone RJ, Walker JR, Maira SM, Garcia Echeverria C, Schultz PG, Reddy VA (2009) The role of PTEN/Akt/PI3K signaling in the maintenance and viability of prostate cancer stem like cell populations. *Proc Natl Acad Sci USA* 106:268–273
- Eccles S, Massey A, Raynaud FI, Sharp SY, Bax G, Valenti M, Patterson L, Ad B, Gowan S, Boxall F, Aherne W, Rowlands M et al (2008) NVP-AUY922: a novel heat shock protein 90 inhibitor active against xenograft tumor growth, angiogenesis, and metastasis. *Cancer Res* 68:2850–2860
- Egorin MJ, Parise RA, Joseph E, Hamburger DR, Lan J, Covey JM, Eiseman JL (2002) Plasma pharmacokinetics and bioavailability of the phosphatidylinositide 3 kinase signalling inhibitor, OMDPI (NSC 710297), in CD2F1 mice. *Proc Am Assoc Cancer Res* 43:abstract 2996
- Eichhorn PJ, Gili M, Scaltriti M, Serra V, Guzman M, Nijkamp W, Beijersbergen RL, Valero V, Seoane J, Bernards R, Baselga J (2008) Phosphatidylinositol 3 kinase hyperactivation results in lapatinib resistance that is reversed by the mTOR/phosphatidylinositol 3 kinase inhibitor NVP-BEZ235. *Cancer Res* 68:9221–9230
- Engelman JA, Chen L, Tan X, Crosby K, Guimaraes AR, Upadhyay R, Maira M, McNamara K, Perera SA, Song Y, Chirieac LR, Kaur R, Lightbown A, Simendinger J, Li T, Padera RF, Garcia Echeverria C, Weissleder R, Mahmood U, Cantley LC, Wong KK (2008) Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. *Nat Med* 14:1351–1356

- Feldman ME, Apsel B, Uotila A, Loewith R, Knight ZA, Ruggero D, Shokat KM (2009) Active site inhibitors of mTOR target rapamycin resistant outputs of mTORC1 and mTORC2. *PLoS Biol* 7:e38
- Flinn IW, Byrd JC, Furman RR, Brown JR, Lin TS, Bello C, Giese NA, Yu AS (2009) Preliminary evidence of clinical activity in a phase I study of CAL 101, a selective inhibitor of the p1108 isoform of phosphatidylinositol 3 kinase (PI3K), in patients with select hematologic malignancies. *J Clin Oncol (Meeting Abstracts)* 27:3543
- Folkes A, Ahmadi K, Alderton WK, Alix S, Baker SJ, Box G, Chuckowree IS, Clarke PA, Depledge P, Eccles SA, Friedman LS, Hayes A et al (2008) The identification of 2 (1H indazol 4 yl) 6 (4 methanesulfonyl 1 piperazin 1 ylmethyl) 4 morpholin 4 yl thieno[3,2 d] pyrimidine (GDC 0941) as a potent, selective, orally bioavailable inhibitor of class I PI3 Kinase for the treatment of cancer. *J Med Chem* 51:5522 5532
- Fougerat A, Gayral S, Gourdy P, Schambourg A, Ruckle T, Schwarz MK, Rommel C, Hirsch E, Arnal JF, Salles JP, Perret B, Breton Douillon M, Wymann MP, Laffargue M (2008) Genetic and pharmacological targeting of phosphoinositide 3 kinase gamma reduces atherosclerosis and favors plaque stability by modulating inflammatory processes. *Circulation* 117:1310 1317
- Foukas LC, Claret M, Pearce W, Okkenhaug K, Meek S, Peskett E, Sancho S, Smith AJ, Withers DJ, Vanhaesebroeck B (2006) Critical role for the p110alpha phosphoinositide 3 OH kinase in growth and metabolic regulation. *Nature* 441:366 370
- Friedman L (2008) Biomarkers of PI3K inhibitor GDC 0941. Presented at AACR special conference in cancer research Targeting the PI3 Kinase pathway in cancer, Cambridge, USA, 11 14 November 2008
- Fujita N, Sato S, Ishida A, Tsuruo T (2002) Involvement of Hsp90 in signaling and stability of 3 phosphoinositide dependent kinase 1. *J Biol Chem* 277:10346 10353
- Garlich JR, De P, Dey N, Su JD, Peng X, Miller A, Murali R, Lu Y, Mills GB, Kundra V, Shu HK, Peng Q, Durden DL (2008) A vascular targeted pan phosphoinositide 3 kinase inhibitor prodrug, SF1126, with antitumor and antiangiogenic activity. *Cancer Res* 68:206 215
- Ge J, Normant E, Porter JR, Dembski MS, Gao Y, Georges AT, Grenier L et al (2008) Design, synthesis, and biological evaluation of hydroquinone derivatives of 17 Amino 17 demethoxygeldanamycin as potent, water soluble inhibitors of Hsp90. *J Med Chem* 49:4606 4615
- Giese NA (2008) Targeting PI3K p110 delta for the treatment of hematological malignancies. Presented at AACR special conference in cancer research Targeting the PI3 Kinase pathway in cancer, Cambridge, USA, 11 14 November 2008
- Greshock J (2008) In vitro sensitivity data identifies tumor types and molecular subtypes that predicts response to the PI3K inhibitor GSK1059615 and the Akt inhibitor GSK690693. Presented at AACR special conference in cancer research Targeting the PI3 Kinase pathway in cancer, Cambridge, USA, 11 14 November 2008
- Guillemet Guibert J, Bjorklof K, Salpekar A, Gonella C, Ramadani F, Bilancio A, Meek S, Smith AJ, Okkenhaug K, Vanhaesebroeck B (2008) The p110beta isoform of phosphoinositide 3 kinase signals downstream of G protein coupled receptors and is functionally redundant with p110gamma. *Proc Natl Acad Sci USA* 105:8292 8297
- Han E H, Levenson JD, McGonigal T, Shah OJ, Woods KW, Hunter T, Giranda VL, Luo Y (2007) Akt inhibitor A 443654 induces rapid Akt Ser 473 phosphorylation independent of mTORC1 inhibition. *Oncogene* 26:5655 5661
- Hartnett JC, Barnett SF, Bilodeau MT, Defeo Jones D, Hartman GD, Huber HE, Jones RE, Kral AM, Robinson RG, Wu Z (2008) Optimization of 2,3,5 trisubstituted pyridine derivatives as potent allosteric Akt1 and Akt2 inhibitors. *Bioorg Med Chem Lett* 18:2194 2197
- Hawkins PT, Anderson KE, Davidson K, Stephens LR (2006) Signalling through Class I PI3Ks in mammalian cells. *Biochem Soc Trans* 34:647 662
- Hoeflich KP, O'Brien C, Boyd Z, Cavet G, Guerrero S, Jung K, Januario T, Savage H, Punnoose E, Truong T, Zhou W, Berry L, Murray L, Amler L, Belvin M, Friedman LS, Lackner MR (2009) *In vivo* Antitumor Activity of MEK and Phosphatidylinositol 3 Kinase Inhibitors in Basal Like Breast Cancer Models. *Clin Cancer Res* 15:4649 4664

- Huang CH, Mandelker D, Schmidt Kittler O, Samuels Y, Velculescu VE, Kinzler KW, Vogelstein B, Gabelli SB, Amzel LM (2007) The structure of a human p110alpha/p85alpha complex elucidates the effects of oncogenic PI3Kalpha mutations. *Science* 318:1744-1748
- Huang CH, Mandelker D, Gabelli SB, Amzel LM (2008) Insights into the oncogenic effects of PIK3CA mutations from the structure of p110alpha/p85alpha. *Cell Cycle* 7:1151-1156
- Huston A, Leleu X, Jia X, Moreau A S, Ngo HT, Runnels J, Anderson J, Alsayed Y, Roccaro A, Vallet S et al (2008) Targeting Akt and heat shock protein 90 produces synergistic multiple myeloma cell cytotoxicity in the bone marrow microenvironment. *Clin Cancer Res* 14:865-874
- Ihle NT, Powis G (2009) Take your PIK: phosphatidylinositol 3 kinase inhibitors race through the clinic and toward cancer therapy. *Mol Cancer Ther* 8:1-9
- Ihle NT, Williams R, Chow S, Chew W, Berggren MI, Paine Murrieta G, Minion DJ, Halter RJ, Wipf P, Abraham R, Kirkpatrick L, Powis G (2004) Molecular pharmacology and antitumor activity of PX 866, a novel inhibitor of phosphoinositide 3 kinase signaling. *Mol Cancer Ther* 3:763-772
- Ihle NT, Paine Murrieta G, Berggren MI, Baker A, Tate WR, Wipf P, Abraham RT, Kirkpatrick DL, Powis G (2005) The phosphatidylinositol 3 kinase inhibitor PX 866 overcomes resistance to the epidermal growth factor receptor inhibitor gefitinib in A 549 human non small cell lung cancer xenografts. *Mol Cancer Ther* 4:1349-1357
- Ihle NT, Lemos R, Schwartz D, Oh J, Halter RJ, Wipf P, Kirkpatrick L, Powis G (2009a) Peroxisome proliferator activated receptor gamma agonist pioglitazone prevents the hyperglycemia caused by phosphatidylinositol 3 kinase pathway inhibition by PX 866 without affecting antitumor activity. *Mol Cancer Ther* 8:94-100
- Ihle NT, Lemos R Jr, Wipf P, Yacoub A, Mitchell C, Siwak D, Mills GB, Dent P, Kirkpatrick DL, Powis G (2009b) Mutations in the phosphatidylinositol 3 kinase pathway predict for antitumor activity of the inhibitor PX 866 whereas oncogenic Ras is a dominant predictor for resistance. *Cancer Res* 69:143-150
- Janin YL (2005) Heat shock protein 90 inhibitors. A text book example of medicinal chemistry? *J Med Chem* 48:7503-7512
- Jensen MR, Schoepfer J, Radimerski T, Massey A, Guy CT, Brueggen J, Quadt C, Buckler A et al (2008) NVP AUY922: a small molecule HSP90 inhibitor with potent antitumor activity in preclinical breast cancer models. *Breast Cancer Res* 10:R33
- Jia W, Yu C, Rahmani M, Krystal G, Sausville EA, Dent P, Grant S (2003) Synergistic antileukemic interactions between 17 AAG and UCN 01 involve interruption of RAF/MEK and AKT related pathways. *Blood* 102:1824-1832
- Jia S, Liu Z, Zhang S, Liu P, Zhang L, Lee SH, Zhang J, Signoretti S, Loda M, Roberts TM, Zhao JJ (2008) Essential roles of PI(3)K p110beta in cell growth, metabolism and tumorigenesis. *Nature* 454:776-779
- Jia S, Roberts TM, Zhao JJ (2009) Should individual PI3 kinase isoforms be targeted in cancer? *Curr Opin Cell Biol* 21:199-208
- Jimeno A, Hong DS, Hecker S, Clement R, Kurzrock R, Pestano LA, Hiscox A, Leos RA, Kirkpatrick DL, Eckhardt SG, Herbst RS (2009) Phase I trial of PX 866, a novel phosphoinositide 3 kinase (PI 3K) inhibitor. *J Clin Oncol (Meeting Abstracts)* 27:3542
- Junttila TT, Akita RW, Parsons K, Fields C, Lewis Phillips GD, Friedman LS, Sampath D, Sliwkowski MX (2009) Ligand independent HER2/HER3/PI3K complex is disrupted by trastuzumab and is effectively inhibited by the PI3K inhibitor GDC 0941. *Cancer Cell* 15:429-440
- Kavorkian L, Watkins C, Jallal H, Payne A, Hutshinson G, Stubberfield C, Zinkewich Peotti K (2008) Combination studies with a potent inhibitor of PI3K, UCB1320437, and effects on cellular signalling and apoptosis (poster). Presented at AACR special conference in cancer research Targeting the PI3 Kinase pathway in cancer, Cambridge, USA, 11-14 November 2008
- Knight ZA, Gonzalez B, Feldman ME, Zunder ER, Goldenberg DD, Williams O, Loewith R, Stokoe D, Balla A, Toth B, Balla T, Weiss WA, Williams RL, Shokat KM (2006) A

- pharmacological map of the PI3 K family defines a role for p110 α in insulin signaling. *Cell* 125:733-747
- Kondapaka SB, Singh SS, Dasmahapatra GP, Sausville EA, Roy KK (2003) Perifosine, a novel alkylphospholipid, inhibits protein kinase B activation. *Mol Cancer Ther* 2:1093-1103
- Kong D, Yamori T (2007) ZSTK474 is an ATP competitive inhibitor of class I phosphatidylinositol 3 kinase isoforms. *Cancer Sci* 98:1638-1642
- Kong D, Yaguchi S, Yamori T (2009) Effect of ZSTK474, a novel phosphatidylinositol 3 kinase inhibitor, on DNA dependent protein kinase. *Biol Pharm Bull* 32:297-300
- Kumar CC, Madison V (2001) Drugs targeted against protein kinases. *Expert Opin Emerg Drugs* 6(2):303-315
- Laffargue M, Calvez R, Finan P, Trifilieff A, Barbier M, Altruda F, Hirsch E, Wymann MP (2002) Phosphoinositide 3 kinase gamma is an essential amplifier of mast cell function. *Immunity* 16:441-451
- Lannutti BJ, Meadows SA, Kashishian A, Steiner B, Chen H, Ulrich RG, Yu A, Puri KD, Giese NA (2009) CAL 101, a specific PI3K p110 δ inhibitor for the treatment of hematological malignancies (poster). Presented at 100th AACR annual meeting, Denver, USA, 18-22 April 2009
- Li Q, Zhu GD (2002) Targeting serine/threonine protein kinase B/Akt and cell cycle checkpoint kinases for treating cancer. *Curr Top Med Chem* 2:939-971
- Li X, Luwor R, Lu Y, Liang K, Fan Z (2006) Enhancement of antitumor activity of the anti EGF receptor monoclonal antibody cetuximab/C225 by perifosine in PTEN deficient cancer cells. *Oncogene* 25:525-535
- Lin T Y, Bear M, Du Z, Foley KP, Ying W, Barsoum J, London C (2008) The novel Hsp90 inhibitor STA 9090 exhibits activity against Kit dependent and independent malignant mast cell tumors. *Exp Hematol* 36:1266-1277
- Lindsley CW, Zhao Z, Leister WH, Robinson RG, Barnett SF, Defeo Jones D, Jones RE, Hartman GD, Huff JR, Huber HE, Duggan ME (2005) Allosteric Akt (PKB) inhibitors: discovery and SAR of isozyme selective inhibitors. *Bioorg Med Chem Lett* 15:761-764
- LoRusso P, Hurwitz H, Chiorean E, Jewell R, Lampkin T, Bachman K, Kumar R, Lebowitz P, Weber B (2008) AKT inhibitor GSK690693: Preliminary results from the first time in human study. *AACR Meeting Abstracts* 2008:LB 68
- LoRusso P, Markman B, Tabernero J, Shazer R, Nguyen L, Heath E, Patnaik A, Papadopoulos K (2009) A phase I dose escalation study of the safety, pharmacokinetics (PK), and pharmacodynamics of XL765, a PI3K/TORC1/TORC2 inhibitor administered orally to patients (pts) with advanced solid tumors. *J Clin Oncol (Meeting Abstracts)* 27:3502
- Lundgren K, Zhang H, Kamal A, Lough R, et al (2007) BII021 is a small molecule inhibitor of the heat shock protein, Hsp90, that shows potent anti tumor activity in preclinical models. In: *AACR NCI EORTC Int Conf Mol Targets Cancer Ther*
- Lundgren K, Zhans H, Brekken J, Huser N, Powell RE, Timple N, Busch DJ, Neely L, Sensintaffar JL, Yang Y, McKenzie A, Friedman J, Scannevin R, Kamal A, Hong K, Ksibhatla SR, Boehm MF, Burrows FJ (2009) BII021, an orally available, fully synthetic small molecule inhibitor of the heat shock protein Hsp90. *Mol Cancer Res* 8:921-929
- Luo Y, Shoemaker AR, Liu X, Woods KW, Thomas SA, de Jong R, Han EK, Li T, Stoll VS, Powlas JA, Oleksijew A, Mitten MJ, Shi Y et al (2005) Potent and selective inhibitors of Akt kinases slow the progress of tumors *in vivo*. *Mol Cancer Res* 4:977-986
- Maira SM, Stauffer F, Brueggen J, Furet P, Schnell C, Fritsch C, Brachmann S, Chene P, De PA, Schoemaker K, Fabbro D, Gabriel D, Simonen M, Murphy L, Finan P, Sellers W, Garcia Echeverria C (2008a) Identification and characterization of NVP BEZ235, a new orally available dual phosphatidylinositol 3 kinase/mammalian target of rapamycin inhibitor with potent *in vivo* antitumor activity. *Mol Cancer Ther* 7:1851-1863
- Maira SM, Voliva C, Garcia Echeverria C (2008b) Class IA phosphatidylinositol 3 kinase: from their biologic implication in human cancers to drug discovery. *Expert Opin Ther Targets* 12:223-238

- Majumder PK, Febbo PG, Bikoff R, Berger R, Xue Q, McMahon LM, Manola J, Brugarolas J, McDonnell TJ, Golub TR, Loda M, Lane HA, Sellers WR (2004) mTOR inhibition reverses Akt dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF 1 dependent pathways. *Nat Med* 10:594 601
- Marion F, Williams DE, Patrick BO, Hollander I, Mallon R, Kim SC, Roll DM, Feldberg L, Van SR, Andersen RJ (2006) Liphagal, a Selective inhibitor of PI3 kinase alpha isolated from the sponge akacoralliphaga: structure elucidation and biomimetic synthesis. *Org Lett* 8:321 324
- Marone R, Cmiljanovic V, Giese B, Wymann MP (2008) Targeting phosphoinositide 3 kinase: moving towards therapy. *Biochim Biophys Acta* 1784:159 185
- Marone R, Erhart D, Mertz AC, Bohnacker T, Schnell C, Cmiljanovic V, Stauffer F, Garcia Echeverria C, Giese B, Maira SM, Wymann MP (2009) Targeting melanoma with dual phosphoinositide 3 kinase/mammalian target of rapamycin inhibitors. *Mol Cancer Res* 7:601 613
- McMillin DW, Ooi M, Delmore J, Negri J, Hayden P, Mitsiades N, Jakubikova J, Maira SM, Garcia Echeverria C, Schlossman R, Munshi NC, Richardson PG, Anderson KC, Mitsiades CS (2009) Antimyeloma activity of the orally bioavailable dual phosphatidylinositol 3 kinase/mammalian target of rapamycin inhibitor NVP BEZ235. *Cancer Res* 69:5835 5842
- Miled N, Yan Y, Hon WC, Perisic O, Zvelebil M, Inbar Y, Schneidman Duhovny D, Wolfson HJ, Backer JM, Williams RL (2007) Mechanism of two classes of cancer mutations in the phosphoinositide 3 kinase catalytic subunit. *Science* 317:239 242
- Miller N (2008) mTORC1/mTORC2 selective inhibitors: Identification and characterization of novel small molecules with antitumor activity. Presented at 20th EORTC NCI AACR symposium on molecular targets and cancer therapeutics, Geneva, Switzerland, 21 24 October 2008
- Modi S, Stopeck AT, Gordon MS, Mendelson D, Solit DB, Bagatell R, Ma W, Wheler J, Rosen N, Norton L, Cropp GF, Johnson RG, Hannah AL, Hudis CA (2007) Combination of trastuzumab and tanespimycin (17 AAG, KOS 593) is safe and active in trastuzumab refractory HER 2 overexpressing breast cancer: a Phase I dose escalation study. *J Clin Oncol* 25:5410 5417
- Okawa Y, Hideshima T, Steed P, Vallet S, Hall S, Huang K, Rice J, Barabasz A, Foley B, Ikeda H, Raje N, Kiziltepe T, Yasui H, Enatsu S, Anderson KC (2009) SNX 2112, a selective Hsp90 inhibitor, potently inhibits tumor cell growth, angiogenesis, and osteoclastogenesis in multiple myeloma and other hematologic tumors by abrogating signaling via Akt and ERK. *Blood* 113:846 855
- Okuzumi T, Fiedler D, Zhang C, Gray DC, Aizenstein B, Hoffman R, Shokat KM (2009) Inhibitor hijacking of Akt activation. *Nat Chem Biol* 5:484 493
- Patrullo E, Notte A, Barberis L, Selvetella G, Maffei A, Brancaccio M, Marengo S, Russo G, Azzolino O, Rybalkin SD, Silengo L, Altruda F, Wetzker R, Wymann MP, Lembo G, Hirsch E (2004) PI3Kgamma modulates the cardiac response to chronic pressure overload by distinct kinase dependent and independent effects. *Cell* 118:375 387
- Powis G, Bonjouklian R, Berggren MM, Gallegos A, Abraham R, Ashendel C, Zalkow L, Matter WF, Dodge J, Grindey G (1994) Wortmannin, a potent and selective inhibitor of phosphatidylinositol 3 kinase. *Cancer Res* 54:2419 2423
- Rhodes N, Heerding DA, Duckett DR, Eberwein DJ, Knick VB, Lansing TJ, McConnell RT, Gilmer TM, Zhang S Y, Robell K et al (2008) Characterization of an Akt kinase inhibitor with potent pharmacodynamic and antitumor activity. *Cancer Res* 68:2366 2374
- Ruiter GA, Zerp SF, Bartelin H, Van Blitterswijk WJ, Verheij M (2003) Anti cancer alkyl lysophospholipids inhibit the phosphatidylinositol 3 kinase Akt/PKB survival pathway. *Anti cancer Drugs* 14:167 173
- Sadhu C, Dick K, Tino WT, Staunton DE (2003) Selective role of PI3Kd in neutrophil inflammatory responses. *Biochem Biophys Res Commun* 308:764 769
- Safa O, Parkin SM, Bibby MC (1998) Morphological changes and cytokine gene expression in tumor xenografts following treatment with alkylphosphocholines hexadecylphosphocholine and perfosine. *Drugs Today* 34:15 26
- Sarbassov D, Guertin DA, Ali SM, Sabatini DM (2005) Phosphorylation and regulation of Akt/PKB by the rictor mTOR complex. *Science* 307:1098 1101

- Sarker D, Kristeleit R, Mazina KE, Ware JA, Yan Y, Dresser M, Derynck MK, De Bono J (2009) A phase I study evaluating the pharmacokinetics (PK) and pharmacodynamics (PD) of the oral pan phosphoinositide 3 kinase (PI3K) inhibitor GDC 0941. *J Clin Oncol (Meeting Abstracts)* 27:3538
- Seipelt I, Claus E, Schuster T, Polymeropoulos E, Teifel M, Guenther E (2007) New generation of anilino substituted pyridopyrazine urea derivatives show highly selective PI3K inhibition. *AACR Meeting Abstracts* 2007:2379
- Seipelt I, Baasner S, Gerlach M, Teifel M, Fensterle J, Blumenstein L, Mueller G, Guenther EG (2009) AEZS 126, a new orally bioavailable PI3K inhibitor in preclinical development (poster). Presented at 100th AACR annual meeting, Denver, USA, 18–22 April 2009
- Serra V, Markman B, Scaltriti M, Eichhorn PJ, Valero V, Guzman M, Botero ML, Llonch E, Atzori F, Di CS, Maira M, Garcia Echeverria C, Parra JL, Arribas J, Baselga J (2008) NVP-BEZ235, a dual PI3K/mTOR inhibitor, prevents PI3K signaling and inhibits the growth of cancer cells with activating PI3K mutations. *Cancer Res* 68:8022–8030
- Shapiro G, Kwak E, Baselga J, Rodon J, Scheffold C, Laird AD, Bedell C, Edelman G (2009) Phase I dose escalation study of XL147, a PI3K inhibitor administered orally to patients with solid tumors. *J Clin Oncol (Meeting Abstracts)* 27:3500
- Shi Y, Liu X, Han E, Guan R, Shoemaker AR, Oleksijew A, Woods KW, Fisher JP, Klinghofer V, Lasko L, McGonigal T, Li Q, Rosenberger SH, Giranda VL, Luo Y (2005) Optimal classes of chemotherapeutic agents sensitized by specific small molecule inhibitors of akt *in vitro* and *in vivo*. *Noeplasia* 7:992–1000
- Solit DB, Basso AD, Olshen AB, Scher HI, Rosen N (2003) Inhibition of Heat Shock Protein 90 Function Down Regulates Akt Kinase and Sensitizes Tumors to Taxol. *Cancer Res* 63:2139–2144
- Stauffer F, Maira SM, Furet P, Garcia Echeverria C (2008) Imidazo[4,5-c]quinolines as inhibitors of the PI3K/PKB pathway. *Bioorg Med Chem Lett* 18:1027–1030
- Stümer T, Zöllinger A, Siegmund D, Chatterjee M, Grella E, Knop S, Kortüm M et al (2008) Signalling profile and antitumour activity of the novel Hsp90 inhibitor NVP-AUY922 in multiple myeloma. *Leukemia* 22:1604–1612
- Sydor J, Normant E, Pien CS, Porter JR, Ge J, Grenier L, Park RH, Ali JA, Dembski MS et al (2006) Development of 17 allylamino 17 demethoxygeldanamycin hydroquinone hydrochloride (IPI 504), an anti cancer agent directed against Hsp90. *Proc Natl Acad Sci USA* 103:17408–17413
- Thoreen CC, Kang SA, Chang JW, Liu Q, Zhang J, Gao Y, Reichling LJ, Sim T, Sabatini DM, Gray NS (2009) An ATP competitive mammalian target of rapamycin inhibitor reveals rapamycin resistant functions of mTORC1. *J Biol Chem* 284:8023–8032
- Tian ZQ, Liu Y, Zhang D, Wang Z, Dong SD, Carreras CW, Zhou Y, Rastelli G, Santi DV, Myles DC (2004) Synthesis and biological activities of novel 17 aminogeldanamycin derivatives. *Bioorg Med Chem* 12:5317–5329
- Vanhaesebroeck B, Leevers SJ, Panayotou G, Waterfield MD (1997) Phosphoinositide 3 kinases: a conserved family of signal transducers. *Trends Biochem Sci* 22:267–272
- Vanhaesebroeck B, Ali K, Bilancio A, Geering B, Foukas LC (2005) Signalling by PI3K isoforms: insights from gene targeted mice. *Trends Biochem Sci* 30:194–204
- Vlahos CJ, Matter WF, Hui KY, Brown RF (1994) A specific inhibitor of phosphatidylinositol 3 kinase, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002). *J Biol Chem* 269:5241–5248
- Wagner AJ, Von Hoff DH, LoRusso PM, Tibes R, Mazina KE, Ware JA, Yan Y, Derynck MK, Demetri GD (2009) A first in human phase I study to evaluate the pan PI3K inhibitor GDC 0941 administered QD or BID in patients with advanced solid tumors. *J Clin Oncol (Meeting Abstracts)* 27:3501
- Walker EH, Perisic O, Ried C, Stephens L, Williams RL (1999) Structural insights into phosphoinositide 3 kinase catalysis and signalling. *Nature* 402:313–320
- Walker EH, Pacold ME, Perisic O, Stephens L, Hawkins PT, Wymann MP, Williams RL (2000) Structural determinants of phosphoinositide 3 kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine. *Mol Cell* 6:909–919

- Ward SG, Finan P (2003) Isoform specific phosphoinositide 3 kinase inhibitors as therapeutic agents. *Curr Opin Pharmacol* 3:426 434
- Wee S, Wiederschain D, Maira SM, Loo A, Miller C, deBeaumont R, Stegmeier F, Yao YM, Lengauer C (2008) PTEN deficient cancers depend on PIK3CB. *Proc Natl Acad Sci USA* 105:13057 13062
- Whitesell L, Lindquist SL (2005) Hsp90 and the chaperoning of cancer. *Nat Rev* 5:761 772
- Williams R, Baker AF, Ihle NT, Winkler AR, Kirkpatrick L, Powis G (2006) The skin and hair as surrogate tissues for measuring the target effect of inhibitors of phosphoinositide 3 kinase signaling. *Cancer Chemother Pharmacol* 58:444 450
- Williams R, Berndt A, Miller S, Hon WC, Zhang X (2009) Form and flexibility in phosphoinositide 3 kinases. *Biochem Soc Trans* 37:615 626
- Yaguchi S, Fukui Y, Koshimizu I, Yoshimi H, Matsuno T, Gouda H, Hirono S, Yamazaki K, Yamori T (2006) Antitumor activity of ZSTK474, a new phosphatidylinositol 3 kinase inhibitor. *J Natl Cancer Inst* 98:545 556
- Yao E, Zhou W, Lee Hoefflich ST, Truong T, Haverty PM, Eastham Anderson J, Lewin Koh N, Gunter B, Belvin M, Murray LJ, Friedman LS, Sliwkowski MX, Hoefflich KP (2009) Suppression of HER2/HER3 mediated growth of breast cancer cells with combinations of GDC 0941 PI3K inhibitor, trastuzumab, and pertuzumab. *Clin Cancer Res* 15:4147 4156
- Yu K, Toral Barza L, Shi C, Zhang WG, Lucas J, Shor B, Kim J, Verheijen J, Curran K, Malwitz DJ, Cole DC, Ellingboe J, Ayril Kaloustian S, Mansour TS, Gibbons JJ, Abraham RT, Nowak P, Zask A (2009) Biochemical, cellular, and *in vivo* activity of novel ATP competitive and selective inhibitors of the mammalian target of rapamycin. *Cancer Res* 69:6232 6240
- Yuan TL, Cantley LC (2008) PI3K pathway alterations in cancer: variations on a theme. *Oncogene* 27:5497 5510
- Zask A, Verheijen JC, Curran K, Kaplan J, Richard DJ, Nowak P, Malwitz DJ, Brooijmans N, Bard J, Svenson K, Lucas J, Toral Barza L, Zhang WG, Hollander I, Gibbons JJ, Abraham RT, Ayril Kaloustian S, Mansour TS, Yu K (2009a) ATP competitive inhibitors of the mammalian target of rapamycin: design and synthesis of highly potent and selective pyrazolopyrimidines. *J Med Chem* 52:5013 5016
- Zask A, Verheijen J, Kaplan J, Curran K, Richard DJ, Brooijmans N, Lucas J, Toral Barza L, Zhang WG, Hollander I, Gibbons JJ, Abraham R (2009b) ATP competitive inhibitors of the mammalian target of rapamycin (mTOR): design and synthesis of novel, highly selective pyrazolopyrimidines with potent *in vivo* antitumor activity. Presented at 100th AACR annual meeting, Denver, USA, 18 22 April 2009
- Zhang TT, Okkenhaug K, Nashed BF, Puri KD, Knight ZA, Shokat KM, Vanhaesebroeck B, Marshall AJ (2008) Genetic or pharmaceutical blockade of p110delta phosphoinositide 3 kinase enhances IgE production. *J Allergy Clin Immunol* 122:811 819
- Zhao L, Vogt PK (2008) Class I PI3K in oncogenic cellular transformation. *Oncogene* 27:5486 5496
- Zhao Z, Leister WH, Robinson RG, Stanley FB, Defeo Jones D, Jones RE, Hartman GD, Huff JR, Huber HE, Duggan ME, Lindsley CW (2005) Discovery of 2,3,5 trisubstituted pyridine derivatives as potent Akt and Akt2 dual inhibitors. *Bioorg Med Chem Lett* 15: 905 909
- Zhao JJ, Cheng H, Jia S, Wang L, Gjoerup OV, Mikami A, Roberts TM (2006) The p110alpha isoform of PI3K is essential for proper growth factor signaling and oncogenic transformation. *Proc Natl Acad Sci USA* 103:16296 16300
- Zhao Z, Robinson RG, Barnett SF, Defeo Jones D, Jones RE, Hartman GD, Huber HE, Duggan ME, Lindsley CW (2008) Development of potent, allosteric dual Akt1 and Akt2 inhibitors with improved physical properties and cell activity. *Bioorg Med Chem Lett* 18:49 53
- Zhu GD, Gandhi VB, Gong J, Thomas S, Woods KW, Song X, Li T, Diebold RB et al (2007) Synthesis of potent, selective, and orally bioavailable indazole pyridine series of protein kinase B/Akt inhibitors with reduced hypotension. *J Med Chem* 50:2990 3003

New Inhibitors of the PI3K-Akt-mTOR Pathway: Insights into mTOR Signaling from a New Generation of Tor Kinase Domain Inhibitors (TORKinibs)

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Abstract mTOR (mammalian Target of Rapamycin) is the hub of the phosphoinositide 3-Kinase (PI3-K)→Akt→mTOR pathway, which is one of the most commonly mutated pathways in cancer. PI3-Ks and mTOR are related kinases which share an evolutionarily related kinase domain, although the former is a lipid kinase and the latter is a protein kinase. As a result of their similar ATP sites, the prototypical PI3-K inhibitors LY294002 and wortmannin inhibit both kinases, although the compounds have been primarily thought of as inhibitors of PI3-Ks. The widespread use of these reagents to understand PI3-K signaling and the likelihood that many of their effects are confounded by dual inhibition of PI3-K and mTOR make it essential to develop selective mTOR inhibitors in part to understand the unique cellular effects of inhibition of this key downstream

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component in the growth factor pathway. Rapamycin has historically provided a means for selective mTOR inhibition, yet it is not a typical ATP competitive inhibitor, making its effects difficult to reconcile with LY294002 and wortmannin. Several groups have recently reported pharmacological agents which inhibit mTOR but not PI3-K, providing a new pharmacological approach to selective mTOR inhibition. The *TOR* kinase domain *inhibitors* of mTOR have been termed TORKinibs to distinguish their mode of action from rapamycin and its analogs (rapalogs). These inhibitors bind to the ATP binding site of the kinase domain of mTOR and as a result inhibit both mTOR complexes, TORC1 (rapamycin sensitive) and TORC2 (rapamycin resistant). These molecules have allowed a reinvestigation of mTOR and in particular a reinvestigation of the mechanistic basis for incomplete proliferative arrest of cells by Rapamycin. A consensus has quickly emerged from the study of various TORKinibs that Rapamycin is ineffective at blocking cell proliferation because it only partially inhibits the activity of mTORC1. The profound anti-proliferative effect of TORKinibs suggests that as the molecules enter the clinic they may be successful in the treatment of cancers where rapamycin has failed.

1 Two TOR Complexes and Rapamycin Studies in *S. Cerevisiae*

Immediately after the discovery of TOR as the target of rapamycin in yeast (Heitman et al. 1991; Cafferkey et al. 1993), it was recognized that some essential functions of TOR are resistant to rapamycin. TOR is a serine threonine kinase related to PI3K. Yeast have two genes coding for TOR, TOR1 and TOR2 (Kunz et al. 1993; Helliwell et al. 1994). Rapamycin blocks the growth of wild-type yeast, yet mutation of a conserved amino acid in either of the two yeast genes for TOR allows them to grow in the presence of rapamycin. The ability of rapamycin to block yeast growth also requires the presence of the proline isomerase FPR1. Rapamycin inhibits wild-type TOR by nucleating the formation of a ternary complex containing FPR1, rapamycin and TOR, and the formation of this complex prevents TOR from phosphorylating its substrates. The resistance alleles of TOR1 and TOR2 prevent the formation of this inhibitory complex (Zheng et al. 1995). Yet TOR1 and TOR2 are not redundant because of the two yeast TOR genes; TOR2 is essential while TOR1 can be deleted. This presents a paradox because mutation of either TOR1 or TOR2 leads to rapamycin resistance, yet only TOR2 is essential. Resolving this paradox led to the recognition that TOR possess rapamycin resistant functions.

Understanding how yeast can have two target of rapamycin genes, TOR1 and TOR2, yet only one of these genes, TOR2, is essential, revealed that some functions of TOR2 are resistant to rapamycin. The logic for this conclusion is as follows. TOR2 is an essential gene in yeast and if rapamycin inhibited all the functions of TOR2, then treating yeast with rapamycin would be equivalent to deletion of TOR2. Yet treating yeast with rapamycin and deleting TOR2 are not equivalent because rapamycin-resistance mutations in TOR1 allows yeast to grow in the

presence of rapamycin, but not in the absence of the essential TOR2. Treating yeast with rapamycin is, therefore, not equivalent to deleting the essential TOR2. Thus, TOR2 must have an essential function that is unaffected by rapamycin. Mutation of TOR1 is sufficient to allow yeast to grow in the presence of rapamycin, because mutant TOR1 can provide the essential TOR functions that are usually sensitive to rapamycin, while wild-type TOR2 continues to provide TOR functions that are resistant to rapamycin.

TOR was found to belong to two protein complexes TORC1 and TORC2 and the rapamycin resistant functions of TOR were ascribed to TORC2. While activity of both TOR complexes is required for yeast growth, rapamycin can only inhibit TORC1 (Loewith et al. 2002). TOR2 is essential because it can participate in either TOR complex, while TOR1 can only belong to the rapamycin sensitive TORC1 and is excluded from TORC2. Rapamycin-FPR1 inhibits TOR by binding to FKBP-Rapamycin Binding (FRB) Domain of TOR. TORC2 is resistant to rapamycin because one of the components of TORC2 likely occludes the FRB domain of TOR and prevents the binding of rapamycin-FPR1. Although these elegant yeast experiments clearly established that TORC2 is resistant to rapamycin, they do not exclude the possibility that TORC1 also has rapamycin resistant functions. Even though it is widely assumed that rapamycin is a complete inhibitor of TORC1, the experiments in yeast that identified rapamycin-resistant functions of TORC2 leave open the possibility that TORC1 also has functions that are resistant to rapamycin, but are nonetheless dependent on catalytic activity rather than a scaffolding function.

2 A Single Mammalian TOR in Two Complexes (mTORC1 and mTORC2)

TOR is conserved in all eukaryotes examined so far, including mammals. Mammals have a single TOR gene called mTOR for mammalian TOR (Brown et al. 1994; Sabatini et al. 1994; Chiu et al. 1994; Chen et al. 1994; Sabers et al. 1995), yet like yeast TOR, mTOR belongs to two protein complexes, mTORC1 and mTORC2 (Loewith et al. 2002; Kim et al. 2002; Sarbassov et al. 2004). The major components of mTORC1 are mTOR, LST8 and Raptor. mTORC2 also contains mTOR and LST8, but instead of Raptor, mTORC2 contains Rictor and the additional component Sin1. Like yeast TORC1, mTORC1 is sensitive to rapamycin because rapamycin mediates the formation of an inhibitory complex between the FRB of mTOR and a proline isomerase FKBP-12, the mammalian ortholog of the yeast FPR1. Rapalogs such as CCI-779 (Rini et al. 2007) and RAD001 (Sedrani et al. 1998) are analogs of rapamycin that exhibit better pharmacokinetic properties than rapamycin, but share the same basic pharmacological mechanism. Because rapamycin resistant functions in yeast are associated with TORC2, and mTORC2 is also clearly resistant to rapamycin, it has been widely assumed, without any experimental evidence, that rapamycin is a complete inhibitor of mTORC1. The key finding made clear by using TORKinibs is that mTORC1 has important functions that are

resistant to rapamycin, and rapamycin-resistance is, therefore, distributed between both mTOR complexes. By analogy, yeast TORC1 may also possess rapamycin resistant functions, though these have not yet been described.

mTORC2 is resistant to inhibition by rapamycin, although, as discussed below, long term treatment with rapamycin can prevent the assembly of mTORC2 in some cell lines (Sarbasov et al. 2006). The inhibition of mTORC2 assembly by rapamycin may explain why mTORC2 is resistant to acute treatment with rapamycin. Upon long-term treatment with rapamycin, it is thought that newly synthesized mTOR binds to rapamycin FKBP before it has a chance to be incorporated into mTORC2. Once bound by rapamycin FKBP, mTOR can no longer be incorporated into mTORC2, probably because binding of rapamycin FKBP to the FRB domain of mTOR prevents the subsequent association of one of the core components of mTORC2 such as Sin1 or Rictor. The binding of rapamycin FKBP to mTOR, therefore, appears to be mutually exclusive to the binding of Sin1 and/or Rictor. Sin1 and/or Rictor probably use the FRB domain as part of their binding surface to mTOR, and therefore they cannot bind to mTOR when the FRB domain is already occupied. Conversely, Sin1 and/or Rictor probably prevent the association of rapamycin FKBP with mTORC2 by covering the FRB domain of mTOR, thereby rendering mTORC2 resistant to rapamycin.

The two mTOR complexes regulate cell growth by phosphorylating members of the AGC (protein kinase A/protein kinase G/protein kinase C) kinase family (Jacinto and Lorberg 2008). mTORC1 also phosphorylates eIF4E-Binding protein (4EBP) (Brunn et al. 1997; Burnett et al. 1998) a regulator of Cap-dependent translation, which is not an AGC kinase. Because rapamycin only inhibits mTORC1, it was widely assumed that active site inhibitors of mTOR (TORKinibs, Fig. 1.) would slow cell growth more effectively than rapamycin through dual inhibition of mTORC1/mTORC2 (Guertin and Sabatini 2007). Surprisingly, TORKinibs show enhanced antiproliferative activity as compared to rapamycin through their affect on mTORC1 (Feldman et al. 2009; Garcia-Martinez et al. 2009; Thoreen et al. 2009). TORKinibs revealed that rapamycin resistant functions of mTOR are not limited to mTORC2, and mTORC1 activity is partially resistant to rapamycin. These rapamycin-resistant activities will be examined below after we discuss the known substrates of mTOR and its regulation as the hub of the PI-3K→Akt→mTOR pathway.

3 Regulation of AGC Kinases Through Hydrophobic Motif Phosphorylation by TOR

Regulation of AGC kinase phosphorylation by mTOR has been thoroughly reviewed (Jacinto and Lorberg 2008), and we will focus our discussion on p70 S6-Kinase (S6K), Akt and Serum and Glucocorticoid induced Kinase (SGK) because these are the best validated AGC kinase substrates of mTOR and furthermore these three kinase are all activated by phosphorylation in response to growth factor stimulation of PI3-K.

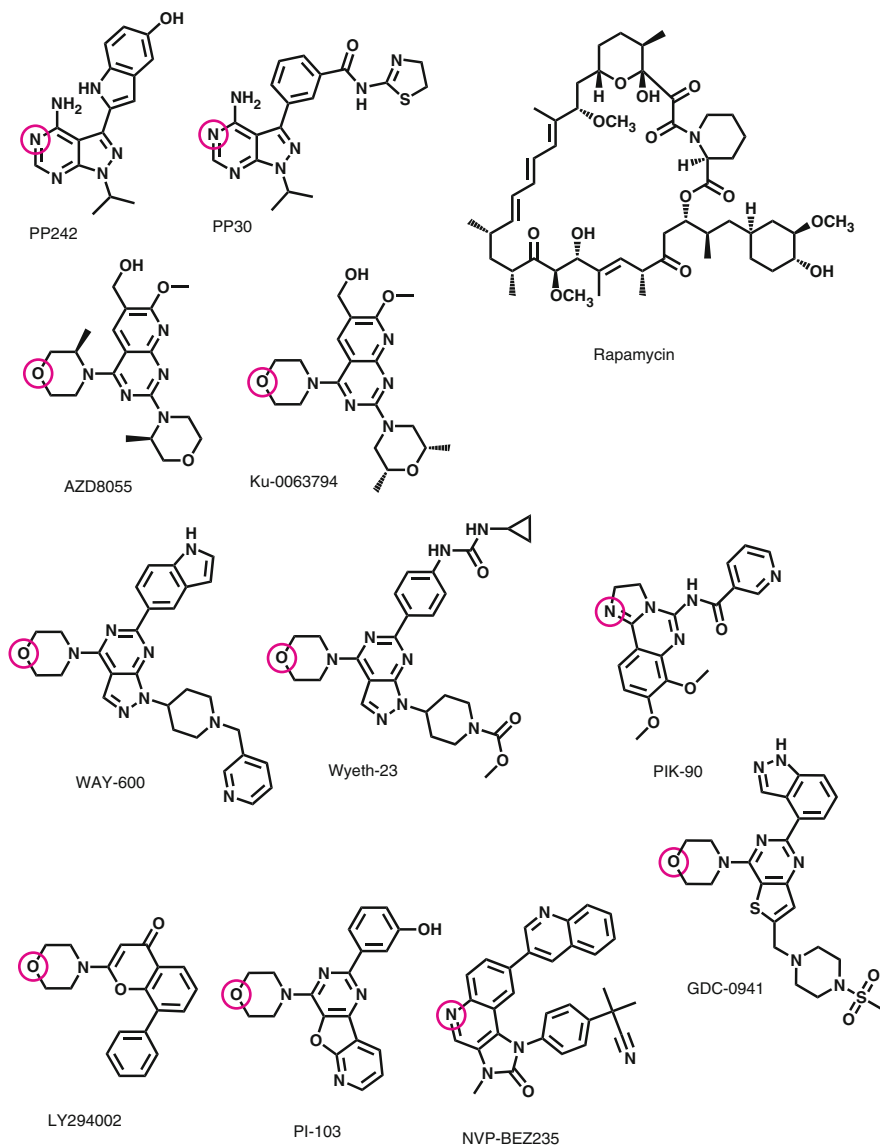


Fig. 1 Representative inhibitors of mTOR and/or PI3 K. Rapamycin is an allosteric inhibitor of mTOR, while the other inhibitors are active site inhibitors of mTOR and/or PI3 K. The hinge binding hydrogen bond acceptor is shown in red (see text). PP242, PP30 (Feldman et al. 2009), AZD8055 (Chresta et al. 2010), Ku 0063794 (Garcia Martinez et al. 2009), WAY 600 (Yu et al. 2009; Nowak et al. 2009) and Wyeth 23 (Zask et al. 2009) are all TORKinibs, that is specific active site inhibitors of mTOR. Torin1 could not be included because its structure has not been released (Thoreen et al. 2009). LY294002 (Brunn et al. 1996; Vlahos et al. 1994), PI 103 (Knight et al. 2006) and NVP BEZ235 (Maira et al. 2008) are dual inhibitors of mTOR and PI3 K. PIK 90 (Knight et al. 2006) and GDC 0941 (Raynaud et al. 2009; Folkes et al. 2008) are inhibitors of PI3 K which do not target mTOR

AGC kinases share a 30-amino acid stretch of sequence homology C-terminal to their kinase domains. At the end of this region of C-terminal homology, AGC kinases often contain a phosphorylation site within a stretch of hydrophobic residues called the hydrophobic motif (HM). Because its phosphorylation and activation is acutely sensitive to rapamycin, S6K was one of the earliest discovered substrates of mTOR. mTOR phosphorylates the HM of S6K at T389 (Pearson et al. 1995). Another important HM phosphorylation is S473 on Akt (Fig. 2). Because the phosphorylation of Akt is not acutely sensitive to rapamycin, it was not initially recognized that mTOR was the kinase for S473-P on Akt and several other putative kinases for S473 on Akt were proposed (Chan and Tsichlis 2001). RNAi targeting of Rictor revealed that the rapamycin-resistant mTOR Complex 2 is the HM kinase for Akt (Sarbasov et al. 2005). Cells from knockout mice lacking mTORC2 have confirmed that phosphorylation of Akt at S473 is dependent on mTORC2 (Jacinto et al. 2006; Guertin et al. 2006; Shiota et al. 2006). SGK is highly related to Akt and it is also phosphorylated by mTORC2 (Garcia-Martinez and Alessi 2008). Further experiments will be required to determine if the HMs of other AGC kinases are also phosphorylated by mTOR. These studies will be greatly helped by the ability to acutely inhibit mTOR using TORKinibs.

HM phosphorylation by mTOR can directly increase the activity of AGC kinases. Once phosphorylated, the HM of an AGC kinase binds to a docking site on the N-lobe of its own kinase domain. Binding of a phosphorylated HM to the kinase N-lobe, orders the kinase active site (Yang et al. 2002) and increases the activity of the kinase by five- to tenfold in the case of Akt (Andjelkovic et al. 1997).

HM phosphorylation is, however, not the most important determinant of kinase activity. Activation loop phosphorylation by PDK1 is more critical for kinase activity than HM phosphorylation. For example, the activity of Akt with T308 (Fig. 2) mutated to alanine is 100-fold lower than the wild-type kinase (Andjelkovic et al. 1997). mTOR, however, cooperates with PDK1 to activate AGC kinases. Unlike most AGC kinases, PDK1 lacks the C-terminal HM. Despite lacking a HM, PDK1 still possesses a binding site for phosphorylated HMs on the N-lobe of its kinase domain. The HM binding site in PDK1 is called the PIF pocket and it can

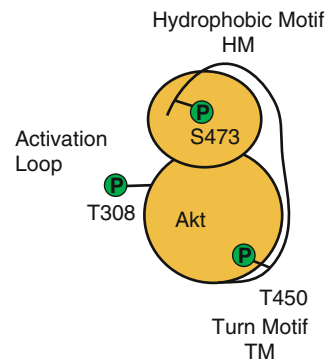


Fig. 2 Important phosphorylation sites on Akt

interact with the phosphorylated HMs of its kinase substrates. For example, HM phosphorylation of S6K by mTOR creates a binding site for PDK1 on S6K, thereby priming S6K for activation loop phosphorylation by PDK1. Using cells in which the PDK1 PIF pocket was mutated to no longer bind to phosphorylated HMs, it was found that S6K, RSK and SGK all require prior HM phosphorylation to prime them for activation loop phosphorylation by PDK1 (Collins et al. 2003). In contrast, phosphorylation of the activation loop of Akt at T308 was retained in cells with the mutant PIF pocket, suggesting that activation loop phosphorylation Akt by PDK1 does not require priming HM phosphorylation by mTOR. The turn motif (TM) is a third conserved phosphorylation site on AGC kinases. The TM is located between the kinase domain and the HM. Phosphorylation of the TM stabilizes the binding of the HM to the kinase N-lobe (Kannan et al. 2007). TM phosphorylation of Akt at T450 (Fig. 2) is absent in cells that lack mTORC2. Lacking TM phosphorylation, Akt is unstable in these cells and associates chaperones such as HSP90. Unlike the highly regulated HM and activation loop phosphorylations, TM phosphorylation is constitutive (Facchinetti et al. 2008; Ikenoue et al. 2008).

4 TORC1 Substrate 4EBP-1

In addition to S6K, mTORC1 is known to phosphorylate 4EBP, a key regulator of cap-dependent translation (Brunn et al. 1997; Burnett et al. 1998). Most proteins are translated from mRNAs through 5' cap-dependent translation rather than internal ribosome entry site (IRES) dependent translation (Sonenberg et al. 2000). The up regulation of cap-dependent translation is emerging as a key feature of the oncogenic program resulting from oncogene/tumor suppressor induced activation of the Ras→MAPK and the PI3-K→Akt→mTOR pathways which are the two most commonly activated signaling pathways in cancer (Ruggero and Sonenberg 2005; Ruggero and Pandolfi 2003; Ruggero et al. 2004). 4EBP binds to the major mRNA 5' cap binding protein eIF4E and inhibits the ability of eIF4E to nucleate the formation of the translation preinitiation complex. Phosphorylation of 4EBP by mTOR releases 4EBP from eIF4E, relieving the inhibition of eIF4E by exposing a surface on eIF4E for the binding of eIF4G. eIF4G is a large scaffolding protein which recruits the remaining preinitiation complex members including eIF3, the 40S subunit of the ribosome and a helicase composed of eIF4A and the helicase cofactor eIF4B. Once formed, the entire preinitiation complex, known as eIF4F, scans forward through the 5'-untranslated region (UTR) of the mRNA to find the start codon and begin translating the mRNA. The helicase activity provided by eIF4A and eIF4B allows the preinitiation complex to unwind the secondary structure of 5'-UTRs that would otherwise stall the scanning process and preventing translation initiation. Some messages contain highly structured 5'-UTRs that are difficult to unwind. For example, the 5'-UTRs of some key oncogenic proteins such as VEGF, ODC, HIF1 α , etc., are highly structured (Richter and Sonenberg 2005). The translation of these oncogenic messages likely requires more translation

initiating activity which may account for the need to upregulate cap-dependent translation as part of the oncogenic program downstream of oncogenic events within the RAS→MAPK and PI3K→Akt→mTOR pathways.

5 mTOR is Both Upstream and Downstream of Akt

The discovery that Akt is phosphorylated by mTORC2 was exciting because mTORC1 was already known to be regulated in part by Akt activity (Fig. 3). The regulation of Akt by mTORC2, therefore, places mTOR both upstream and downstream of Akt within the critical oncogenic PI3-K→Akt→mTOR pathway. Prior to the discovery of Akt's regulation by mTORC2, an analysis of the molecular basis of Tuberous Sclerosis had shown that Akt is a major regulator of mTORC1 (Inoki and Guan 2009). Tuberous sclerosis is a genetic disorder caused by the loss of either of

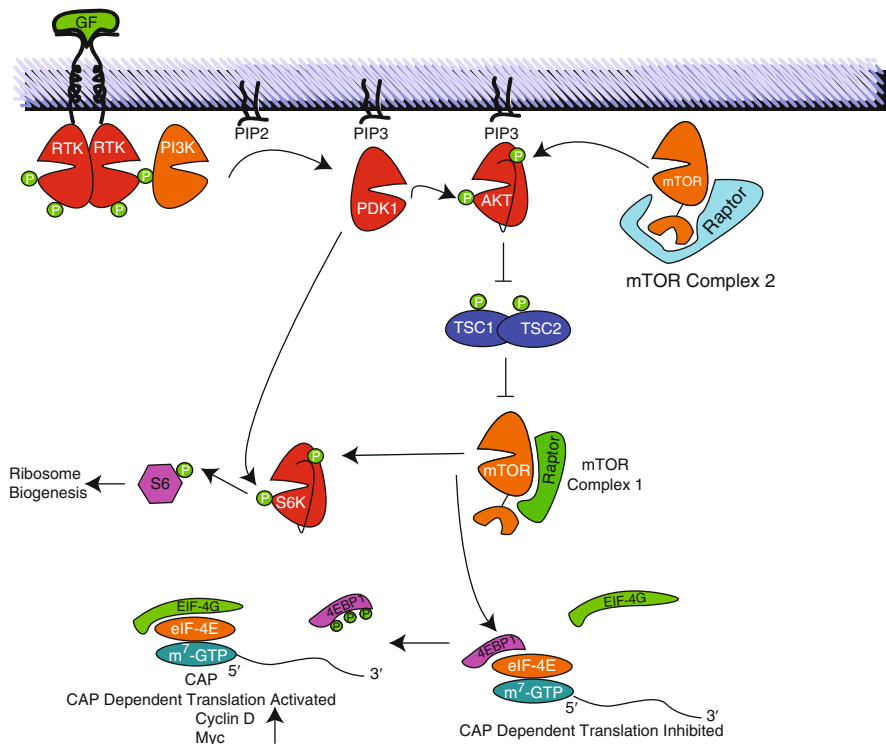


Fig. 3 The PI3 K→Akt→mTOR pathway. Note especially that mTORC2 is upstream of Akt, while mTORC1 is downstream and activated by Akt

the tuberous sclerosis genes TSC1 or TSC2. Loss of TSC1 or TSC2 causes the growth of benign tumors throughout the body and it is characterized at the molecular level by constitutively active mTORC1 leading to the hyperphosphorylation of S6K, S6 and 4EBP. The TSC1/2 complex is, therefore, a negative regulator of mTORC1. TSC2 is a GTPase activating protein (GAP) for the GTPase Rheb which when bound to GTP is an activator of mTORC1. TSC2 promotes the hydrolysis of GTP in Rheb to GDP. TSC2 is not stable on its own, but must form a complex with TSC1 in order to be stable. Loss of either TSC1 or TSC2, therefore, leads to an accumulation of GTP::Rheb which activates mTORC1. TSC2 is a substrate of Akt. Phosphorylation of TSC2 by Akt inhibits the ability of TSC2 to act as a GAP for Rheb and similar to loss of the TSC1/2 complex, leads to an accumulation of GTP::Rheb and activation of mTORC1. In wild-type cells with an intact TSC1/2 complex, Akt activates mTOR by phosphorylating TSC2, while in cells that lack TSC1/2, mTORC1 is constitutively activated even in the absence of growth factor stimulation of Akt through upstream PI3-K activation.

6 Rapamycin Induces Feedback Activation of Akt

In addition to providing insight into the regulation of mTORC1 by Akt, studying Tuberous Sclerosis also revealed a mechanism by which activated mTORC1 inhibits upstream activation of PI3-K and Akt. In cells lacking the TSC1/2 complex, mTORC1 is constitutively active and S6K is constitutively phosphorylated as discussed above. In addition to hyperactivation of mTORC1 and its downstream substrates, cells lacking TSC1/2 show a deficit in Akt phosphorylation and activity (Manning et al. 2005). Conversely, cells treated with the mTORC1 inhibitor rapamycin, which strongly inhibits S6K phosphorylation by mTORC1, often show an increase in the phosphorylation of Akt (Wan et al. 2007). Active S6K phosphorylates IRS1, an important adapter that allows certain receptor tyrosine kinases such as the insulin receptor and the insulin like growth factor receptors (IGF) to activate PI3-K. Serine/Threonine phosphorylation of IRS1 by S6K targets IRS1 for degradation and therefore inhibits the activation of PI3-K by RTKs such as the insulin receptor and IGF-1 (Taniguchi et al. 2006). Highly active S6K in TSC1/2 null cells phosphorylates IRS1, targeting IRS1 for degradation and limiting the ability of some RTKs to activate PI3-K and Akt. By inhibiting mTORC1 and S6K, rapamycin has the opposite effect of relieving feedback inhibition of IRS1 from S6K. Rapamycin treatment, therefore, often results in more efficient activation of PI3-K by RTKs, leading to hyperphosphorylation of Akt. Because IRS1 scaffolds the upstream activators of the MAPK pathway including Grb2, SOS and Ras, rapamycin treatment can also cause hyperactivation of the MAPK pathway (Kinkade et al. 2008; Carracedo et al. 2008). Hyperactivation of both Akt and the MAPK pathway in response to rapamycin treatment for cancer may actually accelerate the progression of the cancer in some cases.

7 mTOR Inhibitors for Cancer

The oncogenic potential of the PI3K→Akt→mTOR pathway became clear as the PIP₃ phosphatase PTEN was identified as the second most commonly mutated tumor suppressor (Li et al. 1997) after p53 and sequencing efforts identified activating mutations in PI3-K driving a wide variety of cancers (Samuels et al. 2004). The activation of mTORC1 downstream of PI3-K, suggested that mTOR inhibitors and in particular inhibitors of mTORC1, such as rapamycin, would be effective anti-cancer therapies. Several findings challenged this assumption. First of all, although rapamycin and analogs of rapamycin developed to alter the pharmacokinetic properties of rapamycin (rapalogs) have been evaluated for the treatment of a broad variety of cancers, so far rapamycin has only been approved for the treatment of renal cell carcinoma. Rapamycin's lack of broad efficacy as a cancer therapeutic was generally thought to stem from its inability to inhibit mTORC2; however, in some cell lines, long-term rapamycin treatment appeared to act as a dual inhibitor of mTORC1/2, by blocking the assembly mTORC2 in addition to directly inhibiting mTORC1 (Sarbasov et al. 2006). The ability of rapamycin to act as a dual inhibitor of mTORC1/2 challenged the explanation that it was a poor anti-cancer therapeutic because it did not inhibit mTORC2 and suggested that despite the compelling logic of the PI3-K→Akt→mTOR pathway, mTOR might not be a good target for cancer treatment. Furthermore the fact that rapamycin is extremely well tolerated when taken as an immunosuppressant (Abraham and Wiederrecht 1996) suggested that it did not possess the type of potent anti-proliferative activities of an anti-cancer therapeutic.

Although the failure of rapamycin to effectively treat many types of cancers suggested that mTOR might not be a good target for cancer therapy, the surprising *in vitro* efficacy of inhibitors targeting both PI3-K and the active site of mTOR challenged this view (Fan et al. 2006; Maira et al. 2008). At the very least, these studies argued that inhibition of mTOR in addition to PI3-K might be important in the treatment of cancer and they left open the possibility that active site inhibitors of mTOR alone might be powerful anti-proliferative agents. Although mTOR is a protein kinase, it is a member of the PI3-K family of lipid kinases and small molecule inhibitors of the active-site of PI3-K often inhibit the active site of mTOR as well. Indeed, the classic pan-PI3-K inhibitor LY294002 (Fig. 1.) inhibits both mTOR and PI3-K with similar potency (Brunn et al. 1996). Many of the cellular functions attributed to PI3-K using LY294002 may, therefore, be due to active-site inhibition of mTOR or at least dual inhibition of PI3-K and mTOR. A structurally similar but much more potent PI3-K inhibitor, PI-103, also inhibits PI3-K and mTOR (Knight et al. 2006) and the clinical PI3-K inhibitor NVP-BEZ235 also targets mTOR (Maira et al. 2008). PI-103 showed surprising efficacy in the inhibition of glioma cell proliferation *in vitro* through its dual inhibition of PI3-K and mTOR (Fan et al. 2006). In this study, PI-103 was better at inhibiting cell proliferation than the pure PI3-K inhibitor PIK-90. It was unclear, however, how a pure active-site inhibitor of mTOR would compare with a pure PI3-K inhibitor.

8 Active-Site Inhibitors of mTOR

The placement of mTORC2 upstream of Akt and mTORC1 downstream of Akt suggested that an active-site inhibitor which targets mTORC1 and mTORC2 should be efficacious in cancer. Although long term treatment with rapamycin can inhibit mTORC2 (Sarbasov et al. 2006), this affect is limited to a minority of cell lines and it is unclear whether it could be relied on to inhibit mTORC2 in cancer cells *in vivo*. Because of the highly compelling pathway logic and as a hedge against the possibility that dual PI3-K/mTOR inhibitors might be poorly tolerated in the clinic, much effort was recently invested to develop specific inhibitors of the mTOR active site. These efforts are coming to light with the recent release of multiple papers documenting the effect of specific active-site inhibitors of mTOR (Feldman et al. 2009; Garcia-Martinez et al. 2009; Thoreen et al. 2009; Chresta et al. 2010; Zask et al. 2009; Yu et al. 2009; Nowak et al. 2009).

Structures of these inhibitors are shown in Fig. 1. Except for the pyrazolopyrimidines, PP242 and PP30, all the ATP site inhibitors of mTOR described so far share the aryl-morpholine pharmacophore of LY294002. The inhibitors from Astra-Zeneca (AZD8055 and Ku-0063794) contain two morpholines. It is interesting that the morpholine continues to be a critical pharmacophore in both the AZ and Wyeth series, which can be traced directly back to Eli Lilly's initial 1994 report of LY294002 (Vlahos et al. 1994). Just two years after the first report of LY294002, Abraham and colleagues reported that LY294002 was also an inhibitor of mTOR (Brunn et al. 1996). The fact that it required almost 13 years for selective mTOR inhibitors to be reported is quite surprising considering the increasing appreciation of the importance of mTOR in the past decade. One potential explanation for this slow pace of inhibitor discovery was the availability of rapamycin and its amazing potency and selectivity for mTOR, and the difficulty of carrying out biochemical assays of mTOR kinase activity in a high throughput assay.

Although no crystal structure has been reported for the kinase domain of mTOR, based on the published structure of LY294002 and other drugs bound to the related PI3-K γ (Walker et al. 2000) we can make a tentative guess about the orientation of each drug in the mTOR binding site. A key feature is an H-bond acceptor (morpholine ether oxygen circled in red) in the AZ and Wyeth series, which is predicted to bind to the N H bond of Val2240 in mammalian mTOR. Interestingly, the morpholines in the AZ series contain alkyl substitutions compared to LY294002 which may enhance binding to mTOR or diminish binding to the PI3K. The binding orientation of PP242 can be predicted based on a similar analysis to structures of the related PP102 bound to PI3-K γ . In this case the pyrimidine ring N-1 supplies the H-bond acceptor function of the morpholine ether oxygen in the other series. In the PP242 series, the hydroxy-indole function exerts critical interactions in the so-called "affinity pocket" of mTOR. Small modifications of this heterocycle, cause severe diminution of binding affinity or selectivity within the PI3K/mTOR family (Apsel et al. 2008).

Initial work with the active site inhibitors *in vitro* quickly led to a re-evaluation of the mechanism of action of rapamycin and a new understanding for the partial effect of rapamycin as an anti-proliferative (Feldman et al. 2009; Garcia-Martinez et al. 2009; Thoreen et al. 2009) and anti-cancer agent (Chresta et al. 2010; Zask et al. 2009; Yu et al. 2009; Nowak et al. 2009). These studies revealed that the problem with rapamycin was not that it missed mTORC2, but that it only partially inhibits mTORC1. This has refocused our attention on the importance of mTORC1, 4EBP1 and protein translation in the treatment of cancer.

9 TORKinibs and Akt

Because it was expected that TORKinibs would differ from rapamycin in their ability to inhibit mTORC2, the effect of TORKinibs on the mTORC2 dependent phosphorylation of Akt phosphorylation at S473 was examined. S473-P is potently inhibited by TORKinibs in all cell lines examined so far (Feldman et al. 2009; Garcia-Martinez et al. 2009; Thoreen et al. 2009; Chresta et al. 2010; Zask et al. 2009; Yu et al. 2009; Nowak et al. 2009). Preliminary *in vivo* experiments, showed inhibition of S473-P in fat and liver of mice following acute administration of PP242 (Feldman et al. 2009). Unexpectedly, S473-P in skeletal muscle appeared resistant to inhibition by PP242. Consistent with the possible resistance of muscle S473-P to TORKinibs, a muscle specific knockout of the rictor, which is required for the formation of mTORC2, shows only partial rather than complete loss of S473-P (Kumar et al. 2008). These results suggest that in muscle a kinase other than mTOR, such as DNA-PK, might play a role in the phosphorylation of Akt on S473, but these tissue specific effects of TORKinibs need to be repeated using multiple inhibitors.

When studies using RNAi discovered that mTORC2 was the kinase for S473-P on Akt, it was seen that disabling mTORC2 using RNAi also caused a loss of T308-P in most of the cell lines examined (Sarbassov et al. 2005; Hresko and Mueckler 2005). In contrast, subsequent genetic knockout of integral mTORC2 components such as Rictor, SIN1 and LST8 led to inhibition of S473-P with no effect on T308-P (Jacinto et al. 2006; Guertin et al. 2006; Shiota et al. 2006). In MEFs derived from mice lacking mTORC2, both basal and growth factor stimulated phosphorylation of T308-P was largely unperturbed. Closer examination revealed that, in addition to S473, these cells also lacked TM phosphorylation of Akt at T450. Loss of TM-P reduced the stability of Akt leading to its association with HSP90 and causing its expression level to be somewhat variable (Facchinetti et al. 2008; Ikenoue et al. 2008).

Whereas all current TORKinib studies see potent *in vitro* inhibition of S473-P, the influence of TORKinibs on T308-P varies. Inhibition of mTOR using the TORKinibs PP242 and PP30, led to a reduction in T308-P, but the EC₅₀ for inhibition of T308-P was fourfold weaker than for inhibition of S473 (Feldman et al. 2009). To confirm that the weaker inhibition of T308-P was not due to an off

target of PP242 or PP30, it was shown that these TORKinibs had no effect on T308-P in *Sin1*^{-/-} cells. *Sin1*^{-/-} cells lack mTORC2 and S473-P, but retain T308-P. Because these cells lack the TORKinib target mTORC2 and already show a complete loss of Akt S473-P, the only way TORKinibs could affect T308-P is through inhibition of an off target. The TORKinibs, PP242 and PP30 had no effect on T308-P in *Sin1*^{-/-} cells, while in matching wild-type cells with mTORC2 and S473-P they inhibited S473-P and T308-P. In a conceptually identical experiment, the TORKinib Torin1 had no effect on the phosphorylation of T308 in *mLST8*^{-/-} cells which like *Sin1*^{-/-}, also lack mTORC2 (Thoreen et al. 2009). Furthermore, another TORKinib, Ku-0063794, had no effect on T308-P in *Rictor*^{-/-}, *mLST8*^{-/-} and *Sin1*^{-/-} cells which all lack mTORC2, but it inhibited T308-P in wild-type MEFs where mTORC2 is intact (Garcia-Martinez et al. 2009). The lack of an effect of TORKinibs on T308-P in cells lacking mTORC2 suggests that in WT cells the inhibition of T308-P is due indirectly to inhibition of mTORC2's phosphorylation of S473-P of Akt.

In wild-type cells where mTORC2 is present, S473-P and T308-P appear to be somewhat “tethered”, such that inhibition of S473-P also inhibits T308-P, though to a lesser extent (Guertin et al. 2009). The partial dependence of T308-P on S473-P might be because PDK1 finds it easier to phosphorylate Akt when it is already phosphorylated on T308, perhaps due to an interaction between the PIF pocket of PDK1 and S473-P. Alternately, S473-P might protect T308-P from dephosphorylation. In either case, in cells that lack mTORC2, the dependence of T308-P on S473-P is apparently lost through an unknown compensatory mechanism.

The pharmacological finding that T308-P is linked to S473-P underscores the importance of deciphering the logic of complex kinase signaling pathways using specific kinase inhibitors rather than genetic knockouts. Genetic knockouts of a key survival kinase such as mTORC2, often generate a complex phenotype that is not due primarily to loss of the kinase activity being studied (Knight and Shokat 2005). Instead the phenotype generated by a kinase knockout is often an amalgam of effects due to loss of the scaffolding role of the kinase protein itself and compensatory signaling changes within the kinase network. Together these effects obscure the phenotype that would be seen if the kinase activity were acutely inhibited. Important aspects of kinase signaling often become apparent only once a network is probed using specific inhibitors. Even studying kinase signaling using specific inhibitors is not without peril because when a kinase inhibitor binds into the active site of a kinase it alters the conformation of the kinase, sometimes leading to unexpected consequences. For instance the binding of inhibitors to the active site of Akt alters the conformation of Akt leading to massive hyperphosphorylation of Akt on both S473 and T308 (Okuzumi et al. 2009). If simply altering the conformation of a kinase using a small molecule can distort the logic of a kinase pathway, removing a kinase entirely may have a correspondingly greater effect on a kinase pathway.

Although the studies mentioned above with PP242, PP30 and Ku-0063794 found a tethering between S473-P and T308-P in a variety of wild-type cell lines (Feldman et al. 2009; Garcia-Martinez et al. 2009) and even *in vivo*

(Feldman et al. 2009), studies using AZD8055 (Chresta et al. 2010) and WAY-600 (Yu et al. 2009) see a striking lack of effect of TORKinibs on T308-P, even at concentrations much higher than required to effect S473-P. Whether the differences are due to inherent differences in the pharmacological properties of the molecules or simply differences in experimental setup such as choice of cell line will require directly comparing all the current TORKinibs in a side by side experiment. Comparing the effects from multiple compounds with different structures that all target a single kinase is a very effective way to avoid pitfalls when using kinase inhibitors. Although the results obtained with a single compound might be spurious because they are due to the inhibition of a known or perhaps unknown off target, the compendium of results obtained using two or more compounds increases the likelihood that the effects seen in the experiment are due to inhibition of the intended target. In this regard it is scientifically irresponsible when research with new pharmacological agents is presented without releasing the structure of these new molecules (Thoreen et al. 2009). The report of the activity of a small molecule, without revealing its structure prevents the fundamental requirement of all science, the replication of results. Luckily for those in the mTOR field, multiple TORKinibs have been structurally reported (Fig. 1), even two from major pharmaceutical companies. Just as most journals require the release of protein structure coordinates, all journals must require the release of the structure of pharmacological agents used in a study. The patent process allows for the free circulation of new inventions while protecting commercial interests. Rather than opposing disclosure of chemical structures in scientific literature, authors should secure patent protection for their inventions prior to publication if they have commercial interests.

Despite differing in their affect on T308-P, all TORKinibs cause some inhibition of Akt substrate phosphorylation. In the case of PP242 and Ku-0063794, their inhibition of Akt substrate phosphorylation generally tracks with their inhibition of Akt at T308 (Feldman et al. 2009; Garcia-Martinez et al. 2009). Using AZD8055 and WAY-600, although no inhibition of Akt T308-P was seen, these molecules inhibited Akt substrate phosphorylation at concentrations slightly higher than those required to inhibit S473 (Chresta et al. 2010; Yu et al. 2009).

10 Cell Proliferation and Rapamycin Resistant mTORC1

Across multiple cell lines, rapamycin causes a potent (EC_{50} 1–10 nM), but only partial (40–60%) inhibition in cell proliferation. Prior to the introduction of TORKinibs, it was assumed that rapamycin could only partially inhibit cell proliferation because it could not inhibit mTORC2. Reassuringly, cell proliferation is in most cases completely inhibited by TORKinibs, at concentrations that are not substantially higher than the biochemical EC_{50} for inhibition mTOR as judged by the phosphorylation of S473 on Akt or T389 on S6K. Surprisingly, however, the proliferation of cells lacking mTORC2, including Sin1^{-/-} (Feldman et al. 2009), Rictor^{-/-} (Thoreen et al. 2009) and mLST8^{-/-} (Garcia-Martinez et al. 2009) MEFs

is only partially sensitive to rapamycin, while TORKinibs fully inhibit the proliferation of these cells (Table 1). The presence of mTORC2 is, therefore, not required for rapamycin and a TORKinib to have a differential effect on cell proliferation, suggesting that rapamycin and TORKinibs differ in their effects on mTORC1, and indicating that important activities of mTORC1 are resistant to rapamycin.

S6K and 4EBP1 are the best characterized substrates of mTOR and naturally their phosphorylation was examined in cells treated with TORKinibs. Surprisingly, whereas S6K-P was potently inhibited by rapamycin and TORKinibs, 4EBP1 phosphorylation was fully inhibited only by TORKinibs, but not rapamycin. A pair of threonine phosphorylations on 4EBP1, T37/46, which were known to be quite resistant to rapamycin (Wang et al. 2005; Gingras et al. 2001), were found to be highly sensitive TORKinibs. It had been previously asserted that because T37/46 were constitutively phosphorylated they were, therefore, partially resistant to rapamycin, perhaps because only a small amount of mTOR activity might be required to maintain their phosphorylation (Gingras et al. 1999). The sensitivity of T37/46-P and S6K-P to TORKinibs is nearly identical, however, suggesting that rapamycin is simply not a good inhibitor of mTOR's phosphorylation of 4EBP1 at T37/46. In this way, rapamycin is acting as a substrate specific inhibitor of mTOR in that it inhibits mTOR's phosphorylation of S6K but not 4EBP. 4EBPs have a major role in the regulation of cap-dependent translation and across a wide range of assays it was found that treating cells with TORKinibs, inhibited cap-dependent translation and total protein synthesis to a much greater extent than rapamycin. The greater inhibition of 4EBP-P and cap-dependent translation could, therefore, account for the much greater ability of TORKinibs to block cell proliferation when compared with rapamycin. It is also possible that other substrates of mTORC1 are, like 4EBP, resistant to rapamycin and the combined inhibition of 4EBP-P as well as other rapamycin-resistant substrates of mTORC1 accounts for the profound antiproliferative effects of TORKinibs. In addition, studies showing that TORKinibs can inhibit cell proliferation to a greater extent than rapamycin even in the absence of mTORC2, were only performed on MEFs. It is likely that in other cell types and especially in cancer cells with activated PI3-K and Akt, that the full inhibition of mTORC1 by a TORKinib will cooperate with inhibition of mTORC2 to fully inhibit cell proliferation. Luckily, by targeting the active site of mTOR, TORKinibs naturally inhibit the all the activity of mTORC1 and mTORC2.

Despite the general finding that rapamycin is only a partial inhibitor of cell proliferation, at very high concentrations, rapamycin is able to completely inhibit proliferation of some cell lines (Shor et al. 2008). Typically, cell proliferation slows by approximately 40% in cells treated with 1 10 nM rapamycin. Increasing the concentration of rapamycin above 10 nM causes no further decrease in cell proliferation until around 10 50 μ M when cell proliferation is suddenly affected once again and cell proliferation is often fully inhibited by these micromolar concentrations of rapamycin. Surprisingly, the inhibition of cell proliferation by micromolar concentrations of rapamycin is independent of FKBP12. Micromolar concentrations of rapamycin, therefore, inhibit mTOR through a distinct mode of action from nanomolar rapamycin which depends on binding FKBP12 to mTOR. Like the

Table 1 Properties of Selected TORKinibs

Compound	Chemical class	In vitro IC ₅₀ μM ([ATP] μM)		Cell proliferation		Ref.
		mTOR	p110α	IC ₅₀ μM	Cell line	
PP242	Pyrazolopyrimidine	0.008 (10)	1.96 (10)	0.6	WT & SIN1 ^{-/-} MEFs	Feldman et al. (2009)
PP30	Pyrazolopyrimidine	0.080 (10)	3 (10)	6	WT & Sin1 ^{-/-} MEFs	Feldman et al. (2009)
Torin1	Unknown	0.003 (10)	1.8 (10)	<0.25	WT & Rictor ^{-/-} MEFs	Thoreen et al. (2009)
Ku-0063794	Morpholino-pyridopyrimidine	0.010 (100)	>10 (1,000)	<3	WT & mLST8 ^{-/-} MEFs	Garcia-Martinez et al. (2009)
AZD8055	Morpholino-pyridopyrimidine	0.00013 (20?)	3.6 (20?)	0.05	U87-MG	Chresta et al. (2010)
Wyeth-23	Morpholino-pyrazolopyrimidine	0.00045 (100)	0.7 (100)	0.05 0.02	A549 H838	Zask et al. (2009)
WAY-600	Morpholino-pyrazolopyrimidine	0.009 (100)	1.96 (100)	0.04 0.6–2.5	LNCap Multiple tumor lines	Yu et al. (2009), Nowak et al. (2009)

inhibition of mTOR by TORKinibs, micromolar, but not nanomolar rapamycin causes a large decrease in protein translation. Micromolar rapamycin and TORKinibs both cause a strong decrease in protein synthesis and cell proliferation suggesting that micromolar rapamycin, like TORKinibs, may be acting as a complete inhibitor of mTORC1. Reaching micromolar concentrations may be possible and actually achieved when cancer patients are treated with rapalogs having enhanced pharmacokinetic properties such as RAD001. It is possible that some of the promising effects observed with rapalogs as anti-cancer agents may depend on reaching micromolar rather than nanomolar concentrations with these agents.

11 Inhibition of mTORC1 by Rapamycin

At nanomolar concentrations, rapamycin is a substrate specific inhibitor of mTORC1, fully inhibiting S6K while only partially inhibiting 4EBP. Furthermore, protein translation is largely unaffected by nanomolar rapamycin and cell proliferation is only partially inhibited. In contrast, TORKinibs and probably micromolar rapamycin act as direct and complete inhibitors of mTORC1. Through complete inhibition of mTORC1, TORKinibs cause full dephosphorylation of 4EBP, strong inhibition of protein synthesis and full inhibition of cell proliferation. It is unclear exactly how rapamycin FKBP binding to the FRB domain of mTOR prevents mTOR from phosphorylating S6K. Similarly, it is unclear how 4EBP phosphorylation can escape inhibition by rapamycin. However, knowing that rapamycin inhibits mTORC1 in a substrate specific fashion, helps to narrow the possible models for how rapamycin inhibits mTOR. Several models are presented below to explain the partial inhibition of mTORC1 by rapamycin.

One model for the inhibition of S6K phosphorylation by mTOR asserts that rather than directly inhibiting the kinase activity of mTOR, binding of rapamycin FKBP to the FRB domain of mTOR occludes the association of mTOR with its substrates (Zheng et al. 1995). Within the framework of this model, the inhibition of S6K, but not 4EBP phosphorylation by rapamycin can be explained if binding of rapamycin FKBP to the FRB domain of mTORC1 only interferes with the binding and phosphorylation of S6K, but has a minimal effect on the phosphorylation of the smaller substrate 4EBP.

Just as Rictor or Sin1 probably protects mTORC2 from inhibition by rapamycin, there may exist a subtype of mTORC1 whose FRB is protected from rapamycin by an as yet undiscovered protein partner. This subtype of mTORC1, which we will hypothetically name mTORC1 β , may be primarily responsible for the phosphorylation of 4EBP, while the hypothetical mTORC1 α , which is fully sensitive to rapamycin, is responsible for the phosphorylation of S6K. This model might be verified through the discovery of new protein co-factors of mTORC1.

Binding of rapamycin to the FRB domain of TOR is conserved through evolution from yeast to mammals. The conservation of rapamycin binding probably not due to an evolutionary need to conserve the ability of mTOR to bind rapamycin.

Instead, the conservation of rapamycin binding probably reflects the need for the FRB domain of mTOR to perform an important cellular role and conservation of this cellular role has constrained the evolution of mTOR and inadvertently conserved its binding to rapamycin. Rapamycin's binding surface with TOR is highly hydrophobic, suggesting that the FRB domain of TOR might be involved in lipid binding. A solution structure of PA bound to the FRB of mTOR has been solved by NMR (Veverka et al. 2008), and experiments suggest that mTORC1 is regulated might be regulated in part through activation by PA (Fang et al. 2001; Foster 2007). The conserved binding site on mTOR for rapamycin, may reflect the constraint that mTOR maintain a binding site for PA through evolution. PA is generated by the hydrolysis of phosphatidyl-choline by phospholipase D, or by the phosphorylation of diacyl-glycerol (DAG), by diacyl-glycerol kinase, or by the acylation of lysophosphatidic acid (LPA) by LPA acyltransferase (LPAAT) (Foster 2007). Phospholipase D is probably responsible for the bulk production of PA and phospholipase D can be inhibited by n-butanol and to lesser extent sec-butanol while it is unaffected by tert-butanol. S6K phosphorylation is inhibited by n-butanol, but less so by sec-butanol and unaffected by tert-butanol, suggesting a pathway in which PA produced by phospholipase D either activates mTOR or cooperates with other inputs to mTOR to facilitate its phosphorylation of S6K. For instance, binding of mTOR to PA might localize it to a membrane compartment where S6K is present and waiting to be phosphorylated. Rapamycin by binding to the FRB domain of mTOR, likely occludes binding of PA and may prevent the PA dependent activation or localization of mTOR. Rapamycin might primarily affect mTOR's phosphorylation of S6K, while having less effect on 4EBP, if the pathway activating mTOR to phosphorylate 4EBP does not rely on PA. For instance, while S6K might require PA binding to mTOR to properly associate mTOR and S6K, mTOR's phosphorylation of 4EBP might not require membrane association. By being regulated independently of PA, mTOR's phosphorylation of 4EBP would escape inhibition by rapamycin.

12 Using Inhibitors of mTOR to Treat Cancer

The assignment of antiproliferative effects from active-site TOR inhibitors to mTORC1 over mTORC2, while interesting, is a rather academic enterprise, because there is no specific inhibitor of mTORC2. A specific inhibitor of mTORC2 would undoubtedly be highly interesting and might well prove useful for some cancers as suggested by genetic studies in which eliminating mTORC2 (Guertin et al. 2009) block the development of cancer in the mouse. But, such an inhibitor would likely require inhibiting protein-protein interactions necessary for the assembly of mTORC2 or allosterically inhibiting mTORC2 without affecting mTORC1. Given that our ability to discover specific inhibitors of protein-protein interactions and allosteric inhibitors is still in its infancy, it is unlikely we will soon see the discovery of a specific inhibitor of mTORC2 with the potency and

pharmacological properties needed for even preclinical work. Instead, the compelling question right now is what type of inhibitor (PI3K, dual PI3K/mTOR, isoform specific PI3-K, TORKinib or Rapamycin) from our current arsenal of potent inhibitors will be the best for treating each subtype of cancer.

Of the many hallmarks of cancer (Hanahan and Weinberg 2000), hyperproliferation is often the basis for targeting cancer using conventional chemotherapy. In this sense, mTOR inhibitors seem to follow a similar logic. However, while conventional chemotherapy targets cancer cells by targeting hyperproliferating cells in general, mTOR inhibitors present a slightly different logic; they seek to inhibit the pathways that drive cell proliferation. By blocking the proliferation of cancer cells, TORKinibs may even antagonize conventional chemotherapy because chemotherapy relies on hyperproliferation to distinguish between cancer and non-cancer cells. Alternately, because the PI3-K→Akt→mTOR pathway drives cell survival, inhibitors of mTOR and/or PI3-K may synergize with chemotherapeutic agents that cause or activate apoptosis. Careful awareness and evaluation of these possibilities is critical as TORKinibs are brought into the clinic.

References

- Abraham RT, Wiederrecht GJ (1996) Immunopharmacology of rapamycin. *Annu Rev Immunol* 14:483–510
- Andjelkovic M et al (1997) Role of translocation in the activation and function of protein kinase B. *J Biol Chem* 272(50):31515–31524
- Apfel B et al (2008) Targeted polypharmacology: discovery of dual inhibitors of tyrosine and phosphoinositide kinases. *Nat Chem Biol* 4(11):691–699
- Brown EJ et al (1994) A mammalian protein targeted by G1-arresting rapamycin receptor complex. *Nature* 369(6483):756–758
- Brunn GJ et al (1996) Direct inhibition of the signaling functions of the mammalian target of rapamycin by the phosphoinositide 3-kinase inhibitors, wortmannin and LY294002. *Embo J* 15(19):5256–5267
- Brunn GJ et al (1997) Phosphorylation of the translational repressor PHAS I by the mammalian target of rapamycin. *Science* 277(5322):99–101
- Burnett PE et al (1998) RAFT1 phosphorylation of the translational regulators p70 S6 kinase and 4E-BP1. *Proc Natl Acad Sci USA* 95(4):1432–1437
- Cafferkey R et al (1993) Dominant missense mutations in a novel yeast protein related to mammalian phosphatidylinositol 3-kinase and VPS34 abrogate rapamycin cytotoxicity. *Mol Cell Biol* 13(10):6012–6023
- Carracedo A et al (2008) Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. *J Clin Invest* 118(9):3065–3074
- Chan TO, Tsichlis PN (2001) PDK2: a complex tail in one Akt. *Sci STKE* 2001(66):PE1
- Chen Y et al (1994) A putative sirolimus (rapamycin) effector protein. *Biochem Biophys Res Commun* 203(1):1–7
- Chiu MI, Katz H, Berlin V (1994) RAPT1, a mammalian homolog of yeast Tor, interacts with the FKBP12/rapamycin complex. *Proc Natl Acad Sci USA* 91(26):12574–12578
- Chresta CM et al (2010) AZD8055 is a potent, selective and orally bioavailable ATP-competitive mTOR kinase inhibitor with *in vitro* and *in vivo* anti-tumor activity. *Cancer Res* 70(1):288–298

- Collins BJ et al (2003) In vivo role of the PIF binding docking site of PDK1 defined by knock in mutation. *Embo J* 22(16):4202 4211
- Facchinetti V et al (2008) The mammalian target of rapamycin complex 2 controls folding and stability of Akt and protein kinase C. *Embo J* 27(14):1932 1943
- Fan QW et al (2006) A dual PI3 kinase/mTOR inhibitor reveals emergent efficacy in glioma. *Cancer Cell* 9(5):341 349
- Fang Y et al (2001) Phosphatidic acid mediated mitogenic activation of mTOR signaling. *Science* 294(5548):1942 1945
- Feldman ME et al (2009) Active site inhibitors of mTOR target rapamycin resistant outputs of mTORC1 and mTORC2. *PLoS Biol* 7(2):e38
- Folkes AJ et al (2008) The identification of 2 (1H indazol 4 yl) 6 (4 methanesulfonyl piperazin 1 ylmethyl) 4 morpholin 4 yl thieno[3,2 d]pyrimidine (GDC 0941) as a potent, selective, orally bioavailable inhibitor of class I PI3 kinase for the treatment of cancer. *J Med Chem* 51(18):5522 5532
- Foster DA (2007) Regulation of mTOR by phosphatidic acid? *Cancer Res* 67(1):1 4
- Garcia Martinez JM, Alessi DR (2008) mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and activation of serum and glucocorticoid induced protein kinase 1 (SGK1). *Biochem J* 416(3):375 385
- Garcia Martinez JM et al (2009) Ku 0063794 is a specific inhibitor of the mammalian target of rapamycin (mTOR). *Biochem J* 421(1):29 42
- Gingras AC et al (1999) Regulation of 4E BP1 phosphorylation: a novel two step mechanism. *Genes Dev* 13(11):1422 1437
- Gingras AC et al (2001) Hierarchical phosphorylation of the translation inhibitor 4E BP1. *Genes Dev* 15(21):2852 2864
- Guertin DA, Sabatini DM (2007) Defining the role of mTOR in cancer. *Cancer Cell* 12(1):9 22
- Guertin DA et al (2006) Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signaling to Akt FOXO and PKCalpha, but not S6K1. *Dev Cell* 11(6):859 871
- Guertin DA et al (2009) mTOR complex 2 is required for the development of prostate cancer induced by Pten loss in mice. *Cancer Cell* 15(2):148 159
- Hanahan D, Weinberg RA (2000) The hallmarks of cancer. *Cell* 100(1):57 70
- Heitman J, Movva NR, Hall MN (1991) Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science* 253(5022):905 909
- Helliwell SB et al (1994) TOR1 and TOR2 are structurally and functionally similar but not identical phosphatidylinositol kinase homologues in yeast. *Mol Biol Cell* 5(1):105 118
- Hresko RC, Mueckler M (2005) mTOR.RICTOR is the Ser473 kinase for Akt/protein kinase B in 3T3 L1 adipocytes. *J Biol Chem* 280(49):40406 40416
- Ikenoue T et al (2008) Essential function of TORC2 in PKC and Akt turn motif phosphorylation, maturation and signalling. *Embo J* 27(14):1919 1931
- Inoki K, Guan KL (2009) Tuberous sclerosis complex, implication from a rare genetic disease to common cancer treatment. *Hum Mol Genet* 18(R1):R94 100
- Jacinto E, Lorberg A (2008) TOR regulation of AGC kinases in yeast and mammals. *Biochem J* 410(1):19 37
- Jacinto E et al (2006) SIN1/MIP1 maintains rictor mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. *Cell* 127(1):125 137
- Kannan N et al (2007) The hallmark of AGC kinase functional divergence is its C terminal tail, a cis acting regulatory module. *Proc Natl Acad Sci USA* 104(4):1272 1277
- Kim DH et al (2002) mTOR interacts with raptor to form a nutrient sensitive complex that signals to the cell growth machinery. *Cell* 110(2):163 175
- Kinkade CW et al (2008) Targeting AKT/mTOR and ERK MAPK signaling inhibits hormone refractory prostate cancer in a preclinical mouse model. *J Clin Invest* 118(9):3051 3064
- Knight ZA, Shokat KM (2005) Features of selective kinase inhibitors. *Chem Biol* 12(6):621 637

- Knight ZA et al (2006) A pharmacological map of the PI3 K family defines a role for p110alpha in insulin signaling. *Cell* 125(4):733 747
- Kumar A et al (2008) Muscle specific deletion of rictor impairs insulin stimulated glucose transport and enhances Basal glycogen synthase activity. *Mol Cell Biol* 28(1):61 70
- Kunz J et al (1993) Target of rapamycin in yeast, TOR2, is an essential phosphatidylinositol kinase homolog required for G1 progression. *Cell* 73(3):585 596
- Li J et al (1997) PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* 275(5308):1943 1947
- Loewith R et al (2002) Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. *Mol Cell* 10(3):457 468
- Maira SM et al (2008) Identification and characterization of NVP BEZ235, a new orally available dual phosphatidylinositol 3 kinase/mammalian target of rapamycin inhibitor with potent *in vivo* antitumor activity. *Mol Cancer Ther* 7(7):1851 1863
- Manning BD et al (2005) Feedback inhibition of Akt signaling limits the growth of tumors lacking Tsc2. *Genes Dev* 19(15):1773 1778
- Nowak P et al (2009) Discovery of potent and selective inhibitors of the mammalian target of rapamycin (mTOR) kinase. *J Med Chem* 52(22):7081 7089
- Okuzumi T et al (2009) Inhibitor hijacking of Akt activation. *Nat Chem Biol* 5(7):484 493
- Pearson RB et al (1995) The principal target of rapamycin induced p70s6k inactivation is a novel phosphorylation site within a conserved hydrophobic domain. *Embo J* 14(21):5279 5287
- Raynaud FI et al (2009) Biological properties of potent inhibitors of class I phosphatidylinositol 3 kinases: from PI 103 through PI 540, PI 620 to the oral agent GDC 0941. *Mol Cancer Ther* 8(7):1725 1738
- Richter JD, Sonenberg N (2005) Regulation of cap dependent translation by eIF4E inhibitory proteins. *Nature* 433(7025):477 480
- Rini B, Kar S, Kirkpatrick P (2007) Temsirolimus. *Nat Rev Drug Discov* 6(8):599 600
- Ruggero D, Pandolfi PP (2003) Does the ribosome translate cancer? *Nat Rev Cancer* 3(3):179 92
- Ruggero D, Sonenberg N (2005) The Akt of translational control. *Oncogene* 24(50):7426 7434
- Ruggero D et al (2004) The translation factor eIF 4E promotes tumor formation and cooperates with c Myc in lymphomagenesis. *Nat Med* 10(5):484 486
- Sabatini DM et al (1994) RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin dependent fashion and is homologous to yeast TORs. *Cell* 78(1):35 43
- Sabers CJ et al (1995) Isolation of a protein target of the FKBP12 rapamycin complex in mammalian cells. *J Biol Chem* 270(2):815 822
- Samuels Y et al (2004) High frequency of mutations of the PIK3CA gene in human cancers. *Science* 304(5670):554
- Sarbassov DD et al (2004) Rictor, a novel binding partner of mTOR, defines a rapamycin insensitive and raptor independent pathway that regulates the cytoskeleton. *Curr Biol* 14(14):1296 1302
- Sarbassov DD et al (2005) Phosphorylation and regulation of Akt/PKB by the rictor mTOR complex. *Science* 307(5712):1098 1101
- Sarbassov DD et al (2006) Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Mol Cell* 22(2):159 168
- Sedrani R et al (1998) Chemical modification of rapamycin: the discovery of SDZ RAD. *Transplant Proc* 30(5):2192 2194
- Shiota C et al (2006) Multiallelic disruption of the rictor gene in mice reveals that mTOR complex 2 is essential for fetal growth and viability. *Dev Cell* 11(4):583 589
- Shor B et al (2008) A new pharmacologic action of CCI 779 involves FKBP12 independent inhibition of mTOR kinase activity and profound repression of global protein synthesis. *Cancer Res* 68(8):2934 2943
- Sonenberg N, Hershey JWB, Mathews M (2000) Translational control of gene expression, 2nd edn. Cold Spring Harbor monograph series, vol 39. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp 1020

- Taniguchi CM, Emanuelli B, Kahn CR (2006) Critical nodes in signalling pathways: insights into insulin action. *Nat Rev Mol Cell Biol* 7(2):85–96
- Thoreen CC et al (2009) An ATP competitive mammalian target of rapamycin inhibitor reveals rapamycin resistant functions of mTORC1. *J Biol Chem* 284(12):8023–8032
- Veverka V et al (2008) Structural characterization of the interaction of mTOR with phosphatidic acid and a novel class of inhibitor: compelling evidence for a central role of the FRB domain in small molecule mediated regulation of mTOR. *Oncogene* 27(5):585–595
- Vlahos CJ et al (1994) A specific inhibitor of phosphatidylinositol 3 kinase, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002). *J Biol Chem* 269(7):5241–5248
- Walker EH et al (2000) Structural determinants of phosphoinositide 3 kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine. *Mol Cell* 6(4):909–919
- Wan X et al (2007) Rapamycin induces feedback activation of Akt signaling through an IGF-1R dependent mechanism. *Oncogene* 26(13):1932–1940
- Wang X et al (2005) Distinct signaling events downstream of mTOR cooperate to mediate the effects of amino acids and insulin on initiation factor 4E binding proteins. *Mol Cell Biol* 25(7):2558–2572
- Yang J et al (2002) Crystal structure of an activated Akt/protein kinase B ternary complex with GSK3 peptide and AMP-PNP. *Nat Struct Biol* 9(12):940–944
- Yu K et al (2009) Biochemical, cellular, and *in vivo* activity of novel ATP competitive and selective inhibitors of the mammalian target of rapamycin. *Cancer Res* 69(15):6232–6240
- Zask A et al (2009) ATP competitive inhibitors of the mammalian target of rapamycin: design and synthesis of highly potent and selective pyrazolopyrimidines. *J Med Chem* 52(16):5013–5016
- Zheng XF et al (1995) TOR kinase domains are required for two distinct functions, only one of which is inhibited by rapamycin. *Cell* 82(1):121–130

Small Molecule Inhibitors of the PI3-Kinase Family

Zachary A. Knight

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Abstract The PI3-K family is one of the most intensely pursued classes of drug targets. This chapter reviews some of the chemical and structural features that determine the selectivity of PI3-K inhibitors, by focusing on a few key compounds that have been instrumental in guiding our understanding of how to design drugs against this family.

1 Introduction

PI3-K was first identified in the late 1980s as an enzyme activity associated with immunoprecipitates of oncogenic tyrosine kinases (Kaplan et al. 1986; Whitman et al. 1985, 1988) and activated growth factor receptors (Kaplan et al. 1987; Ruderman et al. 1990). In 1992, PI3-K (p110 α) was purified and cloned

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(Hiles et al. 1992), and it was shown to have sequence homology to VPS34, a yeast gene required for protein sorting (Robinson et al. 1988). This led to the rapid discovery of a family of 15 kinases, termed the PI3-K family, that share a conserved phosphoinositide kinase (PIK) domain but otherwise vary in their substrate specificity, expression pattern, and modes of regulation.

Most attention has focused on the four class I PI3-Ks, which contain a 110-kDa catalytic domain (termed p110 α , p110 β , p110 δ , or p110 γ) that heterodimerizes with a family of adaptor molecules (termed p85 or p101, among others). The class I enzymes are activated by receptor tyrosine kinases (RTKs) or G-protein coupled receptors (GPCRs) to synthesize phosphatidylinositol-3,4,5-trisphosphate (PIP₃), a lipid second messenger that acts as a recruitment site at the plasma membrane for downstream proteins, including Akt and PDK1. These downstream proteins in turn regulate a wide range of cellular processes that include growth, nutrient uptake, survival, chemotaxis, and proliferation.

In addition to the class I enzymes, the PI3-K family includes four further classes of kinases. These include the class II and class III PI3-Ks, which synthesize phosphatidylinositol-3-phosphate (PI(3)P) or phosphatidylinositol-3,4-bisphosphate (PI(3,4)P₂), and the PI4-Ks, which synthesize phosphatidylinositol-4-phosphate (PI(4)P). The biological roles of these kinases are still being elucidated, but all appear to participate in the regulation of the intracellular trafficking of proteins or vesicles. Finally, the PI3-K family encodes five kinases that phosphorylate proteins rather than lipids. These phosphoinositide 3-kinase-related kinases (PIKKs) include mTOR, which is a key regulator of cell growth, and ATM, ATR, hSmg1, and DNA-PK, which monitor genomic integrity and initiate the DNA damage response.

Recently, there has been considerable excitement about the potential of PI3-Ks as targets for the treatment of cancer and autoimmune disease. In the field of cancer, a key discovery was the finding that PIK3CA, the gene encoding p110 α , is a frequently mutated oncogene (Samuels et al. 2004). Recent estimates suggest that PIK3CA may be mutated at a cumulative frequency of up to 15% across all tumor types (Karakas et al. 2006), which would make p110 α the most frequently mutated kinase in cancer. In addition, the lipid phosphatase PTEN, which inhibits PI3-K signaling, is one of the most commonly inactivated tumor suppressors (Cantley and Neel 1999). Together, these and other data have led to the view that PI3-K is a critical node that controls cancer cell growth and survival (Chang et al. 1997; Shaw and Cantley 2006).

The potential of PI3-Ks as targets for autoimmune disease was suggested by the finding that the expression of p110 γ and p110 δ is restricted (primarily) to leukocytes. Inactivation of these kinases in mice (either by deletion of the gene or by substitution of a kinase-dead allele) demonstrated that they are required for the immune response but otherwise have a limited role in normal physiology (Hirsch et al. 2000; Okkenhaug et al. 2002; Sasaki et al. 2000). This indicated that inhibitors of these kinases might have anti-inflammatory activity with limited side effects, and, indeed, pharmacological inhibition of p110 γ or p110 δ has shown efficacy in preclinical models of arthritis (Camps et al. 2005), lupus (Barber et al. 2005), and the allergic response (Ali et al. 2004).

In response to this data, the pharmaceutical industry has made intense effort in recent years to develop potent and selective PI3-K inhibitors. As recently as 2003, only two PI3-K inhibitors were widely known wortmannin and LY294002. Today, dozens of new chemotypes of PI3-K inhibitors have been described, either in the peer-reviewed or patent literature, and the first such compounds entered clinical trials in late 2007. These new chemotypes have been comprehensively reviewed (Marone et al. 2008).

In this chapter, I summarize what has been learned over the past few years about the chemical determinants of PI3-K inhibitor selectivity. I do this by focusing on a few key compounds that have been instrumental in guiding our understanding of how to design drugs that target this family.

2 LY294002

In the early 1990s, Eli Lilly initiated a screening effort to identify natural products that inhibit PI3-K. This led to the identification of the flavonoid quercetin (Fig. 1) as a PI3-K inhibitor with an IC_{50} of 3.8 μ M (Matter et al. 1992).

Flavonoids often display promiscuous biological activity (Davies et al. 2000; Knight and Shokat 2005), and, by 1992, quercetin had already been shown to inhibit several targets unrelated to PI3-K (Matter et al. 1992). To identify compounds with improved selectivity, analogs of quercetin were synthesized that replaced the catechol moiety with more drug-like substituents (Fig. 1). This led to the discovery in 1994 of LY294002, a reversible, ATP competitive inhibitor of most enzymes in the PI3-K family (Vlahos et al. 1994). LY294002 inhibits the class I PI3-Ks, mTOR, and DNA-PK in vitro with IC_{50} values in the 1–10 μ M range (Brunn et al. 1996; Knight et al. 2004; Vlahos et al. 1994). Unlike quercetin, LY294002 is selective for PI3-K relative to most protein kinases; exceptions include CK2 (Davies et al. 2000) and PLK1 (Liu et al. 2005).

The enhanced PI3-K selectivity of LY294002 relative to quercetin is due to the introduction of the morpholine ring in LY294002 (Fig. 1). In the crystal structure of LY294002 bound to p110 γ , the morpholine ring adopts a chair conformation that enables it to make close hydrophobic contacts with a complementary region of the ATP-binding pocket (Walker et al. 2000). The morpholine oxygen also makes a critical hydrogen bond to the backbone amide of V882. This hydrogen bond is made by the N1 of the adenine ring of ATP, as well as every PI3-K inhibitor that has been described to date, suggesting that this hydrogen bond is required for high-affinity binding. A requirement for a similar hydrogen bond is found among inhibitors of the protein kinase family. Remarkably, although LY294002 was discovered as an analog of quercetin, the crystal structures of these two compounds revealed that they bind to PI3-K in opposite orientations (Fig. 1).

The core pharmacophore in LY294002 is an aromatic ring linked to a morpholine. This “aryl morpholine” pharmacophore is a privileged structure for PI3-K inhibition: it is embedded within many newer classes of PI3-K inhibitors, including

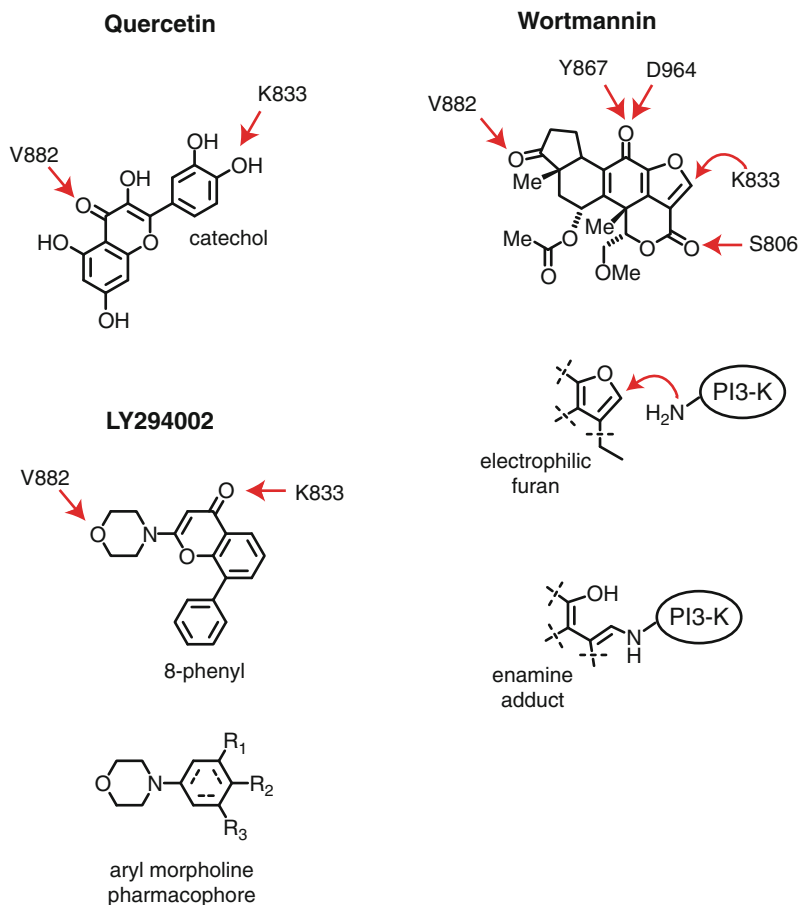


Fig. 1 Classical PI3 K inhibitors. Chemical structure and relative orientation in the ATP binding pocket of p110 γ of the PI3 K inhibitors quercetin, LY294002, and wortmannin. Hydrogen bonds to residues in p110 γ are indicated by *straight arrows*. The nucleophilic attack of K833 on wortmannin is indicated by a *curved arrow*

molecules that target p110 α (Alexander et al. 2008; Folkes et al. 2008; Hayakawa et al. 2006, 2007; Knight et al. 2006; Perry et al. 2008a, b; Raynaud et al. 2007; Yaguchi et al. 2006), p110 β (Jackson et al. 2005; Knight et al. 2004), DNA-PK (Barbeau et al. 2007; Desage-El Murr et al. 2008; Griffin et al. 2005; Hardcastle et al. 2005; Hollick et al. 2007; Knight et al. 2004; Leahy et al. 2004), mTOR (Knight et al. 2006), and ATM (Hickson et al. 2004; Hollick et al. 2007). Structure activity relationship (SAR) data and co-crystal structures indicate that the aryl morpholine in all of these compounds binds in an orientation similar to the aryl morpholine in LY294002. In this sense, these newer compounds are all analogs of LY294002.

LY294002 and its partner wortmannin (discussed below) have been two of the most widely used tool compounds in biological research. Much of what is known about PI3-K signaling was first learned by using these reagents. Nonetheless, it is now clear that the cellular selectivity of LY294002 is limited. To fully inhibit PI3-K activity in cells, LY294002 is often applied at concentrations above 10 μ M. In this concentration range, LY294002 targets several unrelated proteins, including calcium channels (Welling et al. 2005), potassium channels (Sun et al. 2004; Wu et al. 2009), phosphodiesterases (Abbott and Thompson 2004), and the estrogen receptor (Pasapera Limon et al. 2003). Therefore, some of the cellular functions attributed to PI3-Ks based on the use of LY294002 are probably mediated by these secondary targets. For example, LY294002 induces apoptosis in some cell lines and acute toxicity in mice, which are not observed with more potent and selective PI3-K inhibitors.

3 Wortmannin

Wortmannin is a steroid-like natural product that was originally isolated from the fungus *Penicillium wortmanni* (Brian et al. 1957). Early experiments revealed that wortmannin had potent antiproliferative and anti-inflammatory activity (Wiesinger et al. 1974). The specificity of this activity was suggested by the finding that wortmannin blocked, at low nanomolar concentrations, the respiratory burst of neutrophils activated by fMLP, yet did not directly inhibit NADPH oxidase (Baggiolini et al. 1987). This led to the hypothesis that wortmannin may “interfere with the signal transduction sequence initiated by the particulate stimulus” in neutrophils, even though the components of that signaling pathway had not been fully characterized.

In 1993, PI3-K was identified as the direct target of wortmannin responsible for its inhibition of the neutrophil response to fMLP (Arcaro and Wymann 1993). Within the PI3-K family, the most potent targets of wortmannin are the class I enzymes, which are inhibited with IC_{50} values in the 1–10 nM range. Wortmannin also potently inhibits the class II enzyme PI3K-C2 β (IC_{50} = 1.6 nM), but is somewhat less active against PI3K-C2 α (IC_{50} = 420 nM), PI4-KIII β (IC_{50} = 320 nM), mTOR (IC_{50} = 200 nM), and DNA-PK (IC_{50} = 150 nM) (Arcaro et al. 1998; Balla et al. 2008b; Brunn et al. 1996; Domin et al. 1997; Hartley et al. 1995). Interestingly, the wortmannin sensitivity of VPS34, the class III PI3-K, is species dependent: the human and fly enzymes are sensitive (IC_{50} = 10 nM), whereas the yeast enzyme is resistant (IC_{50} = 3 μ M) (Fruman et al. 1998; Stack and Emr 1994). Despite this striking difference in sensitivity between orthologs, efforts to use this information to alter the wortmannin sensitivity of p110 γ by mutagenesis were unsuccessful (Walker et al. 2000).

Wortmannin inhibits PI3-Ks by covalent inactivation of the enzyme (Walker et al. 2000; Wymann et al. 1996): the electrophilic furan ring of wortmannin is attacked by a lysine residue in the kinase active site (K833 in p110 γ), resulting in

ring opening and formation of a stable enamine (Fig. 1). This lysine residue is conserved in all PI3-Ks as a result of its role in catalysis, and this conservation may contribute to the potent inhibition of several PI3-Ks by wortmannin. Nonetheless, noncovalent interactions are also critical for the high-affinity binding of this molecule. The co-crystal structure of wortmannin bound to p110 γ revealed a network of five hydrogen bonds and extensive hydrophobic interactions between the natural product and the enzyme (Fig. 1) (Walker et al. 2000).

The electrophilic furan ring of wortmannin is sensitive to cellular nucleophiles, and wortmannin rapidly decomposes in tissue culture media (half-life \sim 10 min) (Holleran et al. 2003). Nonetheless, wortmannin treatment of cells results in durable PI3-K inhibition. This “wortmannin paradox” may reflect the slow reversibility of the covalent reaction of wortmannin with some cellular nucleophiles, which results in the gradual regeneration of active drug (Yuan et al. 2007). This observation has enabled the design wortmannin pro-drugs with improved pharmacological properties (Yuan et al. 2006).

4 p110 δ Inhibitors and the Selectivity Pocket

In 2003, scientists from ICOS described IC87114 (Fig. 2), an isoquinolinone purine that inhibits p110 δ with an IC₅₀ value of 0.5 μ M (Sadhu et al. 2003b). IC87114 displays remarkable selectivity for p110 δ relative to the rest of the PI3-K family: it is 100-fold more potent for p110 δ than p110 β or p110 γ , and it is essentially inactive against all other PI3-K family members. This molecule was the first selective PI3-K inhibitor to be described, and it has been widely used to probe the role of p110 δ in processes such as the allergic response (Ali et al. 2004, 2008; Zhang et al. 2008), neutrophil activation (Sadhu et al. 2003a, b), and leukemic cell proliferation (Billottet et al. 2006; Sujobert et al. 2005).

The impressive selectivity of IC87114 raised the question of how this molecule discriminates between the class I PI3-K isoforms. IC87114 is ATP competitive, but the ATP-binding pocket of the class I PI3-Ks is highly conserved: among the residues that directly contact the adenine of ATP, there is only one difference among the class I PI3-Ks (a conservative substitution of a valine for isoleucine in p110 γ). While these kinases do diverge in sequence on the periphery of the ATP-binding pocket, it is unclear how these differences could be targeted by a small molecule to achieve selectivity. Like most kinase inhibitors, IC87114 was discovered through chemical optimization of a screening hit, rather than through structure-based design.

In 2006, the crystal structure of an analog of IC87114 (called PIK-39) was reported in complex with p110 γ , along with crystal structures of several other novel PI3-K inhibitors (Knight et al. 2006). Comparison of the binding mode of PIK-39 with that of other inhibitors suggested a rationale for its unique selectivity. Most PI3-K inhibitors bind to the kinase in a flat orientation, in which the drug sits primarily within the plane defined by the adenine of ATP. By contrast, the PIK-39

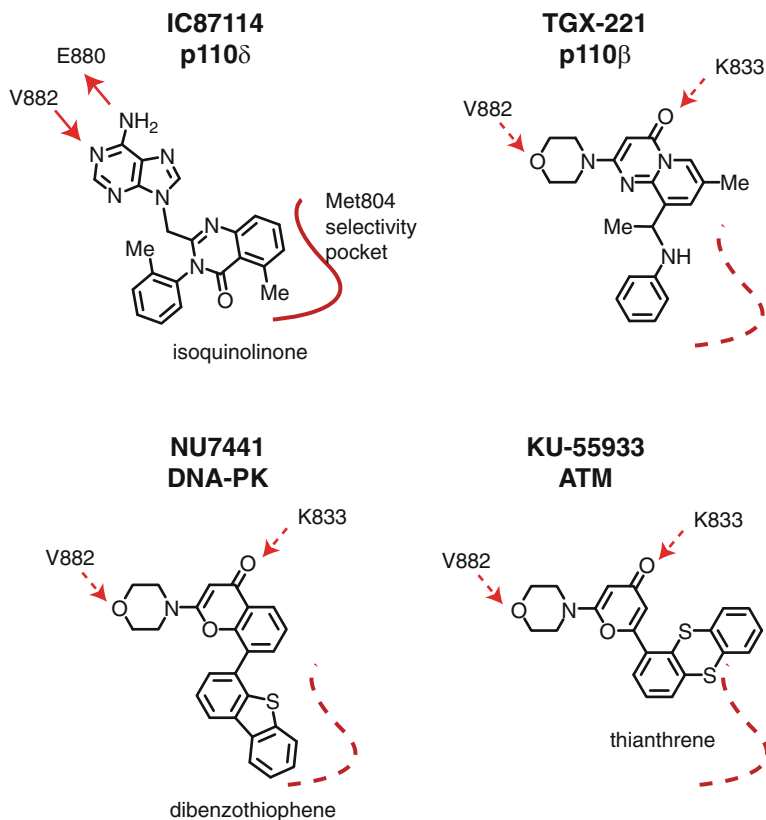


Fig. 2 Isoform selective PI3 K inhibitors. Chemical structure and relative orientation in the PI3 K ATP binding site of four selective inhibitors. Hydrogen bonds observed in crystal structures are indicated by *solid red arrows*; those predicted from models are indicated by *dashed red arrows*

structure revealed that the isoquinolinone moiety of the drug projects upward toward the roof of the ATP-binding pocket (Fig. 3). To accommodate the drug and avoid a steric clash, the kinase undergoes a conformational rearrangement in which the side chain of Met804 moves downward, creating a new hydrophobic pocket at the entrance to the ATP binding site. This inducible pocket, which is not observed in any other PI3-K crystal structure, buries approximately 180 Å² of solvent-accessible surface area of the drug.

These structural data revealed the existence of an inducible drug-binding pocket, gated by Met804, located at the entrance to the PI3-K active site. To test whether this inducible pocket is required for the selectivity of PIK-39, resistance mutants were designed in which Met804 was mutated to a β -branched residue (isoleucine or valine); modeling indicated that a β -branched side chain would be unable to move and thereby accommodate the drug. These resistance mutations blocked the binding of PIK-39 (and IC87114) to p110 δ but had no effect on other classes of inhibitors

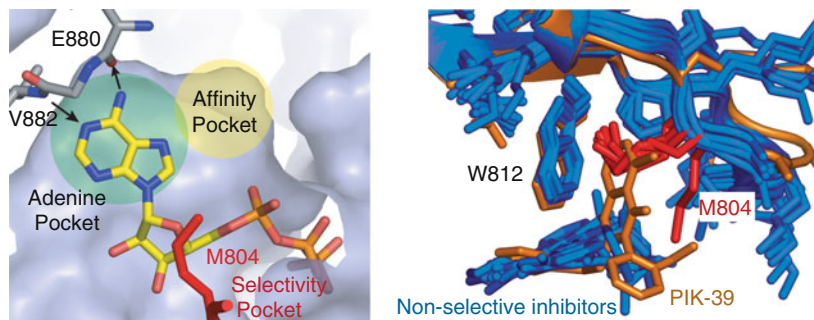


Fig. 3 Structure of the ATP binding pocket of p110 γ . *Left*, crystal structure of ATP bound to p110 γ , indicating hydrogen bonds to the backbone of residues E880 and V882, and the relative location of the regions occupied by the adenine of ATP, the inducible selectivity pocket, and a deeper region occupied by potent inhibitors (affinity pocket). *Right*, an overlay of the crystal structures of structurally diverse PI3 K inhibitors bound to p110 γ . Multitargeted compounds are shown in *blue* and PIK 39 is shown in *orange*. Met804 (*red*) is highlighted to show the conformational change that occurs upon binding of PIK 39. These figures are reproduced with permission from (Knight et al. 2006)

(Knight et al. 2006). This confirmed that the inducible pocket is required for the unique selectivity of the isoquinolinone chemotype.

It is likely that different PI3-K isoforms vary in their ability to form this inducible pocket, as a result of sequence differences that are distal to the ATP-binding site (e.g., second and third shell interactions). If so, this provides an explanation for how PIK-39 can discriminate between PI3-K isoforms even though the residues that directly contact the drug are the same. This prediction has been supported by the recent crystal structure of p110 α , which showed that the loop containing the residue equivalent to Met804 in p110 α adopts an orientation that would preclude the conformational rearrangement of Met804 observed in the PIK-39 structure (Amzel et al. 2008). Finally, it is worth noting that this mechanism of drug selectivity, which is based on differences in conformational flexibility between targets, is well characterized in the field of protein kinase inhibitors. The first example of this was the drug imatinib, which distinguishes between the closely related kinases Abl and Src through binding to a differentially accessible inactive conformation (Schindler et al. 2000).

5 p110 β , DNA-PK, and ATM Inhibitors: A Shared Selectivity Mechanism?

In 2004 and 2005, a series of selective inhibitors of p110 β were described (Jackson et al. 2005; Knight et al. 2004). These compounds are analogs of LY294002 that replace the 8-phenyl substituent of LY294002 with bulkier and more extended groups (Fig. 2). A representative compound is TGX221, which is a potent inhibitor

of p110 β (IC_{50} = 0.005 μ M) and displays impressive selectivity for p110 β over p110 α and p110 γ (\sim 1,000-fold). The selectivity of these compounds for p110 β over p110 δ is much less (two- to tenfold) (Knight et al. 2004). However, the residual p110 δ activity of these compounds has not limited their usefulness, because highly selective p110 δ inhibitors such as IC87114 have been available. Thus, it has been possible to dissect the specific contribution of p110 β in cell-based experiments by using these two classes of inhibitors in combination. Compounds from the TGX series have been employed in this way to assign a role for p110 β in thrombus formation by platelets (Jackson et al. 2005), in fine-tuning insulin signaling in muscle cells (Knight et al. 2006), and in driving cell proliferation in PTEN-null tumors (Torbett et al. 2008), among other functions.

During the same time period, scientists from KuDOS published a series of papers describing their efforts to identify selective DNA-PK inhibitors (Griffin et al. 2005; Hollick et al. 2003, 2007; Leahy et al. 2004). These molecules were identified by synthesizing focused libraries that introduced diversity around the 8-phenyl group of LY294002 and then screening these compounds for selectivity within the PI3-K family. One of the most selective compounds to emerge from this effort was NU7441, which contains a sterically demanding dibenzothiophene in place of the 8-phenyl moiety of LY294002 (Fig. 2). This compound potently inhibits DNA-PK (IC_{50} = 0.020 μ M) and displays 100- to 1000-fold selectivity against other kinases in the PI3-K family (Leahy et al. 2004).

KU-55933 is a second important compound that originated from KuDOS (Fig. 2) (Hickson et al. 2004). KU-55933 is a potent inhibitor of ATM (IC_{50} = 0.013 μ M) and displays a high degree of selectivity relative to all other PI3-K family members (\sim 1,000-fold). Like TGX-221 and NU7441, KU-55933 introduces a bulky substituent (a thianthrene ring) into the approximate region occupied by the 8-phenyl of LY294002. This compound has become a widely used probe for ATM signaling. One early application of this compound demonstrated the potential of ATM as a therapeutic target for HIV, based on the fact that ATM mediates a DNA damage response required for viral integration (Lau et al. 2005).

Crystal structures have not been reported for NU7441, KU-55933, or the TGX series of p110 β inhibitors. Therefore, the precise structural basis for their selectivity is unknown. However, based on SAR data, it is clear that the aryl morpholine moiety in these drugs binds to PI3-Ks in the same orientation as LY294002. This has made it possible to generate models for how these drugs bind to PI3-Ks, by using the LY294002 crystal structure as a template (Knight et al. 2006). These models indicate that all three drugs project a large hydrophobic substituent out of the plane occupied by ATP and toward the region of the kinase that forms the inducible selectivity pocket in the PIK-39 structure (Fig. 2). In each case, it appears that a conformational change in the kinase would be necessary to accommodate the drug (although in the case of ATM, sequence differences in the residues in this region may also be relevant (Knight et al. 2006)). Thus, it is likely that these varied inhibitors achieve their selectivity, in part, by exploiting differences between PI3-Ks in conformational flexibility around the region that moves in the PIK-39 structure.

Data from mutagenesis of p110 β is consistent with this hypothesis (Frazzetto et al. 2008).

6 p110 γ Inhibitors

Serono has described a series of selective p110 γ inhibitors (Camps et al. 2005; Pomel et al. 2006). A representative compound is AS-605240 (Fig. 4), which has low nanomolar activity against p110 γ and \sim 30-fold selectivity relative to p110 β and p110 δ . The selectivity of AS-605240 for p110 γ over p110 α is less (\sim 10-fold), which is consistent with data from other chemotypes that it is unusually difficult to identify small molecules that discriminate between these two isoforms (Knight et al. 2006).

Compounds from this chemical series have two functional parts: (1) a hetero-aromatic ring that binds in the region of the kinase active site occupied by the adenine of ATP and makes a hydrogen bond to the backbone amide of V882; and (2) a thiazolidinedione that occupies a deeper region of the ATP-binding pocket and makes a hydrogen bond to K833 in p110 γ (Fig. 4). Crystal structures of several

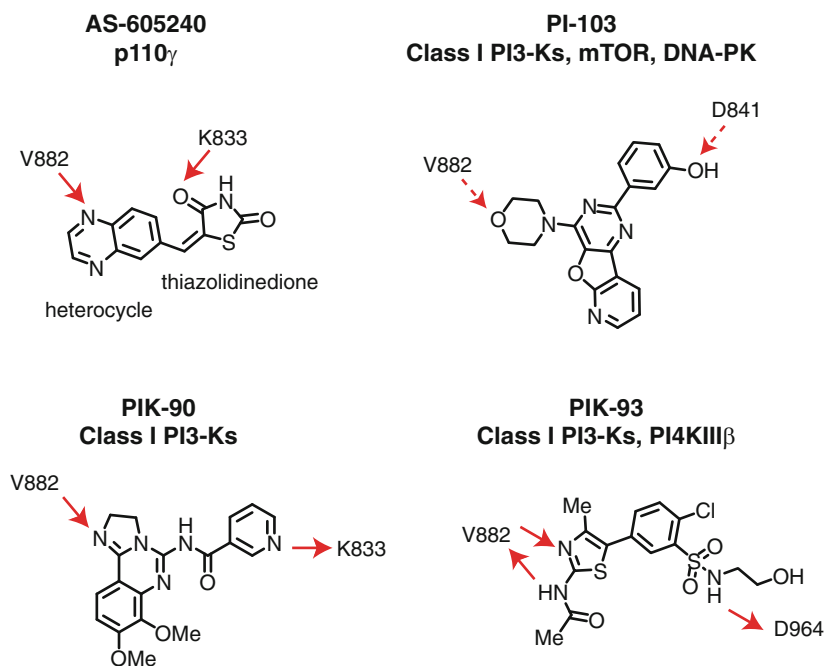


Fig. 4 Inhibitors of p110 γ and class I PI3 Ks. Chemical structure and relative orientation within the ATP binding site of several classes of PI3 K inhibitors. Hydrogen bonds observed in crystal structures are indicated by *solid red arrows*; those predicted from models are indicated by *dashed red arrows*

compounds from this series have been reported, but the interactions that determine the p110 γ selectivity of these compounds are not apparent (Camps et al. 2005). Unlike many classes of PI3-K inhibitors that have been reported, the p110 γ inhibitors from Serono have favorable pharmacological properties, including low molecular weight, high aqueous solubility, and oral bioavailability in mouse.

7 Class I PI3-K Inhibitors

Several classes of multitargeted drugs have been described that inhibit most or all of the class I PI3-Ks. Representative chemotypes are shown in Fig. 4. These compounds often also inhibit DNA-PK and, in some cases, have activity against mTOR. One compound, PIK-93, has unusually potent activity against PI4KIII β and has become a useful probe for that kinase (Balla et al. 2008a, b). By contrast, potent inhibition of ATM, ATR, or hSmg-1 by these drugs is uncommon.

An important early representative of this class was PI-103, an aryl morpholine developed by Piramed (Knight et al. 2006; Raynaud et al. 2007). PI-103 inhibits the class I PI3-Ks, DNA-PK, and mTOR at low- to mid-nanomolar concentrations *in vitro*. As a result of this broad selectivity, PI-103 effectively inhibits almost all PI3-K-dependent growth factor signaling, and PI-103 displays potent antiproliferative activity in most cells, typically as a result of a G₀G₁ cell cycle arrest rather than apoptosis (Fan et al. 2006; Raynaud et al. 2007).

The antiproliferative activity of PI-103 requires direct inhibition of mTOR in addition to the class I PI3-Ks in many cell types. This was unexpected, because it was widely assumed that potent inhibition of PI3-Ks would be sufficient to block tumor cell proliferation. However, a systematic comparison of a panel of structurally diverse PI3-K inhibitors with varying target selectivities revealed that PI-103 was uniquely active against several glioma cell lines (Fan et al. 2006). Compounds such as PIK-90 (Fig. 4) that inhibit the class I PI3-Ks but are less potent against mTOR were found to also have significantly less antiproliferative activity. By combining PIK-90 with the mTOR inhibitor rapamycin, it was possible to phenocopy the cell cycle arrest induced by PI-103, suggesting that inhibition of both PI3-Ks and mTOR is required (Fan et al. 2006). This result has been reproduced in a range of tumor cell lines (Apsel et al. 2008; Fan et al. 2007; Kharas et al. 2008; Park et al. 2008; Torbett et al. 2008) and is also observed with newer inhibitors that are structurally unrelated to PI-103 or PIK-90 (Apsel et al. 2008), arguing that direct mTOR inhibition may be generally required for blockade of cell proliferation by PI3-K family inhibitors. However, it remains unclear which mTOR targets mediate the additional antiproliferative effect caused by direct mTOR inhibition, since upstream inhibition of PI3-K is typically sufficient to block signaling through known mTOR pathway components. In addition, it is unknown to what extent these differences in the drug sensitivity of cell lines *in vitro* will predict the response to PI3-K inhibitors of tumors *in vivo*.

PI-103 has demonstrated activity *in vivo* against tumor xenografts (Fan et al. 2006; Raynaud et al. 2007) and in studies of insulin signaling (Knight et al. 2006).

However, the use of this compound *in vivo* is limited by its low aqueous solubility and rapid metabolism (Raynaud et al. 2007). Piramed has described medicinal chemistry efforts to optimize the pharmacological properties of this compound, resulting in an analog (GDC-0941) that has entered human clinical trials (Folkes et al. 2008). This compound joins class I PI3-K inhibitors such as NVP-BEZ235 (Maira et al. 2008; Serra et al. 2008), XL-147, and XL-765 that have advanced to clinical testing. These compounds are described in greater detail in a separate chapter in this book.

8 Conclusions

Much has been learned over the past 5 years about the chemical principles that control the selectivity PI3-K inhibitors, but some basic questions remain unanswered. Thus far, no highly specific inhibitor of p110 α has been described, despite the fact that this kinase is the primary target of most drug discovery efforts focusing on the PI3-K family. This may reflect the fact that p110 α is challenging to selectively inhibit with a small molecule; for example, SAR and structural data indicate that the selectivity pocket exploited by p110 δ inhibitors is particularly inaccessible in p110 α . Alternatively, it may be that highly selective inhibition of p110 α is not an optimal strategy for the treatment of cancer. Preclinical data clearly indicate that less selective PI3-K inhibitors, such as those that also target mTOR, have enhanced antitumor activity. This has led to efforts to identify small molecules whose selectivity has been expanded even further to target both tyrosine kinases and PI3-Ks (Apsel et al. 2008). Moreover, it seems unlikely that enhanced selectivity for p110 α would prevent the feared side effects of PI3-K inhibitors on glucose metabolism, since p110 α is the primary kinase responsible for insulin signaling (Foukas et al. 2006; Knight et al. 2006).

These questions can be resolved only through clinical testing, and, fortunately, several chemotypes of PI3-K inhibitors are now being evaluated in clinical trials. These human experiments should provide, at last, a verdict on the therapeutic potential of PI3-K inhibitors, at least for the treatment of cancer. Judging from the recent experience of other targeted therapies, however, these clinical data are also likely to supply surprises that will require re-examination of our understanding of PI3-K signaling.

References

- Abbott BM, Thompson PE (2004) *Bioorg Med Chem Lett* 14:2847–2851
- Alexander R, Balasundaram A, Batchelor M, Brookings D, Crepy K, Crabbe T, Deltent MF, Driessens F, Gill A, Harris S, Hutchinson G, Kulisa C, Merriman M, Mistry P, Parton T, Turner J, Whitcombe I, Wright S (2008) *Bioorg Med Chem Lett* 18:4316–4320

- Ali K, Bilancio A, Thomas M, Pearce W, Gilfillan AM, Tkaczyk C, Kuehn N, Gray A, Giddings J, Peskett E, Fox R, Bruce I, Walker C, Sawyer C, Okkenhaug K, Finan P, Vanhaesebroeck B (2004) *Nature* 431:1007 1011
- Ali K, Camps M, Pearce WP, Ji H, Ruckle T, Kuehn N, Pasquali C, Chabert C, Rommel C, Vanhaesebroeck B (2008) *J Immunol* 180:2538 2544
- Amzel LM, Huang CH, Mandelker D, Lengauer C, Gabelli SB, Vogelstein B (2008) *Nat Rev Cancer* 8:665 669
- Apfel B, Blair JA, Gonzalez B, Nazif TM, Feldman ME, Aizenstein B, Hoffman R, Williams RL, Shokat KM, Knight ZA (2008) *Nat Chem Biol* 4:691 699
- Arcaro A, Wymann MP (1993) *Biochem J* 296(Pt 2):297 301
- Arcaro A, Volinia S, Zvelebil MJ, Stein R, Watton SJ, Layton MJ, Gout I, Ahmadi K, Downward J, Waterfield MD (1998) *J Biol Chem* 273:33082 33090
- Baggiolini M, Dewald B, Schnyder J, Ruch W, Cooper PH, Payne TG (1987) *Exp Cell Res* 169:408 418
- Balla A, Kim YJ, Varnai P, Szentpetery Z, Knight Z, Shokat KM, Balla T (2008a) *Mol Biol Cell* 19:711 721
- Balla A, Tuymetova G, Toth B, Szentpetery Z, Zhao X, Knight ZA, Shokat K, Steinbach PJ, Balla T (2008b) *Biochemistry* 47:1599 1607
- Barbeau OR, Cano Soumillac C, Griffin RJ, Hardcastle IR, Smith GC, Richardson C, Clegg W, Harrington RW, Golding BT (2007) *Org Biomol Chem* 5:2670 2677
- Barber DF, Bartolome A, Hernandez C, Flores JM, Redondo C, Fernandez Arias C, Camps M, Ruckle T, Schwarz MK, Rodriguez S, Martinez AC, Balomenos D, Rommel C, Carrera AC (2005) *Nat Med* 11:933 935
- Billett C, Grandage VL, Gale RE, Quattropiani A, Rommel C, Vanhaesebroeck B, Khwaja A (2006) *Oncogene* 25:6648 6659
- Brian PW, Curtis PJ, Hemming HG, Norris GLF (1957) *Transactions of the British Mycological Society* 40:365 368
- Brunn GJ, Williams J, Sabers C, Wiederrecht G, Lawrence JC Jr, Abraham RT (1996) *Embo J* 15:5256 5267
- Camps M, Ruckle T, Ji H, Ardisson V, Rintelen F, Shaw J, Ferrandi C, Chabert C, Gillieron C, Francon B, Martin T, Gretener D, Perrin D, Leroy D, Vitte PA, Hirsch E, Wymann MP, Cirillo R, Schwarz MK, Rommel C (2005) *Nat Med* 11:936 943
- Cantley LC, Neel BG (1999) *Proc Natl Acad Sci USA* 96:4240 4245
- Chang HW, Aoki M, Fruman D, Auger KR, Bellacosa A, Tsichlis PN, Cantley LC, Roberts TM, Vogt PK (1997) *Science* 276:1848 1850
- Davies SP, Reddy H, Caivano M, Cohen P (2000) *Biochem J* 351:95 105
- Desage El Murr M, Cano C, Golding BT, Hardcastle IR, Hummersome M, Frigerio M, Curtin NJ, Menear K, Richardson C, Smith GC, Griffin RJ (2008) *Bioorg Med Chem Lett* 18:4885 4890
- Domin J, Pages F, Volinia S, Rittenhouse SE, Zvelebil MJ, Stein RC, Waterfield MD (1997) *Biochem J* 326(Pt 1):139 147
- Fan QW, Knight ZA, Goldenberg DD, Yu W, Mostov KE, Stokoe D, Shokat KM, Weiss WA (2006) *Cancer Cell* 9:341 349
- Fan QW, Cheng CK, Nicolaides TP, Hackett CS, Knight ZA, Shokat KM, Weiss WA (2007) *Cancer Res* 67:7960 7965
- Folkes AJ, Ahmadi K, Alderton WK, Alix S, Baker SJ, Box G, Chuckowree IS, Clarke PA, Depledge P, Eccles SA, Friedman LS, Hayes A, Hancox TC, Kugendradas A, Lensun L, Moore P, Olivero AG, Pang J, Patel S, Pergl Wilson GH, Raynaud FI, Robson A, Saghiri N, Salphati L, Sohal S, Ultsch MH, Valenti M, Wallweber HJ, Wan NC, Wiesmann C, Workman P, Zhyvoloup A, Zvelebil MJ, Shuttleworth SJ (2008) *J Med Chem* 51:5522 5532
- Foukas LC, Claret M, Pearce W, Okkenhaug K, Meek S, Peskett E, Sancho S, Smith AJ, Withers DJ, Vanhaesebroeck B (2006) *Nature* 441:366 370
- Frazzetto M, Suphioglu C, Zhu J, Schmidt Kittler O, Jennings IG, Cranmer SL, Jackson SP, Kinzler KW, Vogelstein B, Thompson PE (2008) *Biochem J* 414:383 390

- Fruman DA, Meyers RE, Cantley LC (1998) *Annu Rev Biochem* 67:481 507
- Griffin RJ, Fontana G, Golding BT, Guiard S, Hardcastle IR, Leahy JJ, Martin N, Richardson C, Rigoreau L, Stockley M, Smith GC (2005) *J Med Chem* 48:569 585
- Hardcastle IR, Cockcroft X, Curtin NJ, El Murr MD, Leahy JJ, Stockley M, Golding BT, Rigoreau L, Richardson C, Smith GC, Griffin RJ (2005) *J Med Chem* 48:7829 7846
- Hartley KO, Gell D, Smith GC, Zhang H, Divecha N, Connelly MA, Admon A, Lees Miller SP, Anderson CW, Jackson SP (1995) *Cell* 82:849 856
- Hayakawa M, Kaizawa H, Moritomo H, Koizumi T, Ohishi T, Okada M, Ohta M, Tsukamoto S, Parker P, Workman P, Waterfield M (2006) *Bioorg Med Chem* 14:6847 6858
- Hayakawa M, Kaizawa H, Moritomo H, Koizumi T, Ohishi T, Yamano M, Okada M, Ohta M, Tsukamoto S, Raynaud FI, Workman P, Waterfield MD, Parker P (2007) *Bioorg Med Chem Lett* 17:2438 2442
- Hickson I, Zhao Y, Richardson CJ, Green SJ, Martin NM, Orr AI, Reaper PM, Jackson SP, Curtin NJ, Smith GC (2004) *Cancer Res* 64:9152 9159
- Hiles ID, Otsu M, Volinia S, Fry MJ, Gout I, Dhand R, Panayotou G, Ruiz Larrea F, Thompson A, Totty NF et al (1992) *Cell* 70:419 429
- Hirsch E, Katanaev VL, Garlanda C, Azzolino O, Pirola L, Silengo L, Sozzani S, Mantovani A, Altruda F, Wymann MP (2000) *Science* 287:1049 1053
- Holleran JL, Egorin MJ, Zuhowski EG, Parise RA, Musser SM, Pan SS (2003) *Anal Biochem* 323:19 25
- Hollick JJ, Golding BT, Hardcastle IR, Martin N, Richardson C, Rigoreau LJ, Smith GC, Griffin RJ (2003) *Bioorg Med Chem Lett* 13:3083 3086
- Hollick JJ, Rigoreau LJ, Cano Soumillac C, Cockcroft X, Curtin NJ, Frigerio M, Golding BT, Guiard S, Hardcastle IR, Hickson I, Hummersone MG, Menear KA, Martin NM, Matthews I, Newell DR, Ord R, Richardson CJ, Smith GC, Griffin RJ (2007) *J Med Chem* 50:1958 1972
- Jackson SP, Schoenwaelder SM, Goncalves I, Nesbitt WS, Yap CL, Wright CE, Kenche V, Anderson KE, Dopheide SM, Yuan Y, Sturgeon SA, Prabakaran H, Thompson PE, Smith GD, Shepherd PR, Daniele N, Kulkarni S, Abbott B, Saylik D, Jones C, Lu L, Giuliano S, Hughan SC, Angus JA, Robertson AD, Salem HH (2005) *Nat Med* 11:507 514
- Kaplan DR, Whitman M, Schaffhausen B, Raptis L, Garcea RL, Pallas D, Roberts TM, Cantley L (1986) *Proc Natl Acad Sci USA* 83:3624 3628
- Kaplan DR, Whitman M, Schaffhausen B, Pallas DC, White M, Cantley L, Roberts TM (1987) *Cell* 50:1021 1029
- Karakas B, Bachman KE, Park BH (2006) *Br J Cancer* 94:455 459
- Kharas MG, Janes MR, Scarfone VM, Lilly MB, Knight ZA, Shokat KM, Fruman DA (2008) *J Clin Invest* 118:3038 3050
- Knight ZA, Shokat KM (2005) *Chem Biol* 12:621 637
- Knight ZA, Chiang GG, Alaimo PJ, Kenski DM, Ho CB, Coan K, Abraham RT, Shokat KM (2004) *Bioorg Med Chem* 12:4749 4759
- Knight ZA, Gonzalez B, Feldman ME, Zunder ER, Goldenberg DD, Williams O, Loewith R, Stokoe D, Balla A, Toth B, Balla T, Weiss WA, Williams RL, Shokat KM (2006) *Cell* 125:733 747
- Lau A, Swinbank KM, Ahmed PS, Taylor DL, Jackson SP, Smith GC, O'Connor MJ (2005) *Nat Cell Biol* 7:493 500
- Leahy JJ, Golding BT, Griffin RJ, Hardcastle IR, Richardson C, Rigoreau L, Smith GC (2004) *Bioorg Med Chem Lett* 14:6083 6087
- Liu Y, Shreder KR, Gai W, Corral S, Ferris DK, Rosenblum JS (2005) *Chem Biol* 12:99 107
- Maira SM, Stauffer F, Bruegggen J, Furet P, Schnell C, Fritsch C, Brachmann S, Chene P, De Pover A, Schoemaker K, Fabbro D, Gabriel D, Simonen M, Murphy L, Finan P, Sellers W, Garcia Echeverria C (2008) *Mol Cancer Ther* 7:1851 1863
- Marone R, Cmiljanovic V, Giese B, Wymann MP (2008) *Biochim Biophys Acta* 1784:159 185
- Matter WF, Brown RF, Vlahos CJ (1992) *Biochem Biophys Res Commun* 186:624 631

- Okkenhaug K, Bilancio A, Farjot G, Priddle H, Sancho S, Peskett E, Pearce W, Meek SE, Salpekar A, Waterfield MD, Smith AJ, Vanhaesebroeck B (2002) *Science* 297:1031 1034
- Park S, Chapuis N, Bardet V, Tamburini J, Gallay N, Willems L, Knight ZA, Shokat KM, Azar N, Viguie F, Ifrah N, Dreyfus F, Mayeux P, Lacombe C, Bouscary D (2008) *Leukemia* 22: 1698 1706
- Pasapera Limon AM, Herrera Munoz J, Gutierrez Sagal R, Ulloa Aguirre A (2003) *Mol Cell Endocrinol* 200:199 202
- Perry B, Alexander R, Bennett G, Buckley G, Ceska T, Crabbe T, Dale V, Gowers L, Horsley H, James L, Jenkins K, Crepy K, Kulisa C, Lightfoot H, Lock C, Mack S, Morgan T, Nicolas AL, Pitt W, Sabin V, Wright S (2008a) *Bioorg Med Chem Lett* 18:4700 4704
- Perry B, Beevers R, Bennett G, Buckley G, Crabbe T, Gowers L, James L, Jenkins K, Lock C, Sabin V, Wright S (2008b) *Bioorg Med Chem Lett* 18:5299 5302
- Pomel V, Klicic J, Covini D, Church DD, Shaw JP, Roulin K, Burgat Charvillon F, Valognes D, Camps M, Chabert C, Gillieron C, Francon B, Perrin D, Leroy D, Gretener D, Nichols A, Vitte PA, Carboni S, Rommel C, Schwarz MK, Ruckle T (2006) *J Med Chem* 49:3857 3871
- Raynaud FI, Eccles S, Clarke PA, Hayes A, Nutley B, Alix S, Henley A, Di Stefano F, Ahmad Z, Guillard S, Bjerke LM, Kelland L, Valenti M, Patterson L, Gowan S, de Haven Brandon A, Hayakawa M, Kaizawa H, Koizumi T, Ohishi T, Patel S, Saghir N, Parker P, Waterfield M, Workman P (2007) *Cancer Res* 67:5840 5850
- Robinson JS, Klionsky DJ, Banta LM, Emr SD (1988) *Mol Cell Biol* 8:4936 4948
- Ruderman NB, Kapeller R, White MF, Cantley LC (1990) *Proc Natl Acad Sci USA* 87:1411 1415
- Sadhu C, Dick K, Tino WT, Staunton DE (2003a) *Biochem Biophys Res Commun* 308:764 769
- Sadhu C, Masinovsky B, Dick K, Sowell CG, Staunton DE (2003b) *J Immunol* 170:2647 2654
- Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell SM, Riggins GJ, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Velculescu VE (2004) *Science* 304:554
- Sasaki T, Irie Sasaki J, Jones RG, Oliveira dos Santos AJ, Stanford WL, Bolon B, Wakeham A, Itie A, Bouchard D, Kozieradzki I, Joza N, Mak TW, Ohashi PS, Suzuki A, Penninger JM (2000) *Science* 287:1040 1046
- Schindler T, Bornmann W, Pellicena P, Miller WT, Clarkson B, Kuriyan J (2000) *Science* 289:1938 1942
- Serra V, Markman B, Scaltriti M, Eichhorn PJ, Valero V, Guzman M, Botero ML, Llonch E, Atzori F, Di Cosimo S, Maira M, Garcia Echeverria C, Parra JL, Arribas J, Baselga J (2008) *Cancer Res* 68:8022 8030
- Shaw RJ, Cantley LC (2006) *Nature* 441:424 430
- Stack JH, Emr SD (1994) *J Biol Chem* 269:31552 31562
- Sujobert P, Bardet V, Cornillet Lefebvre P, Hayflick JS, Prie N, Verdier F, Vanhaesebroeck B, Muller O, Pesce F, Ifrah N, Hunault Berger M, Berthou C, Villemagne B, Jourdan E, Audhuy B, Solary E, Witz B, Harousseau JL, Hemberlin C, Lamy T, Lioure B, Cahn JY, Dreyfus F, Mayeux P, Lacombe C, Bouscary D (2005) *Blood* 106:1063 1066
- Sun H, Oudit GY, Ramirez RJ, Costantini D, Backx PH (2004) *Cardiovasc Res* 62:509 520
- Torbett NE, Luna Moran A, Knight ZA, Houk A, Moasser M, Weiss W, Shokat KM, Stokoe D (2008) *Biochem J* 415:97 110
- Vlahos CJ, Matter WF, Hui KY, Brown RF (1994) *J Biol Chem* 269:5241 5248
- Walker EH, Pacold ME, Perisic O, Stephens L, Hawkins PT, Wymann MP, Williams RL (2000) *Mol Cell* 6:909 919
- Welling A, Hofmann F, Wegener JW (2005) *Mol Pharmacol* 67:541 544
- Whitman M, Kaplan DR, Schaffhausen B, Cantley L, Roberts TM (1985) *Nature* 315:239 242
- Whitman M, Downes CP, Keeler M, Keller T, Cantley L (1988) *Nature* 332:644 646
- Wiesinger D, Gubler HU, Haefliger W, Hauser D (1974) *Experientia* 30:135 136
- Wu J, Ding WG, Matsuura H, Tsuji K, Zang WJ, Horie M (2009) *Br J Pharmacol* 156:377 387
- Wymann MP, Bulgarelli Leva G, Zvelebil MJ, Pirola L, Vanhaesebroeck B, Waterfield MD, Panayotou G (1996) *Mol Cell Biol* 16:1722 1733

- Yaguchi S, Fukui Y, Koshimizu I, Yoshimi H, Matsuno T, Gouda H, Hirono S, Yamazaki K, Yamori T (2006) *J Natl Cancer Inst* 98:545-556
- Yuan H, Luo J, Weissleder R, Cantley L, Josephson L (2006) *J Med Chem* 49:740-747
- Yuan H, Barnes KR, Weissleder R, Cantley L, Josephson L (2007) *Chem Biol* 14:321-328
- Zhang TT, Okkenhaug K, Nashed BF, Puri KD, Knight ZA, Shokat KM, Vanhaesebroeck B, Marshall AJ (2008) *J Allergy Clin Immunol* 122:811-819, e2

Targeting the RTK-PI3K-mTOR Axis in Malignant Glioma: Overcoming Resistance

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Abstract Gliomas represent the most common primary brain tumor and among the most aggressive of cancers. Patients with glioma typically relapse within a year

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of initial diagnosis. Recurrent glioma is associated with acquired therapeutic resistance. Although neurosurgical resection, radiation and chemotherapy provide clear benefit, survival remains disappointing. It is, therefore, critical that we identify effective medical therapies and appropriate tumor biomarkers in patients at initial presentation, to promote durable responses in glioma. Pathways linking receptor tyrosine kinases, PI3 kinase, Akt, and mTOR feature prominently in this disease and represent therapeutic targets. Small molecules that inhibit one or more of these kinases are now being introduced into the clinic and may have some activity. Disappointingly, however, preclinical studies demonstrate these agents to be primarily cytostatic rather than cytotoxic to glioma cells. Here, we detail activation of the EGFR-PI3K-Akt-mTOR signaling network in glioma, review class I PI3K inhibitors, discuss roles for Akt, PKC and mTOR, and the importance of biomarkers. We further delineate attempts to target both single and multiple components within the EGFR-PI3K-Akt-mTOR axes. Lastly, we discuss the need to combine targeted therapies with cytotoxic chemotherapy, radiation and with inhibitors of survival signaling to improve outcomes in glioma.

Abbreviations

CML	Chronic myelogenous leukemia
EGFR	Epidermal growth factor receptor
GBM	Glioblastoma multiforme
mTOR	Mammalian target of rapamycin
PKC	Protein kinase C
PI3K	Phosphatidylinositol 3'kinase
RTK	Receptor tyrosine kinase

1 Introduction

Gliomas represent the most common primary brain tumor and are among the most lethal of all cancers. Prognosis for glioma differs from most other cancer types in that grade (mitotic features, microvascular proliferation, and necrotic tissue surrounded by anaplastic cells, so-called pseudopalisading necrosis) is much more important than stage (extent of disease). Astrocytomas are the most frequently occurring type of glioma. The vast majority of patients (~90%) present at diagnosis with high-grade glioblastoma multiforme tumors (GBM). Both GBM (grade IV) and grade III astrocytomas (high-grade without pseudopalisading necrosis) comprise “malignant gliomas”. Standard-of-care therapy for GBM includes surgery and radiation therapy, resulting in a median survival of approximately 1 year from the time of diagnosis (reviewed in Persson et al. [2007](#)). Over the past decade,

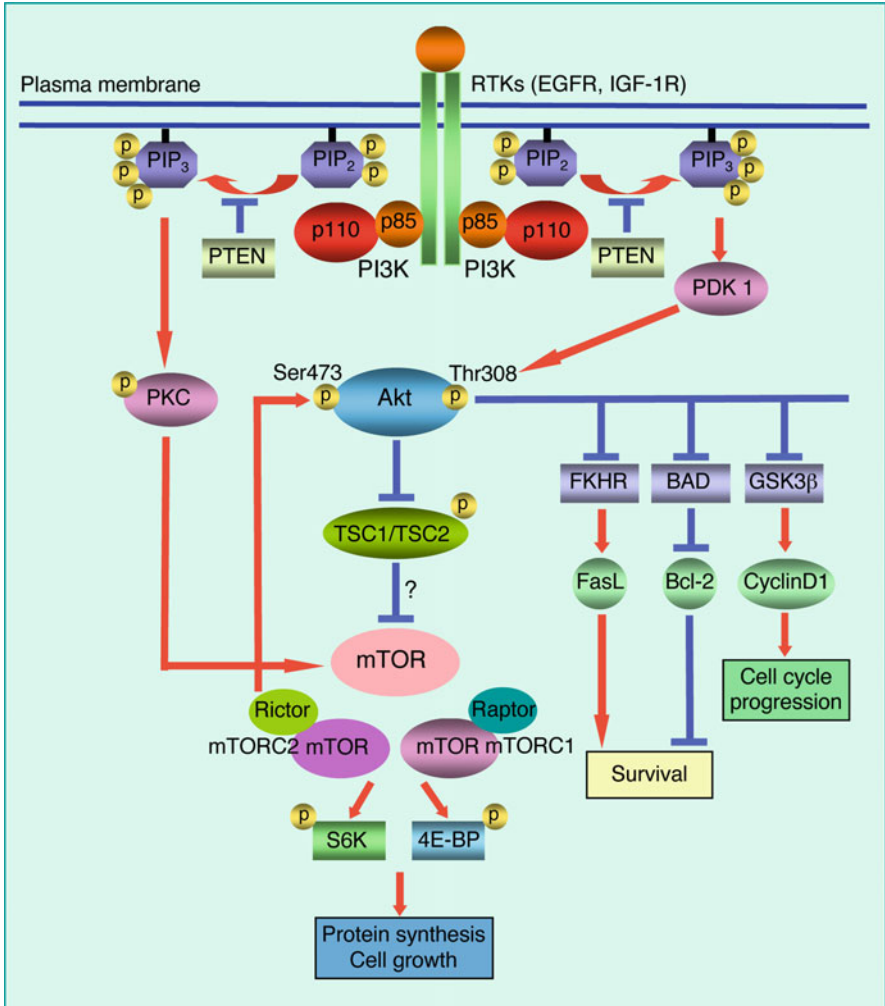


Fig. 1 PI3 kinase signaling pathway in glioma. Class I PI3 kinases are activated by upstream signals from receptor tyrosine kinases (RTKs) including EGFR and other RTKs. PI3 kinase catalyzes production of the second messenger PIP₃, which activates both Akt and PKC. Akt and PKC phosphorylate multiple downstream substrates. We found Akt was dispensable for mitogenic signaling between EGFR and mTOR in glioma cells, whereas PKC was critical (33). PIP₃ is negatively regulated by the tumor suppressor PTEN, a phosphatase driving dephosphorylation of PIP₃

addition of the alkylating agent temozolomide, administered both during and after radiotherapy, has been justifiably viewed as a major advance in the care of these patients, improving survival by approximately 3 m overall (Stupp et al. 2005).

Genetic alterations in GBM typically deregulate pathways involving tumor suppressors p53 (87%), RB (78%), and receptor-tyrosine kinase (RTK)/RAS/PI3K (88%) (Cancer Genome Atlas Research Network 2008). Among these, the

RTK/RAS/PI3K pathway is distinguished in requiring a number of key kinase intermediates, and currently represents the pathway most amenable to pharmacologic intervention. Mutations such as amplification of *EGFR* (45%), gain of function in *PIK3CA* (15%), or loss of *PTEN* (36%) all activate the lipid kinase PI3K and its downstream target, the plekstrin-homology-domain serine threonine kinase Akt. Akt has over 40 downstream targets (Manning and Cantley 2007). Prominent among these are GSK-3, PRAS40, FOXO, BAD, mTOR, and the TSC1/2 proteins (Fig. 1). Although EGFR and downstream signaling components all represent attractive targets for therapy, initial clinical studies focused on inhibiting EGFR have been disappointing in glioma (Prados et al. 2006; Rich et al. 2004). In addition, preclinical studies inhibiting EGFR and other RTKs, as well as PI3K and mTOR have shown only modest efficacy in GBM. Can an understanding of the molecular and genetic abnormalities in GBM lead to improved therapies using single agents or combination protocols, enabling these pathways to be targeted effectively in patients?

2 The Epidermal Growth Factor Receptor Pathway

EGFR is commonly mutated in GBM, leading to overexpression and activation of downstream signaling pathways. The *EGFR* gene is amplified in 40–50% tumors, and overexpressed in a majority of GBM. Approximately 40% of tumors with *EGFR* amplification also have gene rearrangements, most commonly deleting the ligand binding domain, resulting in a constitutively active *EGFRvIII* allele (Cancer Genome Atlas Research Network 2008; Jones et al. 2008). EGFR signals through a complex network of intermediates including PI3K, AKT, MAPK and PLC γ . Overactivity of the EGFR pathway results in proliferation, invasiveness, motility, angiogenesis and inhibition of apoptosis, and is associated with resistance to radiation and chemotherapy (reviewed in Brandes et al. 2008).

Since EGFR is a driving oncogene in malignant glioma, it was anticipated that inhibition of EGFR signaling would represent an effective therapeutic strategy. Two small-molecule tyrosine kinase inhibitors of the EGFR (erlotinib and gefitinib) were evaluated in malignant gliomas. Initial results with EGFR inhibitors in GBM have been disappointing however, with most patients not responding. Only patients with high expression of wild-type EGFR and low levels of phosphorylated Akt in one study (Haas-Kogan et al. 2005), and coexpression of EGFRvIII and wild-type *PTEN* in another study (Mellinghoff et al. 2005) showed a radiographic response to EGFR kinase inhibitors. It was not clear that these changes were durable, and such patients represented a minority population (~10%).

Gefitinib (ZD1839, Iressa) is a small molecule inhibitor of the EGFR tyrosine kinase that has been tested in a phase II study in recurrent GBM. Median event-free survival was 8.1 weeks. No radiographic responses were observed and the 6-month median progression-free survival (PFS) was 17% (Rich et al. 2004). Another phase II trial also reported the ineffectiveness of gefitinib in patients with high-grade

glioma (Franceschi et al. 2007). Gefitinib is rarely used currently in the treatment of GBM.

Erlotinib (OSI-774, Tarceva) inhibits the tyrosine kinase activity of EGFR and EGFRvIII. Partial response rates of 6% were reported in a phase II study, in which progression free survival for patients was 12 weeks. All patients progressed by 24 weeks (de Groot et al. 2008). It is unclear whether erlotinib is more effective than gefitinib for radiographic response rate in high-grade glioma. A recently published Phase II study demonstrated that erlotinib in combination with temozolomide chemotherapy resulted in improved survival, again correlating with PTEN immunopositivity (Prados et al. 2009).

Amplification of *EGFR* is prominent in glioma. It was, therefore, quite disappointing although perhaps not surprising that blockade of this kinase had such a modest effect in patients. At least two observations can help to explain this apparent paradox. First, EGFR is one among many kinases activated in glioma. The abundance of RTKs expressed in GBM suggests a redundancy that may preclude observing clinical improvement in response to targeting any single RTK in this disease (Stommel et al. 2007). This observation is somewhat at odds with one of many lessons learned from CML patients treated with imatinib however; kinases activated by mutation are generally better targets than kinases activated in the absence of genetic mutation (Sawyers 2004). With so many RTKs apparently over-expressed in glioma, why does mutational activation of *EGFR* occur so much more commonly than mutational activation of other RTKs?

A second contributor to the failure of EGFR inhibitors in glioma relates to EGFR-independent mutational activation in coupled signaling pathways, leading to sustained activation of downstream signaling even in the setting of effective upstream blockade. To fully understand this issue requires a brief review of lipid kinase signaling downstream of EGFR.

3 The PI3K/Akt/mTOR Axis in Glioma

PI3Ks are lipid kinases activated by a wide range of RTKs to generate the second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP₃). PIP₃ couples PI3K to downstream effectors such as Akt, a serine-threonine kinase that suppresses apoptosis, promotes growth and drives proliferation. PIP₃ also indirectly activates the protein kinase mTOR, which is critical for cell growth and contains a PI3K homology domain (making mTOR a PIK related Kinase PIKK), although mTOR itself has no lipid kinase activity. The lipid phosphatase Pten acts on PIP₃ to antagonize PI3K signaling and shows frequent inactivation, deletion, or epigenetic silencing in GBM. Inactivation of *PTEN* and activating mutations in PI3K itself collectively occur in a substantial fraction of GBM tumors, effectively uncoupling PI3K from upstream control by EGFR.

It is perhaps not surprising then that inhibition of EGFR in *PTEN* mutant glioma has shown little therapeutic effect, an observation made preclinically by us and

others (Fan et al. 2003, 2007; Wang et al. 2006) and subsequently borne out clinically in studies described above. That responses to clinical inhibitors of EGFR were seen only in patients with tumors that were wild-type for *PTEN*, or that showed low levels of phosphorylated Akt (a histological surrogate for *PTEN* proficiency), suggests that responses were limited to tumors in which inhibition of EGFR led to blockade of downstream PI3K signaling. The role played by *PTEN* mutation in uncoupling EGFR and PI3K also suggests that the efficacy of EGFR inhibition in *PTEN* mutant glioma should be augmented by addition of a PI3K inhibitor. In preclinical studies, we therefore transduced *PTEN* mutant human glioma cells with the constitutively active, tumor-derived *EGFR_{vIII}* allele, established flank xenografts, and treated these with the EGFR inhibitor gefitinib or the pan-PI3K inhibitor LY294002. While low doses of either monotherapy had no effect on tumor burden, combination low-dose therapy efficiently blocked further growth of established tumor xenografts. These and other preclinical studies (Fan et al. 2003; Wang et al. 2006) support the use of such a combination approach in *EGFR*-driven, *PTEN* mutant glioma. However, the availability of clinical PI3K inhibitors, now in clinical trials, has to date precluded clinical trials that test this combination.

4 Isoform Specific Inhibitors of Class I PI3K Inhibitors

The PI3K-Akt-mTOR signaling pathway is currently one of the most attractive therapeutic targets in GBM. The eight mammalian PI3Ks are divided into three classes according to their structure, regulation, and substrate specificity. Most PI3K enzymes consist of a p110 catalytic subunit that heterodimerizes with a separate regulatory subunit. Of particular interest are the four Class I PI3K isoforms (α , β , δ , and γ), which are activated by receptor tyrosine kinases or by heterotrimeric G-proteins. Although gain-of function mutations in the p110 α gene (*PIK3CA*) are found uniquely in human cancers, other p110 isoforms also have oncogenic potential when overexpressed. PI3K β is associated with development of thrombotic diseases through activation of platelets (Jackson et al. 2005). Both PI3K δ and PI3K γ play major roles in the immune system. Knockout of either the δ or γ isoforms of PI3K led to impaired immune responses (Okkenhaug et al. 2002; Sasaki et al. 2000).

Two recent studies show that the PI3K β also has a role in glucose metabolism that was not required for Akt activation. In conditional knock out mice, ablation of PI3K β in the livers led to impaired insulin sensitivity and glucose homeostasis with little change in phosphorylation of Akt (Jia et al. 2008). Interestingly, deletion of PI3K β (but not PI3K α) blocked prostate tumor formation in genetically engineered mice deleted for *PTEN*, and was associated with decreased levels of p-Akt in this setting. Mice expressing a catalytically inactive PI3K β (K805R) developed mild insulin resistance detectable from 6 months of age, and were protected from breast tumor formation driven by a Her2 transgene

(Ciraolo et al. 2008). Comparison of wild-type MEFs and MEFs homozygous for the deleted or inactive PI3K β kinase revealed that the catalytic function of PI3K β was not required for Akt activation shortly after growth factor stimulation, but was required for signaling via GPCRs.

Much of our understanding of the biology of PI3Ks has resulted from experiments using panselective PI3K inhibitors such as LY294002 and wortmannin, which inhibit a broad range of p110 enzymes. However, this same diversity of functions within the PI3K family has limited the utility of panselective inhibitors of PI3Ks to validate particular PI3K isoforms as potential therapeutic targets. In addition, use of LY294002 and wortmannin has been restricted to preclinical studies because of lack of selectivity, toxic effects, and poor pharmaceutical properties. Despite these inadequacies, both compounds have proven invaluable for the early study of PI3K inhibition and serve as pathfinders in the development of PI3K inhibitors.

To assess the impact of inhibiting individual PI3Ks, efforts in both academia and industry have recently developed several isoform-selective inhibitors of class I PI3Ks that show significant selectivity. All are ATP-competitive inhibitors. Because ATP-binding pockets of different kinases are structurally similar, these inhibitors generally show activities against a number of PI3 kinases. Several isoform-selective inhibitors of the PI3Ks are therefore being evaluated in malignant gliomas.

To test the efficacy of these agents, we screened a genetically heterogeneous panel of glioma cell lines using a panel of small molecule PI3K inhibitors, including chemotypes of compounds likely to be further developed as clinical inhibitors (Fan et al. 2006; Knight et al. 2006). We observed biochemical activity (against phospho-Akt) using a number of agents that blocked PI3K alpha and PI3K beta, but not in response to a more limited number of agents that blocked PI3K gamma or PI3K delta. Although agents blocking PI3K beta were equivalent to agents that blocked PI3K alpha in terms of their activity against Akt, only PI3K alpha inhibitors were effective as antiproliferative agents. These observations suggest that PI3K alpha is the principal PI3K driving proliferation in glioma. That inhibitors of PI3K beta showed activity against p-Akt but were ineffective in blocking proliferation suggests that Akt is a poor biomarker for the antiproliferative activity of PI3K inhibitors in glioma (discussed in further detail below).

Interestingly, the Piramed inhibitor PI-103 was unique among inhibitors tested in showing the most potent antiproliferative activity. We traced the antiproliferative activity of PI-103 to its independent inhibitory activities against both PI3K α and mTOR. This combined inhibition of p110 α and mTOR eliminated the increased Akt signaling often observed using allosteric inhibitors of mTOR as monotherapy (Fig. 2) and likely antagonizing the potential efficacy of mTOR inhibitors (Fan et al. 2006). Interestingly, the ability of this and other compounds to inhibit mTOR was largely overlooked during initial development of these drugs, likely because mTOR inhibition was presumed to be a consequence of PI3K blockade. We subsequently demonstrated that inhibitors of PI3K do not block mTOR in glioma (Fan et al. 2009), suggesting that dual inhibitors of PI3K and mTOR in this disease affect two parallel pathways, rather than a single linear one (see below).

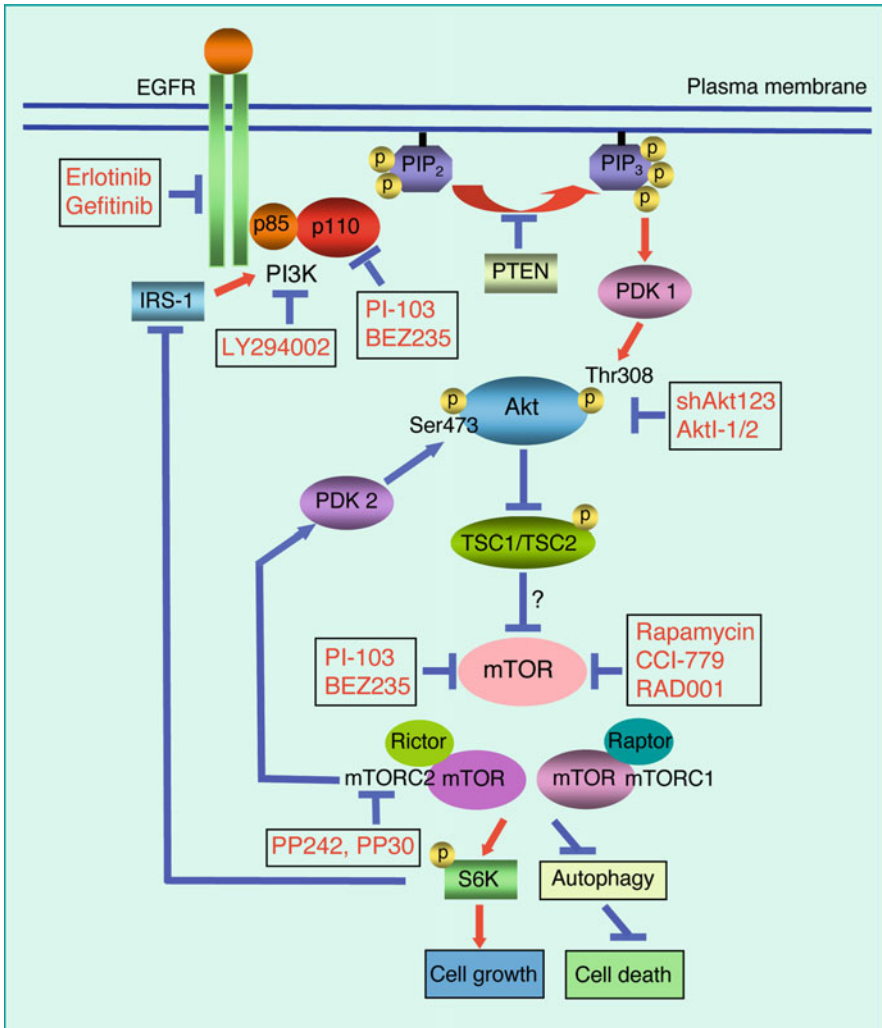


Fig. 2 Sites of action for inhibitors and shRNAs in the EGFR PI3K Akt mTOR pathway. Agents that inhibit the EGFR PI3K Akt mTOR pathway at multiple sites may contribute to anticancer effects in malignant glioma. Agents that inhibit only one target within the EGFR PI3K Akt mTOR axis generally show only modest efficacy and fail to induce appreciable apoptosis. This disappointing efficacy stems in part from multiple nodes of activation in the EGFR PI3K Akt mTOR axis. For example, loss of PTEN activates PI3K signaling. A feedback loop between S6K and IRS 1 also lead to PI3K activation. Inhibition of the PI3K Akt mTOR pathway may also induce autophagy, enabling cancer cells to survive some small molecule inhibitors of this pathway

5 Targeting mTOR Signaling

The mTOR kinase (also known as FRAP, RAFT or RAPT) is intimately linked to PI3K/Akt signaling and to the regulation of protein synthesis, cell growth and survival. Activation of mTOR in response to growth factor signals is thought to be regulated through the PI3K/Akt pathway. Stimulation of PI3K leads to activation of Akt, with subsequent phosphorylation of mTOR on ser-2448 by phospho-Akt. In addition, phospho-Akt is able to phosphorylate the Tsc1/2 (hamartin-tuberin) complex on the Thr-1462 of tuberin that is inhibitory to mTOR.

Rapamycin and analogs temsirolimus (**CCI-779**), everolimus (**RAD001**), and deforolimus (**AP23573**) represent allosteric mTOR inhibitors that possess antiproliferative and antitumor activity as single agents both *in vitro* and *in vivo*, and have been evaluated in a limited clinical setting in malignant glioma. Two recently published phase II studies of temsirolimus monotherapy in recurrent GBM demonstrated a range of radiographic improvement without survival benefit (Chang et al. 2005; Galanis et al. 2005), suggesting that the efficacy of rapamycin analogs might be augmented through combination therapy approaches. A recent Phase I trial tested gefitinib and CCI-779 in combination and showed very few responses, all of which were limited to patients with amplification of EGFR and high levels of p-Akt (Reardon et al. 2006)

The mTOR kinase exists as a component of two distinct protein complexes. mTORC1 is activated by growth factors and nutrients, phosphorylates substrates including S6K at Thr389 and EIF4E at Ser209, and is sensitive to rapamycin. In contrast, the role played by growth factors and/or nutrients in regulating mTORC2 is less certain. The mTORC2 complex itself phosphorylates Akt at S473. Although S473 is generally resistant to rapamycin and rapamycin-like inhibitors, some cancer cell lines do show inhibition in response to rapamycin (Copp et al. 2009). Rapamycin and its analogs inhibit mTOR by altering the conformation of this kinase, inactivating mTORC1 (with limited effect on mTORC2) and until recently defining the activity of the mTORC1 kinase complex. In contrast, dual inhibitors of PI3K and mTOR compete with ATP in binding the active site of these kinases, and inactivate PI3K, mTORC1 and mTORC2. Two recent reports have characterized ATP-competitive inhibitors of mTOR kinase that do not concomitantly inhibit PI3K (Feldman et al. 2009; Thoreen et al. 2009). In contrast to rapamycin, ATP competitive inhibitors of mTOR blocked the phosphorylation of Akt at S473 and prevented its activation (an mTORC2 function). Preclinically, these compounds were more effective antiproliferative agents than rapamycin. Studying MEFs deficient in components of mTORC2 enabled a direct comparison of allosteric inhibition of mTORC1 versus ATP-competitive inhibition of this same complex. Surprisingly, the improved antiproliferative proficiency of ATP-competitive inhibitors was traced to inhibition of mTORC1 and to the improved activities of ATP competitive inhibitors in blocking cap-dependent translation and in inducing autophagy (Feldman et al. 2009; Thoreen et al. 2009). Thus, ATP-competitive inhibitors of mTOR, alone or as a component of dual inhibitors of PI3K and mTOR provide a new class of agents and therapeutics for glioma and other cancers.

6 Targeting the EGFR-PI3K-Akt-mTOR Axis: The Importance of Akt

The AKT family of serine threonine kinases consists of three members: Akt 1 (PKB α), Akt 2 (PKB β), and Akt 3 (PKB γ), all of which have been implicated in cancer. Akt is a central node in the complex cascade of PI3K signaling, with cross-talk and feedback loops influencing regulation of this kinase. Akt is also well known for its antiapoptotic activity when overexpressed. Surprisingly, however, inhibiting components of the PI3K-Akt pathway in glioma often does not typically induce apoptosis (reviewed in Cheng et al. 2009). Several small molecule Akt inhibitors with varying potencies and specificities for the different Akt isoforms have now been developed.

Because inhibitors of PI3K should signal through Akt to mTOR, it is perhaps surprising that dual inhibitors of PI3K and mTOR show enhanced efficacy when compared with mono-specific inhibitors of PI3K. We presumed initially that mTOR had additional upstream inputs, so that inhibition of PI3K would only partially impair mTOR function. To test this hypothesis, we took advantage of the observation that *EGFR*-driven glioma cell lines wild-type for *PTEN* showed proliferative arrest in response to inhibitors of EGFR, and that comparable cell lines mutant for *PTEN* did not. In *PTEN* mutant lines, levels of phospho-Akt decreased significantly in response to EGFR inhibition. These data were aligned with our earlier demonstration that inhibitors of PI3K beta showed pronounced activity against p-Akt in the absence of affecting proliferation, and collectively suggest that p-Akt represents a poor biomarker for the antiproliferative activity of EGFR and PI3K inhibitors (Fan et al. 2006). In contrast, levels of p-mTOR and downstream kinases, including p-rpS6 kinase were robust biomarkers for the antiproliferative activity of both EGFR and PI3K inhibitors (Fan et al. 2007, 2009).

We went on to show that neither blockade nor knockdown of Akt1, 2 or 3 (alone or in combination) impacted proliferation or levels of p-mTOR and downstream targets, even in a setting where the canonical Akt targets Tsc2 and Gsk3 were potently inhibited (Fan et al. 2009). In addition, a constitutively activated allele of Akt affected neither proliferation nor response to the EGFR inhibitor erlotinib in glioma cells. Our observations suggest that Akt is not a central regulator of proliferation in glioma. We subsequently identified protein kinase C as a key intermediate linking EGFR and mTOR in glioma, and showed that PKC alpha contributed prominently to this signaling. Since PKC signals downstream of EGFR and PTEN, and upstream of mTOR, we hypothesized that inhibitors of PKC might show efficacy even in *PTEN* mutant cells. Using the tool compound bis-indolylmaleic acid, a pan-PKC inhibitor, we showed that this agent blocked viability in glioma irrespective of *PTEN* status. These data raise questions regarding whether and how to incorporate inhibitors of Akt into a combination treatment regimen for GBM, an important area of experimental therapeutic investigation more fully detailed below.

7 Combination Strategies Within the EGFR-PI3K-mTOR Axis to Improve Therapeutic Efficacy

Current targeted therapies that inhibit EGFR and downstream PI3K signaling pathways in malignant gliomas have shown modest benefits. At least one component contributing to the relatively disappointing anti-cancer efficacy of these agents stems from multiple nodes of activation in the EGFR-PI3K axis, through which EGFR inhibitors fail to block PI3K signaling as a function of *PTEN* or *PIK3CA* mutational status. Given the complexity of cellular signal transduction and the ability of cancer cells to compensate for acute changes in signaling (in-part through feedback loops activated in response to treatment with kinase inhibitors) it is not surprising that agents that target only one or a few kinases in the EGFR-PI3K-mTOR signaling pathway may be disappointing as they enter clinical trials (Fig. 2). Thus, effective therapy in GBM and other RTK-PI3K-driven cancers likely requires targeting multiple components in EGFR-PI3K-mTOR axis, with the caveat that the toxicities associated with a complex cocktail of agents remain acceptable to patients and clinicians.

Preclinical examples of such approaches include combination therapies using EGFR inhibitors with dual PI3K/mTOR inhibitors, which were superior to either monotherapy or therapy combining an EGFR inhibitor with either a PI3K inhibitor or an mTOR inhibitor in blocking proliferation (but not in inducing apoptosis) in glioma cell lines (Fan et al. 2007). The concept of combining inhibitors of EGFR and mTOR is also translating into clinical trials using EGFR inhibitors with allosteric inhibitors of mTOR in patients (Doherty et al. 2006).

What will the future hold? ATP-competitive inhibitors of EGFR and allosteric inhibitors of mTOR are now in clinical use. Irreversible inhibitors of EGFR, ATP-competitive inhibitors of PI3K and dual inhibitors of PI3K and mTOR are now in clinical trials. ATP-competitive inhibitors of mTOR are likely to be tested clinically in the near future. With such an array of compounds now available or soon to be available, is there a logical approach to testing these alone and in combination in GBM? In this section, we provide a rationale for preclinical therapeutic studies over the next few years, ultimately with the goal of guide clinicians in prioritizing agents for clinical studies.

8 A Role for EGFR Inhibitors in Combination Therapy

Inhibitors of EGFR present a paradox. Multiple RTKs are typically activated in glioma (Stommel et al. 2007). EGFR is the most prominent of these, showing mutational in addition to epigenetic activation. A simple solution to the problem of multiple RTK activation would be to target downstream nodes into which these signals coalesce; however aside from inhibitors of PI3K, such therapeutic reagents are only slowly entering clinical use. At present therefore, there is a clear rationale for continued use of EGFR inhibitors in order to block MAP kinase and other

pathways not directly impacted by PI3K blockade, and for which specific targeted therapies are not yet available. It is likely that irreversible inhibitors of EGFR will be more active than the reversible agents currently available, and that these irreversible inhibitors will show improved activity in GBM. Since many GBM tumors are associated with RTK-independent activation of PI3K, it is likely that inhibitors of EGFR should be combined with inhibitors of PI3K in GBM (Fig. 2).

9 Inhibitors of mTOR, PI3K and Dual PI3K/mTOR Inhibitors

Allosteric inhibitors of mTOR, inhibitors of PI3K and inhibitors of PI3K/mTOR are now all in clinical trials. As mentioned above, inhibitors of PI3K fail to impact mTOR in preclinical studies of GBM, providing a clear rationale for the clinical use of dual inhibitors of PI3K and mTOR. Is there a setting where mono-specific inhibitors of PI3K or allosteric inhibitors of mTOR should be used instead of dual PI3K/mTOR inhibitors? Toxicity issues aside (which are as yet uncertain for PI3K or PI3K/mTOR inhibitors), it is difficult to visualize a scenario in which these mono-selective inhibitors would provide a therapeutic advantage over dual inhibitors.

If combination therapy with EGFR and PI3K/mTOR inhibitors represents the future for glioma therapeutics, is there an additional preclinical rationale for use of Akt inhibitors and/or ATP-competitive inhibitors of mTOR in this disease? In our preclinical studies, inhibition of Akt had little effect on proliferation in glioma. These observations do not preclude the importance of Akt inhibition as a regulator of apoptosis however. A second issue is whether Akt blockade can be accomplished using agents that inhibit kinases upstream of Akt. The ability of PI3K/mTOR inhibitors to block Akt is well-established and raises questions as to whether additional benefit will be derived from use of Akt-selective inhibitors (either instead of or in combination with dual inhibitors of PI3K/mTOR). Further, the two ATP-competitive inhibitors of mTOR studied to date also quite effectively shut down Akt signaling, consistent with the idea that phosphorylation of Akt at S-473 (by mTORC2) is a priming phosphorylation required in order to secondarily phosphorylate T-308 (Feldman et al. 2009; Thoreen et al. 2009). Further studies are needed to determine whether this class of agents shows activities equivalent to or distinct from those displayed by PI3K/mTOR inhibitors, and comparing the efficacy of these agents to that of Akt inhibitors.

10 Inhibitors of PKC

Is there a role for inhibitors of PKC in *PTEN*-mutant glioma and will such agents be more active than EGFR inhibitors in *PTEN*-wild-type glioma? There are currently few clinical agents in development against individual PKC isoforms, although enzastaurin, a potent inhibitor of both PKC alpha and PKC beta (Graff et al. 2005), is currently

being tested clinically in this disease. Whether this and future agents show efficacy in glioma, and whether this class of targeted therapy offer any potential advantage over inhibitors of mTOR remains to be determined.

11 Future Directions

11.1 Therapeutic Strategies to Promote Cytotoxicity in Glioma

The PI3K signaling pathway has risen to prominence as a key regulator of survival in cancer cells, with downstream components Akt and mTOR having well-established anti-apoptotic activities. However, inhibiting components of the PI3K-Akt pathway generally fails to induce appreciable apoptosis in glioma. This is exemplified in our recently study using the dual PI3K/mTOR inhibitor PI-103. PI-103 efficiently inhibited phosphorylation of Akt and mTOR, concomitantly blocking proliferation of glioma cell lines and xenografts (Fan et al. 2006). Even when used in combination with EGFR inhibitors however, PI-103 blocked proliferation without the induction of apoptosis (Fan et al. 2007). NVP-BEZ235, a dual PI3K/mTOR inhibitor currently in phase I clinical trials, also showed strong antiproliferative activity with no obvious affect on viability of U87MG cells (Maira et al. 2008). Further, clinical trials using inhibitors of EGFR in GBM have shown only limited clinical responses, even in those patients whose tumors are driven by *EGFR* and wild-type for *PTEN*. Why does efficient inhibition of survival signaling in a number of cancers generally fail to induce apoptosis in cancer cells? One possibility is that PI3K inhibition is inducing one or more alternative survival pathways, enabling cancer cells to evade pro-apoptotic signals mediated by PI3K/Akt/mTOR blockade.

Inhibition of PI3K/mTOR pathway typically induces autophagy, raising the possibility that autophagy represents a candidate survival signal leading to cytosolysis rather than cytotoxicity in response to inhibitors targeting the PI3K-Akt-mTOR pathway. Autophagy drives a pathway that enables cells to survive periods of catabolic stress by cannibalizing themselves to preserve adenosine triphosphate (ATP) stores. During autophagy, cells degrade their own proteins and organelles through formation of a double-membraned vesicle (autophagosome) that fuses with a lysosome, leading to self-digestion of cytoplasmic organelles and other constituents in the lysosomal compartments. Although autophagy may not always allow cells to survive prolonged periods of stress ultimately resulting in cell death this process allows cells to recycle proteins and organelles as a source of new macromolecules, thereby evading apoptosis at least for a limited time. Inhibiting autophagy can thus either promote or impair cell death depending on the conditions and reagents used (Kroemer and Levine 2008). Can blockade of autophagy in the setting of PI3K or PI3K/mTOR enhance the cytotoxicity of these agents in glioma? In support of this model, inhibition of Akt activity by siRNA-mediated knockdown or using Akt inhibitors did not induce significant apoptosis, but rather markedly increased autophagy in the *PTEN*-null

human prostate cancer cell line PC3 and in the glioma cell line U87MG (Degtyarev et al. 2008). A number of lysosomotropic agents such as chloroquine or bafilomycin A1 cooperated with Akt siRNA or Akt inhibitors to precipitate PC3 cell death in vitro and in vivo, although details of signaling required to achieve cell death in this descriptive study remain unclear. Nevertheless, these results suggest that blockade of autophagy preclinically may enhance the anticancer efficacy of at least some PI3K-Akt-mTOR pathway inhibitors.

PI3K inhibition has also been shown to sensitize cells to chemically induced cell death. Apoptotic cell death proceeds through one of two pathways: the extrinsic-death ligand/receptor-mediated pathway; and the intrinsic-mitochondrial-mediated pathway. Sensitivity of glioma cells to apoptosis induced through either of these pathways can be enhanced by PI3K inhibition (Kao et al. 2007; Opel et al. 2008). PI-103 a dual PI3K/mTOR inhibitor has been shown synergistically or additively to promote the cytotoxic effects of either chemotherapy (BCNU and temozolomide) or radiation therapy (Chen et al. 2008; Prevo et al. 2008). Take together, these results suggest that inhibitors of PI3K may be useful in concert with traditional therapies to enhance apoptosis in GBM.

12 Biomarkers to Stratify Patients and to Measure Responses

A second challenge in glioma is to improve our ability in identifying subgroups of patients most likely to benefit from inhibition of the RTK-PI3K-Akt-mTOR axis, and to develop biomarkers of response. While essentially all patients with GBM have tumors resected as standard of care, additional surgeries are not always performed at relapse, and routine sampling of tumors during therapy is essentially not done. A potential model for future therapy in this disease was recently presented by Cloughesy and colleagues who gave an allosteric mTOR inhibitor to GBM patients with PTEN deficient tumors who had suffered a radiological relapsed after standard treatment, and then biopsied relapsed tumors 1 week after starting rapamycin (Cloughesy et al. 2008). This strategy allowed investigators to assess the biochemical response to mTOR inhibition, while also limiting the trial to patients with PI3K-driven tumors. While such an approach is clearly a move in the right direction, future studies in glioma will likely be improved by development and routine incorporation of non-invasive methods of assessment (metabolic PET imaging, MRI with MR spectroscopy or serum markers) in order to clarify those patients most likely to respond to these agents, and to monitor response to targeted therapies.

13 Conclusion

Advances in neurosurgical techniques and in radiation therapy continue to benefit patients with glioma. In contrast, the comparatively modest effects of medical therapy pose a challenge. In light of the relative failure of medical therapy in this

Table 1 Combination strategies through EGFR PI3K mTOR axis to improve therapeutic efficacy

Agent	Targets	Biological Effects
Erlotinib + mTOR	EGFR, mTOR	Antiproliferative
Erlotinib + PI3K/mTOR	EGFR, p110 α , mTOR	Antiproliferative
shAkt123 + hydroxychloroquine	Akt, lysosomotropic	Antiproliferative, apoptosis?
PI3K/mTOR + temozolomide	p110 α , mTOR, nonspecific	Antiproliferative, apoptosis?
PI3K/mTOR + radiation	p110 α , mTOR, nonspecific	Antiproliferative, apoptosis?

Combination strategies to target multiple nodes within the EGFR PI3K mTOR network. Combining kinase inhibitors with cytotoxic chemotherapy, radiation, and with inhibitors of autophagy may improve the therapeutic efficacy. Whether these approaches show improved efficacy and whether their use is associated with acceptable levels of toxicity are areas of active basic and clinical investigation. A number of allosteric inhibitors of mTORC1 and dual inhibitors of PI3K and mTOR are currently being tested clinically. See text for details

disease, the need for effective targeted therapy in GBM is formidable. Agents only recently introduced into clinical use, which mainly inhibit one component of the EGFR-PI3K-Akt-mTOR pathway, have shown some activity, however agents that significantly improve survival are sorely needed in this disease. As GBM at recurrence is generally associated with enhanced therapeutic resistance, it is critical to identify optimal effective medical therapies that up-front, can induce durable responses in appropriate patient populations. Our ability to identify appropriate patients, to target multiple components within the RTK-PI3K-Akt-mTOR axes, and to combine these therapies with cytotoxic chemotherapy, radiation (Table 1), and potentially with inhibitors of survival signaling including inhibitors of autophagy, all represent challenges for the future. As the cocktail of agents grows increasingly complex, a parallel challenge is to identify effective combinations that yield cytotoxicity specifically in cancer cells, and that are tolerated by patients.

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References

- Brandes AA, Franceschi E, Tosoni A, Hegi ME, Stupp R (2008) Epidermal growth factor receptor inhibitors in neuro oncology: hopes and disappointments. *Clin Cancer Res* 14:957-960
- Cancer Genome Atlas Research Network (2008) Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 455:1061-1068
- Chang SM, Wen P, Cloughesy T, Greenberg H, Schiff D, Conrad C, Fink K, Robins HI, De Angelis L, Raizer J et al (2005) Phase II study of CCI 779 in patients with recurrent glioblastoma multiforme. *Invest New Drugs* 23:357-361
- Chen JS, Zhou LJ, Entin-Meer M, Yang X, Donker M, Knight ZA, Weiss W, Shokat KM, Haas Kogan D, Stokoe D (2008) Characterization of structurally distinct, isoform selective phosphoinositide 3' kinase inhibitors in combination with radiation in the treatment of glioblastoma. *Mol Cancer Ther* 7:841-850

- Cheng CK, Fan QW, Weiss WA (2009) PI3K signaling in glioma animal models and therapeutic challenges. *Brain Pathol* 19:112-120
- Ciraolo E, Iezzi M, Marone R, Marengo S, Curcio C, Costa C, Azzolino O, Gonella C, Rubinetto C, Wu H et al (2008) Phosphoinositide 3 kinase p110beta activity: key role in metabolism and mammary gland cancer but not development. *Sci Signal* 1:ra3
- Cloughesy TF, Yoshimoto K, Nghiemphu P, Brown K, Dang J, Zhu S, Hsueh T, Chen Y, Wang W, Youngkin D et al (2008) Antitumor activity of rapamycin in a Phase I trial for patients with recurrent PTEN deficient glioblastoma. *PLoS Med* 5:e8
- Copp J, Manning G, Hunter T (2009) TORC specific phosphorylation of mammalian target of rapamycin (mTOR): phospho Ser2481 is a marker for intact mTOR signaling complex 2. *Cancer Res* 69:1821-1827
- de Groot JF, Gilbert MR, Aldape K, Hess KR, Hanna TA, Ictech S, Groves MD, Conrad C, Colman H, Puduvalli VK et al (2008) Phase II study of carboplatin and erlotinib (Tarceva, OSI 774) in patients with recurrent glioblastoma. *J Neurooncol* 90:89-97
- Degtyarev M, De Maziere A, Orr C, Lin J, Lee BB, Tien JY, Prior WW, van Dijk S, Wu H, Gray DC et al (2008) Akt inhibition promotes autophagy and sensitizes PTEN null tumors to lysosomotropic agents. *J Cell Biol* 183:101-116
- Doherty L, Gigas DC, Kesari S, Drappatz J, Kim R, Zimmerman J, Ostrowsky L, Wen PY (2006) Pilot study of the combination of EGFR and mTOR inhibitors in recurrent malignant gliomas. *Neurology* 67:156-158
- Fan QW, Specht KM, Zhang C, Goldenberg DD, Shokat KM, Weiss WA (2003) Combinatorial efficacy achieved through two point blockade within a signaling pathway a chemical genetic approach. *Cancer Res* 63:8930-8938
- Fan QW, Knight ZA, Goldenberg DD, Yu W, Mostov KE, Stokoe D, Shokat KM, Weiss WA (2006) A dual PI3 kinase/mTOR inhibitor reveals emergent efficacy in glioma. *Cancer Cell* 9:341-349
- Fan QW, Cheng CK, Nicolaides TP, Hackett CS, Knight ZA, Shokat KM, Weiss WA (2007) A dual phosphoinositide 3 kinase alpha/mTOR inhibitor cooperates with blockade of epidermal growth factor receptor in PTEN mutant glioma. *Cancer Res* 67:7960-7965
- Fan QW, Cheng C, Knight ZA, Haas Kogan D, Stokoe D, James CD, McCormick F, Shokat KM, Weiss WA (2009) EGFR signals to mTOR through PKC and independently of Akt in glioma. *Sci Signal* 2:ra4
- Feldman ME, Apsel B, Uotila A, Loewith R, Knight ZA, Ruggero D, Shokat KM (2009) Active Site Inhibitors of mTOR Target Rapamycin Resistant Outputs of mTORC1 and mTORC2. *PLoS Biol* 7:e38
- Franceschi E, Cavallo G, Lonardi S, Magrini E, Tosoni A, Grosso D, Scopece L, Blatt V, Urbini B, Pession A et al (2007) Gefitinib in patients with progressive high grade gliomas: a multicentre phase II study by Gruppo Italiano Cooperativo di Neuro Oncologia (GICNO). *Br J Cancer* 96:1047-1051
- Galanis E, Buckner JC, Maurer MJ, Kreisberg JI, Ballman K, Boni J, Peralba JM, Jenkins RB, Dakhil SR, Morton RF et al (2005) Phase II trial of temsirolimus (CCI 779) in recurrent glioblastoma multiforme: a North Central Cancer Treatment Group Study. *J Clin Oncol* 23:5294-5304
- Graff JR, McNulty AM, Hanna KR, Konicek BW, Lynch RL, Bailey SN, Banks C, Capen A, Goode R, Lewis JE et al (2005) The protein kinase Cbeta selective inhibitor, Enzastaurin (LY317615.HCl), suppresses signaling through the AKT pathway, induces apoptosis, and suppresses growth of human colon cancer and glioblastoma xenografts. *Cancer Res* 65:7462-7469
- Haas Kogan DA, Prados MD, Tihan T, Eberhard DA, Jelluma N, Arvold ND, Bamber R, Lamborn KR, Kapadia A, Malec M et al (2005) Epidermal growth factor receptor, protein kinase B/Akt, and glioma response to erlotinib. *J Natl Cancer Inst* 97:880-887
- Jackson SP, Schoenwaelder SM, Goncalves I, Nesbitt WS, Yap CL, Wright CE, Kenche V, Anderson KE, Doppeide SM, Yuan Y et al (2005) PI 3 kinase p110beta: a new target for antithrombotic therapy. *Nat Med* 11:507-514

- Jia S, Liu Z, Zhang S, Liu P, Zhang L, Lee SH, Zhang J, Signoretti S, Loda M, Roberts TM, Zhao JJ (2008) Essential roles of PI(3)K p110beta in cell growth, metabolism and tumorigenesis. *Nature* 454:776 779
- Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A et al (2008) Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* 321:1801 1806
- Kao GD, Jiang Z, Fernandes AM, Gupta AK, Maity A (2007) Inhibition of phosphatidylinositol 3 OH kinase/Akt signaling impairs DNA repair in glioblastoma cells following ionizing radiation. *J Biol Chem* 282:21206 21212
- Knight ZA, Gonzalez B, Feldman ME, Zunder ER, Goldenberg DD, Williams O, Loewith R, Stokoe D, Balla A, Toth B et al (2006) A pharmacological map of the PI3 K family defines a role for p110alpha in insulin signaling. *Cell* 125:733 747
- Kroemer G, Levine B (2008) Autophagic cell death: the story of a misnomer. *Nat Rev Mol Cell Biol* 9:1004 1010
- Maira SM, Stauffer F, Brueggen J, Furet P, Schnell C, Fritsch C, Brachmann S, Chene P, De Pover A, Schoemaker K et al (2008) Identification and characterization of NVP BEZ235, a new orally available dual phosphatidylinositol 3 kinase/mammalian target of rapamycin inhibitor with potent in vivo antitumor activity. *Mol Cancer Ther* 7:1851 1863
- Manning BD, Cantley LC (2007) AKT/PKB signaling: navigating downstream. *Cell* 129:1261 1274
- Mellinghoff IK, Wang MY, Vivanco I, Haas Kogan DA, Zhu S, Dia EQ, Lu KV, Yoshimoto K, Huang JH, Chute DJ et al (2005) Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. *N Engl J Med* 353:2012 2024
- Okkenhaug K, Bilancio A, Farjot G, Priddle H, Sancho S, Peskett E, Pearce W, Meek SE, Salpekar A, Waterfield MD et al (2002) Impaired B and T cell antigen receptor signaling in p110delta PI 3 kinase mutant mice. *Science* 297:1031 1034
- Opel D, Westhoff MA, Bender A, Braun V, Debatin KM, Fulda S (2008) Phosphatidylinositol 3 kinase inhibition broadly sensitizes glioblastoma cells to death receptor and drug induced apoptosis. *Cancer Res* 68:6271 6280
- Persson A, Fan Q W, Phillips J, Weiss WA (2007) Glioma. Elsevier, San Diego
- Prados MD, Lamborn KR, Chang S, Burton E, Butowski N, Malec M, Kapadia A, Rabbitt J, Page MS, Fedoroff A et al (2006) Phase I study of erlotinib HCl alone and combined with temozolomide in patients with stable or recurrent malignant glioma. *Neuro Oncol* 8:67 78
- Prados MD, Chang SM, Butowski N, DeBoer R, Parvataneni R, Carliner H, Kabuubi P, Ayers Ringler J, Rabbitt J, Page M et al (2009) Phase II study of erlotinib plus temozolomide during and after radiation therapy in patients with newly diagnosed glioblastoma multiforme or gliosarcoma. *J Clin Oncol* 27:579 584
- Prevo R, Deutsch E, Sampson O, Diplexcito J, Cengel K, Harper J, O'Neill P, McKenna WG, Patel S, Bernhard EJ (2008) Class I PI3 kinase inhibition by the pyridinylfuranopyrimidine inhibitor PI 103 enhances tumor radiosensitivity. *Cancer Res* 68:5915 5923
- Reardon DA, Quinn JA, Vredenburgh JJ, Gururangan S, Friedman AH, Desjardins A, Sathornsumetee S, Herndon JE II, Dowell JM, McLendon RE et al (2006) Phase I trial of gefitinib plus sirolimus in adults with recurrent malignant glioma. *Clin Cancer Res* 12:860 868
- Rich JN, Reardon DA, Peery T, Dowell JM, Quinn JA, Penne KL, Wikstrand CJ, Van Duyn LB, Dancey JE, McLendon RE et al (2004) Phase II trial of gefitinib in recurrent glioblastoma. *J Clin Oncol* 22:133 142
- Sasaki T, Irie Sasaki J, Jones RG, Oliveira dos Santos AJ, Stanford WL, Bolon B, Wakeham A, Itie A, Bouchard D, Kozieradzki I et al (2000) Function of PI3Kgamma in thymocyte development, T cell activation, and neutrophil migration. *Science* 287:1040 1046
- Sawyers C (2004) Targeted cancer therapy. *Nature* 432:294 297
- Stommel JM, Kimmelman AC, Ying H, Nabioullin R, Ponugoti AH, Wiedemeyer R, Stegh AH, Bradner JE, Ligon KL, Brennan C et al (2007) Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. *Science* 318:287 290

- Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U et al (2005) Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 352:987-996
- Thoreen CC, Kang SA, Chang JW, Liu Q, Zhang J, Gao Y, Reichling LJ, Sim T, Sabatini DM, Gray NS (2009) An ATP competitive mTOR inhibitor reveals rapamycin insensitive functions of mTORC1. *J Biol Chem* 284:8023-8032
- Wang MY, Lu KV, Zhu S, Dia EQ, Vivanco I, Shackelford GM, Cavenee WK, Mellinghoff IK, Cloughesy TF, Sawyers CL, Mischel PS (2006) Mammalian target of rapamycin inhibition promotes response to epidermal growth factor receptor kinase inhibitors in PTEN deficient and PTEN intact glioblastoma cells. *Cancer Res* 66:7864-7869

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