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Inflammation and Gastrointestinal Cancers

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Inflammation and Gastrointestinal Cancers

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Prof. Dr. Janusz A. Z. Jankowski
Department of Oncology
University of Oxford
Old Road Campus
Headington
OX3 7DG
UK
e-mail: janusz.jankowski@clinpharm.ox.ac.uk

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Contents

Basic Concepts of Inflammation and its Role in Carcinogenesis

Stephen G. Maher and John V. Reynolds

Abstract

While the normal inflammatory cascade is self-limiting and crucial for host protection against invading pathogens and in the repair of damaged tissue, a wealth of evidence suggests that chronic inflammation is the engine driving carcinogenesis. Over a period of almost 150 years the link between inflammation and cancer development has been well established. In this chapter we discuss the fundamental concepts and mechanisms behind normal inflammation as it pertains to wound healing. We further discuss the association of inflammation and its role in carcinogenesis, highlighting the different stages of cancer development, namely tumour initiation, promotion and progression. With both the innate and adaptive arms of the immune system being central to the inflammatory process, we examine the role of a number of immune effectors in contributing to the carcinogenic process. In addition, we highlight the influences of host genetics in altering cancer risk.

Contents

S. G. Maher · J. V. Reynolds (✉)
Department of Surgery, Institute of Molecular Medicine,
Trinity College Dublin and St. James's Hospital,
Dublin 8, Ireland
e-mail: reynoldsjv@stjames.ie

S. G. Maher
e-mail: maherst@tcd.ie

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1 Introduction

The nineteenth century saw much advancement in the biological sciences. Darwin published his theories on evolution in *The Origin of Species*, Koch developed his postulates on microbial infection, Pasteur developed the first vaccine, and Mendel studied the genetics of inheritance. Although less heralded, it was also an era when the first report of an association between inflammation and cancer was recorded. In 1863, Rudolf Virchow hypothesised that immune cell infiltrate reflected the origins of cancer at sites of chronic inflammation (Balkwill and Mantovani 2001).

The cellular basis for inflammation and cancer is now well understood. Under normal physiological conditions, inflammation is usually associated with tissue injury and arises via innate immunity. The innate immune system, comprised of macrophages, mast cells, dendritic cells, granulocytes (neutrophils, eosinophils and basophils) and the innate lymphocytes (natural killer (NK) and $\gamma\delta$ T cells), is a non-specific first line defence against invading pathogens. Following wounding, the cells of the innate immune system converge at the site of damage. Macrophages engulf and consume pathogens, mast cells release histamine, and the granulocytes produce cytokines and chemokines that promote inflammation. Dendritic cells in the area pick up antigens from pathogens and present them to naïve T cells in the lymph nodes for the development of an adaptive immune response, while NK cells destroy host cells that have been infected with the invading pathogen (Fig. 1). The physiological response to cancer has many parallels with inflammation and wound healing. During normal tissue injury, cell proliferation is enhanced while the tissue regenerates; the proliferation and inflammation subside once the assaulting agent/pathogen is removed or the tissue is repaired. In contrast, as occurs with tumours, proliferating cells that sustain DNA damage and/or mutagenic assault continue to proliferate in microenvironments rich in inflammatory cells and growth/survival factors that support their growth. Because of this, tumours are likened to wounds that fail to heal.

2 Overview of Inflammation

Inflammation is a complex biological response. To understand the role that inflammation plays in carcinogenesis it is important to understand what inflammation is, and how it contributes to physiological and pathological processes,

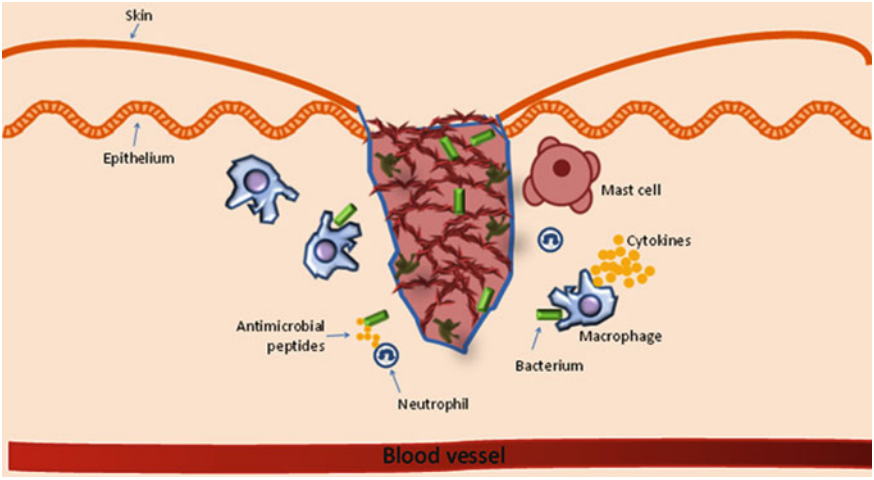

Fig. 1 Normal wound healing. During the wound healing process activated platelets from the circulatory system produce clotting proteins and form a plug. Platelets release chemotactic factors, recruiting leucocyte populations from the periphery to the site of injury. Mast cells release vasoactive amines, which mediate vasodilation and vascular constriction. Neutrophils secrete antimicrobial peptides and together with macrophages phagocytose invading pathogens. Tissue-resident macrophages secrete an array of cytokines that attract immune cells to the wound and activate cells involved in the repair process. The inflammatory process continues until invading pathogens are neutralised and the wound is repaired

including wound healing and infection. Inflammation can be defined by the four Latin words *calor*, *dolor*, *rubor* and *tumor*, meaning heat, pain, redness, and swelling, all of which reflect the effects of cytokines and other inflammatory mediators on the local blood vessels. Dilation and increased permeability of the blood vessels during inflammation lead to increased core blood flow and vascular leakage, and account for the heat, redness and swelling. Cytokines alter the adhesive properties of the endothelium, causing circulating leucocytes to stick to the blood vessel wall and migrate between the endothelial cells to the site of injury along chemokine gradients. The migration of cells into the tissue and their local actions account for the pain.

Inflammation is a fundamental adaptation to the loss of cellular and tissue homeostasis with many important roles, including host defence, but also tissue remodelling and repair, and the regulation of metabolism (Hotamisligil 2006; Medzhitov 2008; Nathan 2002). The complexity of the inflammatory response requires that many of its functions are controlled in a coordinate manner in some situations, but independently in others. This is achieved via multiple mechanisms, including alterations in the composition of immune cells in tissues, changes in cellular responsiveness to inflammatory stimuli, regulation of signalling pathways and control at the level of gene expression (Medzhitov and Horng 2009). Thus, the different mechanisms regulating the inflammatory responses can be divided into cell-specific, signal-specific and gene-specific mechanisms.

Upon injury to tissue, a multifactorial network of chemical signals initiate and maintain a host response designed to destroy invading pathogens at the site and repair the damaged tissue. This involves the activation and directed migration of leucocytes (neutrophils, monocytes and eosinophils) from the venous system to the site of damage. Chemokines, which possess a relatively high degree of specificity for chemoattraction of specific leucocyte populations, recruit downstream effector cells and dictate the natural evolution of the inflammatory response. Mast cells and tissue-resident macrophages are the first host tissue cells to become activated in response to injury, and orchestrate the series of events that follow (Coussens and Werb 2002; Mantovani et al. 2008). Residing along blood vessel walls, mast cells are activated via Fc receptors and by components of the complement system (C3a and C5a) and danger signals that are generated by the presence of bacteria or immune complexes. Activated mast cells release preformed and newly synthesised inflammatory mediators, such as vasoactive amines (e.g., histamine), cytokines and proteases complexed to highly sulphated proteoglycans, as well as lipid mediators, which promote the early vascular response observed in inflammation (Marshall 2004). This response is characterised by transient arteriolar constriction followed by dilatation of veins and capillaries, and an increase in vascular permeability (Le Bitoux and Stamenkovic 2008).

Macrophages are also activated by Fc crosslinking and C3a and C5a fragments, and by mediators released by damaged cells and the chemotactic fMLP (N-formyl-methionyl-leucyl-phenylalanine) peptide, a component of bacterial cell walls. Macrophages produce an array of proinflammatory cytokines, including IL-1 and TNF-α. These cytokines work in synergy, with similar effects of the vasculature, including endothelial cell (EC) activation and expression of members of the selectin family of adhesion proteins, such as L-, E- and P-selectin as well as ICAM-1 on the EC surface. L-, E-, P-selectin recognise sialylated and fucosylated oligosaccharides on the E-selectin ligand (ESL) and P-selectin glycoprotein ligand-1 (PSGL-1) expressed on the surface of neutrophils, monocytes, and certain lymphocyte subsets (Hidalgo et al. 2007; Le Bitoux and Stamenkovic 2008). The interaction between the selectins on ECs and their cognate ligands facilitates leucocyte adhesion through $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins binding to endothelial vascular cell-adhesion molecule-1 (VCAM-1) and MadCAM-1, respectively, and rolling across the vascular endothelium. Neutrophils and monocytes are activated by chemokines, CXCL8 being one example, through G-protein coupled receptors on their surface. These molecules originate from the activated ECs and macrophages, which are stimulated to produce them in response to proximal cell-cell contact. The activated neutrophils arrest on the endothelium and subsequently extravasate into the tissue by a process known as diapedesis (Muller 2009). Following extravasation, the activated leucocytes migrate towards the site of injury and to the provisional extracellular matrix that forms a scaffold upon which fibroblasts and endothelial cells proliferate and migrate, thus providing a nexus for reconstitution of the normal microenvironment (Coussens and Werb 2002). At the wound, neutrophils engulf and breakdown invading pathogens. Within hours, the local activated endothelial adhesion receptors and chemokine/cytokine repertoire adapt

to recruit additional leucocyte subpopulations. Following the initial wave of neutrophils, infiltration by monocytes, which differentiate into macrophages in tissues, is guided by chemotactic factors (Kamei and Carman 2010). Activated macrophages are the main source of lipid mediators, growth factors and cytokines, including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and transforming growth factor (TGF) β, which profoundly affect endothelial, epithelial and mesenchymal cells in the local tissue/wound microenvironment. The infiltrate also releases proteolytic factors, such as matrix metalloproteinases (MMPs) and cathepsins, which participate in the subsequent breakdown of extracellular matrix components.

By nature, the inflammatory cascade is self-limiting, and as the etiologic agents are gradually diminished, the balance of cytokines, chemokines, growth factors and lipid mediators, shifts from inflammation-driven tissue remodelling to tissue repair. A key factor in this process is a switch from the production of proinflammatory prostaglandins to antiinflammatory lipoxins (Serhan and Savill 2005). The production of lipoxins results in the recruitment of monocytes, which, following differentiation into macrophages, remove cellular debris at the site of injury and coordinate repair.

During a normal inflammatory reaction, if the acute inflammatory response fails to eliminate the cause of injury, the inflammatory response may persist but acquires new features. Monocytes and macrophages, and in some cases lymphocytes, replace the infiltrating neutrophils. If this remains insufficient to clear the tissue of the pathogen a chronic inflammatory state develops. Depending on the pathogen and type of host response, the inflammation may be granulomatous or lymphocytic in nature, with the formation of tertiary lymphoid tissues. The profile of cytokine/chemokines persisting at an inflammatory site is important in the development of chronic disease. Being well established in the literature, chronic inflammation is associated with carcinogenesis.

3 Cancer and Inflammation

Cancer results from the expansion of a clonal population of cells from within a tissue. Over the last number of decades, studies on different types of cancer have lead to the development of models characterising their growth. This characterisation process has revealed the essential features of cancer development, termed the 'hallmarks of cancer' (Hanahan and Weinberg 2000). Exposure of tissue to physical injury, ischemia, toxins, or infectious agents, induces inflammation. Inflammatory reactions can stimulate antitumour immune responses, but also have the capacity to promote tumour development. There is a vast amount of epidemiological and clinical data supporting an increased risk of certain cancers in the setting of chronic inflammation. Basic research has shown that many of the processes involved in inflammation (e.g., leucocyte migration, local vascular dilation and enhanced permeability and angiogenesis), when found in association with tumours, are more likely to contribute to

tumour growth, progression and metastasis, than to elicit an effective host anti-tumour immune response.

Through epidemiological studies it is now estimated that about 15–20% of all cancers are attributable, or are associated with, chronic inflammation due to infectious agents, chemical and physical agents and autoimmune reactions (Karin 2008; Kuper et al. 2000). Primary examples are hepatocellular carcinoma and viral hepatitis (Fattovich et al. 2004), gastric adenocarcinoma and gastric lymphoma with *Helicobacter pylori* infection (Roder 2002), colitis-associated cancer and oesophageal adenocarcinoma from Barrett's oesophagus (Ekbom 1998) (Table 1). Intriguingly, inflammation is an integral component at all three stages of tumour development: initiation, promotion and progression (Fig. 2). Inflammation, resulting from tissue injury, contributes to tumour initiation events by stimulating the release of a variety of chemokines and cytokines, which alert the vasculature to allow inflammatory cells and mediators access into the tissue microenvironment. These inflammatory mediators may cause oxidative damage, induce DNA mutations, and other changes in the microenvironment, making it more conducive to cellular transformation, increased cell survival and proliferation. However, the precise mechanism whereby chronic inflammation contributes to tumour progression remains unclear. Tumour cells produce various chemotactic factors that attract inflammatory cells, which then secrete an array of soluble mediators, stimulating further proliferation of the initiated cell, tissue disruption in the stroma, and tumour growth. Leucocyte infiltration, and particularly macrophages, can lead to enhanced angiogenesis, predominantly via VEGF, which is associated with a poor prognosis for a number of tumours. The role of inflammation in the metastatic spread of a tumour is much less well-defined than its role in cancer initiation and progression. Soluble mediators produced by tumour-associated leucocytes promote cell motility, induce angiogenesis, vascular dilatation and the extravasation of tumour cells. Inflammation continues to play a role at metastatic sites by creating a new cytokine milieu conducive to tumour growth.

While the body has a strong reaction to wounding and infection in terms of the humoral and cellular responses, overall its response to tumours is relatively weak. This may be linked to the fact that most tumour antigens are recognised as 'self'. There are a number of factors that determine the repertoire of immune infiltrate found in the tumour microenvironment, with hypoxia being a dominant driving force. Once tumours grow 2 mm beyond the nearest blood supply the oxygen tension drops dramatically, and the tumour cells begin to activate and express hypoxia-responsive genes (Denko et al. 2003). The hypoxic tumour environment favours the infiltration of immune infiltrate dependent on the glycolytic pathway for survival, such as macrophages and granulocytes. These infiltrating cells generate reactive oxygen species (ROS) upon local activation. These ROS are immunosuppressive, mediating their effects through nuclear factor kappa B (NFκB) activation, the major inflammatory transcription factor. The activation of NFκB in the tumour cells themselves can lead to the expression of cytokines, TNF-α among others, which can ultimately drive tumour growth (Balkwill and Coussens 2004; Greten et al. 2004; Pikarsky et al. 2004). As tumours become

Table 1 Agents associated with inflammation and cancer. Adapted from Coussens and Werb (2002) and Hold and El-Omar (2008)

Agents	Inflammation/Cancer Association
Viral	
HIV	Kaposi's sarcoma Squamous cell carcinoma Non-Hodgkin's lymphoma
HBV/HCV	Hepatitis/hepatocellular carcinoma
EBV	Nasopharyngeal carcinoma Malignant lymphoma
HHV8	Kaposi's sarcoma Squamous cell carcinoma Non-Hodgkin's lymphoma
HTLV-1	T cell lymphoma Leukaemia
HPV	Cervical carcinoma Ovarian carcinoma Anogenital carcinoma Penile carcinoma
CMV	Gastric carcinoma
Bacterial	
H. pylori	Gastritis/gastric carcinoma
C. trachomatis	Cervical carcinoma
E. coli	Prostate dysplasia/atypical hyperplasia
Parasitic	
Chinese liver fluke	Cholangiocarcinoma
Schistosomiasis	Chronic cystitis/bladder carcinoma
Liver fluke (*Opisthorchis viverrini*)	Cholangiocarcinoma/colon cancer
Chemical	
Asbestos	Malignant mesothelioma
Fumes, smoking, etc.	Lung cancer
Other inflammatory	
Chronic pancreatitis	Pancreatic carcinoma
Chronic prostatitis	Prostate carcinoma
Endometriosis	Endometrial adenocarcinoma
Pelvic inflammatory disease	Ovarian cancer
Inflammatory bowel disease	Colorectal cancer
Thyroiditis	Papillary thyroid carcinoma
Primary sclerosing cholangitis	Cholangiocarcinoma
Chronic cholecystitis	Gall bladder carcinoma

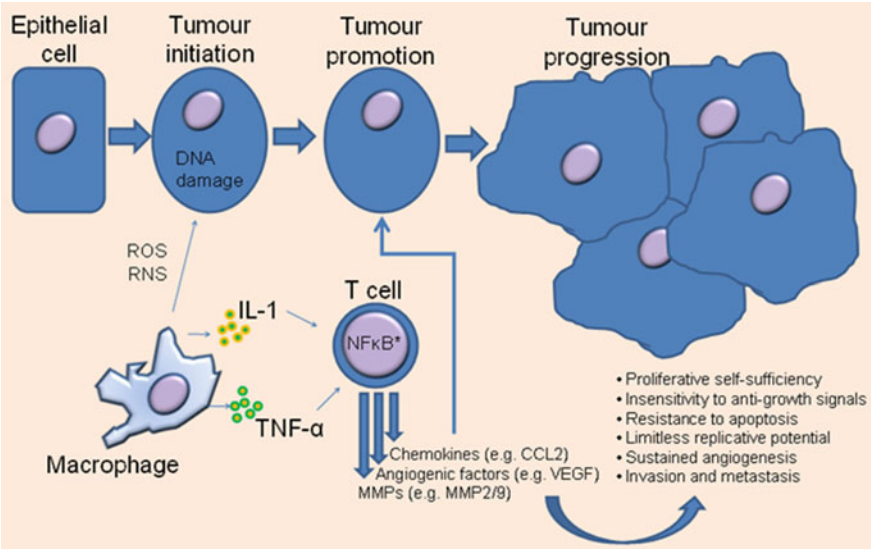

Fig. 2 The stages of tumourigenesis. During chronic inflammation, cells of the innate immune system, such as the macrophage, mediate tumour initiation events via production of DNA-damaging reactive oxygen and nitrogen species (ROS/RNS). Macrophages also produce proinflammatory cytokines, such as IL-1 and TNF-α, which can recruit and stimulate other immune effectors through the activation of the NFκB transcription factor. These immune effectors, such as T cells, produce an array of factors, facilitating angiogenesis and tissue remodelling. As healthy cells progress through the tumour initiation stage and onto the promotion stage they acquire new mutations and dysregulations, leading to tumour progression, which is associated with the 6 hallmarks of cancer. Adapted from Waldner and Neurath (2009)

dependent on these soluble factors the microenvironment is adjusted or programmed so that the release of these factors is continual. Thus, cell proliferation, differentiation, angiogenesis, cell migration and matrix remodelling are all 'reprogrammed' to benefit the tumour.

3.1 Cancer Initiation Events

The vast majority of tumours arise due to one or several genetic mutations that lead to the production of proteins with altered tertiary structure, frequently leading to dysregulation of normal biological function. Although inflammation is a vital response for the resolution of infection, repair of damaged tissue and in the suppression of tumour formation, chronic inflammation is also clearly correlated with increased risk of cancer development. Peyton Rous was the first to recognise that cancers develop from *subthreshold neoplastic states* induced by viral or chemical carcinogens that induce somatic changes (Rous and Kidd 1941). These states, now known as 'initiation' events, involve DNA alterations, which are irreversible and

can exist in a benign state in otherwise normal tissue indefinitely, until the occurrence of a second type of stimulatory event, the 'promotion' event. Hereditary mutations can also act as initiation events. While normal inflammatory processes are self-limiting, there are a number of reasons why acute inflammation may develop into a chronic state: the inflammatory stimulus may persist (such as that in colonic ulcerative colitis, Crohn's disease or gastroesophageal reflux disease), or there is dysregulation in the control mechanisms that normally switch off the proinflammatory reaction. In addition, it may also be due to the persistence of initiation factors.

One of the major advances in beginning to understand the relationship between inflammation and cancer came from murine models of chemically-induced cancer, showing that the carcinogenic process could be broken into a number of sequential steps including initiation, promotion and progression (Foulds 1954; Hecker 1967; Philip et al. 2004; Rous and Beard 1935; Rous and Kidd 1941). From this data, a working hypothesis was proposed. During the course of life, humans accumulate somatic mutations in different cell types within the various tissues. These mutations are induced by exposure to undetectable or trace amounts of carcinogens, or can arise spontaneously. These are referred to as 'initiation events'. Subsequently, inflammation can act as a 'promoter' to either induce more mutations in these cells, for example through exposure to ROS, or drive the mutated cells to proliferate. Inflammation may also provide the damaged cells with a growth advantage, this has been referred to as a 'Darwinian selection' (Klein and Klein 1985).

The molecular basis for the increased risk of cancer, in the background of chronic inflammation, is largely considered to be two-fold. Firstly, macrophages in the tissue microenvironment produce a number of factors, including ROS and reactive nitrogen species (RNS). These reactive species lead to DNA damage in the surrounding epithelial cells. Secondly, enhanced proliferation signals, necessary for tissue regeneration, mediated by cytokines released from immune cells increase the number of cells at risk of mutation. Combining both of these signals, DNA damage and proliferation, provides an environment favourable to carcinogenesis. ROS and RNS can induce significant permanent genetic damage, stimulating recombination events, resulting in the production of aberrant protein species and dysregulated cellular signalling (Fig. 3). Under normal conditions it is estimated that reactive species induce approximately 10,000 oxidative lesions per cell per day, with any number of these being potentially mutagenic (Loft and Poulsen 1996). ROS can also activate certain signalling cascades and transcription factors, including NFκB, which triggers pro-survival and anti-apoptotic mechanisms.

3.2 Cancer Promotion Events

The precise mechanisms and signals stimulated by inflammation for the promotion of tumour progression are largely unclear. Tumour promotion can result from exposure of 'initiated' cells to damage, be this via chemical irritants, factors

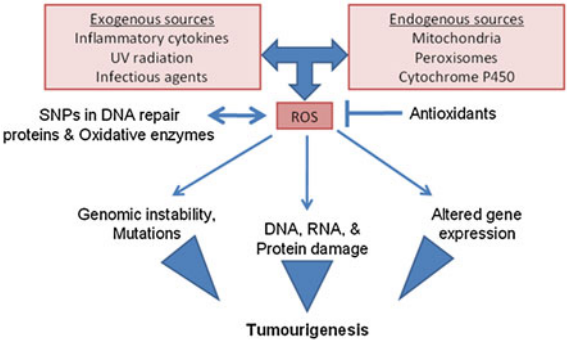

Fig. 3 The role of reactive oxygen species in tumourigenesis. Various exogenous and endogenous stimuli can result in the generation of DNA- and protein-damaging reactive oxygen species (ROS). Polymorphisms in DNA repair and oxidative enzyme genes, encoding proteins with altered functional activity, can alter cellular efficacy in regulating ROS-mediated damage. ROS can promote genomic instability and mutation, as well as damage RNA and protein, resulting in altered gene expression. When these factors combine, inflamed pre-malignant tissue acquires a propensity to tumourigenesis. Adapted from Klaunig et al. (2010) and Kundu and Surh (2008)

released at the site of tissue damage, partial organ resection, hormones, chronic irritation or inflammation. Functionally, many tumour promoters induce cell proliferation, recruit inflammatory cells, increase production of ROS leading to oxidative DNA damage, and reduce DNA repair. Induced oncogenes such as c-Myc have also been reported by TNF-α stimulates in Barrett's metaplasia (Tselepis et al. 2003). Subversion of cell death and/or repair programmes occurs in chronically inflamed tissues, thus resulting in DNA replication and proliferation of cells that have lost normal growth control. Chronic inflammation appears to be due to persistence of the initiating factors or a failure of the mechanisms required to resolve the inflammatory response. It is this sustained inflammatory response that ultimately confers increased risk for carcinogenic progression.

4 Inflammatory Infiltrate in the Tumour Microenvironment

The presence of inflammatory infiltrate within tumour microenvironments is taken as evidence that the host is not ignorant of a developing tumour, and attempts to interfere with its progression (Whiteside 2008; Zitvogel et al. 2006). There have been numerous studies linking inflammatory infiltrate with improved prognosis or increased survival (Baxevanis et al. 1994; Galon et al. 2006; Kornstein et al. 1983; Naito et al. 1998; Pages et al. 2005). The microenvironment of tumours, including the stroma scaffolding, contains a myriad of leucocytes. These leucocytes produce proinflammatory mediators; cytokines, chemokines and growth factors that promote tumour growth and alter the inflammatory status of the tumour, and some of the key players are discussed below.

4.1 Macrophages

Macrophages make up the major component of the total cellular infiltrate within most tumours (Mantovani et al. 1992). These tumour-associated macrophages (TAMs) are originally derived from monocytes, which enter into the tumour via chemotaxis mediated mainly by monocyte chemotactic proteins (MCPs) released from the microenvironment. Many tumours produce colony-stimulating factors that enhance the survival of TAMs. This was shown by Pollard et al. who found that knocking out the CSF-1 gene in mice engineered to develop breast cancer, significantly decreased the rate of progression to malignancy and metastatic spread in these animals (Lin et al. 2001). IL-2-, interferon-γ and IL-12-activated TAMs can promote tumour cell death, or disrupt the tumour vasculature. However, TAMs also produce growth factors and angiogenic factors as well as extracellular matrix (ECM)-degrading proteases. As early as the 1970s it was noted that TAMs promoted tumour growth both *in vitro* and *in vivo* and that a high frequency infiltration of macrophages into tumours was associated with a poor prognosis. For example, genes associated with leucocyte or macrophage infiltration, such as *CD68*, herald poor prognosis for breast carcinomas and lymphomas (Dave et al. 2004). In addition, functional single nucleotide polymorphisms in IL-1 and TNF-α are associated with an increased risk of cancer progression (Balkwill and Mantovani 2001). Consequently, TAMs can promote tumour cell proliferation, matrix-remodelling, angiogenesis, invasion and metastasis.

Toll-like receptors (TLRs) are central to direct pathogen recognition by innate immune cells, and represent a critical link between innate and adaptive immunity. Macrophages, and mast cells, express a range of TLRs, which include TLR-1, -2, -3, -4, -6 and -9. Macrophages are directly activated by TLRs upon interaction with pathogen-associated molecular patterns (PAMPS), such as lipopolysaccharide (LPS) on gram-negative bacteria via TLR-4, peptidoglycan on gram-positive bacteria via TLR-2 and viral dsRNA via TLR-3. Polarised macrophages can be classed as either M1 or M2, similar to the T_h nomenclature used for cells of the T cell lineage. M1 macrophages are stimulated by IFN-γ or bacterial wall products like LPS, as well as cytokines such as TNF-α and GMCSF. M2 macrophages are induced by IL-4 and IL-13. The M1 macrophage phenotype is IL-12^{high}, IL-23^{high}, IL-10^{low}, and they efficiently produce TNF-α, IL-1, IL-6, ROS and RNS. The M2 phenotype is IL-12^{low}, IL-23^{low}, IL-10^{high}. M1 macrophages act as inducers and effectors in T cell Th_1 responses and mediate resistance against tumours. Conversely, M2 macrophages promote tumour proliferation and progression, stromal deposition, tissue repair and remodelling, angiogenesis, lymphangiogenesis, and have suppressive effects on adaptive immune capacity (Albini et al. 2005; De Palma et al. 2005; Nakao et al. 2005; Wyckoff et al. 2004). Most macrophages found in the tumour microenvironment are skewed to the M2 phenotype (Biswas et al. 2006).

In the development of melanoma, activated macrophages produce TGF-β, TNF-α and IL-1-α and ECM-degrading proteases (Torisu et al. 2000). TAMs also

produce cytokines such as IL-10, which downregulates the anti-tumour responses of cytotoxic lymphocytes (CTLs) and is correlated with poor prognosis (Bingle et al. 2002). Other studies have shown that tumour accumulation of TAMs is associated with the increased production of proangiogenic factors including VEGF and PDGF (Birkwell and Mantovani 2001). Hypoxic areas within tumour microenvironments stimulate macrophage infiltration and result in macrophage-mediated production of proangiogenic factors, such as VEGF, TNF-α, bFGF, and CXCL8. The role of CXCL8 in angiogenesis remains unclear, with some reports of anti-angiogenic activity, as well as some describing pro-angiogenic activity.

In gastrointestinal cancers, there are a number of essential elements for carcinogenesis and the metastatic phenotype, which include TNF-α, IL-1, macrophage CSF-1 and CCL2, COX-2, the master inflammatory transcription factor NFκB, and a number of enzymes involved in tissue remodelling (Balkwill et al. 2005; Balkwill and Mantovani 2001; Coussens and Werb 2002; Koehne and Dubois 2004; Mantovani et al. 2008; Pikarsky et al. 2004; Voronov et al. 2003; Wyckoff et al. 2004).

VEGF stimulates lymphangiogenesis by stimulating its cognate receptors expressed on the surface of endothelial cells. Consistent with what is known about TAMs, it has been shown recently in renal cell carcinoma, that VEGF levels were higher in more aggressive (high grade, larger tumours, or symptomatic) RCC. Indeed, patients with symptoms, large tumours, or high grade tumours displayed higher levels of TAMs (Toge et al. 2009). VEGF levels and TAM numbers were higher in patients with recurrence compared to those without recurrence. While VEGF, TAMs, CD34 expression, tumour stage and grade were identified as prognostic factors in a univariate analysis, only TAMs acted as an independent prognostic factor by multivariate analysis (Toge et al. 2009). Using a CT26 colon cancer cell line and RAW 264.7 macrophage model it has been shown that CT26-stimulated macrophages upregulate SDF-1alpha and VEGF, and that these cytokines contribute to CT26 migration *in vitro* (Green et al. 2009). CSF-1 was revealed as the major chemoattractant for RAW 264.7 macrophages in this system. Interestingly, Green et al. found in the CAM (chick chorioallantoic membrane) model of tumour progression and angiogenesis that RAW 264.7 macrophages localised specifically to the tumour periphery, where they were found to increase CT26 tumour growth, microvessel density, vascular disruption and lung metastasis. They suggest that these cells home to actively invading areas of the tumour, but not the hypoxic core of the mass. Supporting this observation, they found that hypoxia downregulated CSF-1 production in several cell lines and decreased RAW 264.7 macrophage migration *in vitro* (Green et al. 2009). In breast cancer, TAM infiltration is associated with the levels of macrophage chemoattractant protein-1 (MCP-1) (Ueno et al. 2000). MCP-1 expression levels also correlated with the expression of serval other proangiogenic factors, including VEGF, TNF-α, CXCL8 and thymidine phosphorylase (TP).

Proinflammatory TNF-α is a key downstream mediator in inflammation. Despite the name, TNF-α is important in early events in tumours, regulating a cascade of cytokines, chemokines, adhesion molecules, MMPs and pro-angiogenic activities.

While high-dose, locally-adminsitered TNF-α can result in an acute and dramatic regression of tumours (Lejeune et al. 1998), it may be surprising that two independent studies demonstrated that the TNF signalling pathway was required for the induction of skin tumours (Moore et al. 1999; Suganuma et al. 1999). Using a syngeneic TNF knockout mouse model, Suganama et al. showed that all the animals were virtually protected against two-stage carcinogenesis when DMBA was used as a tumour initiator and when TPA was used as a promoter. Thus, TNF-α may be one of the ways in which inflammation acts as a tumour promoter. The role of TNF-α in the promotion of tumour progression is a classic example of how tumours usurp a normal inflammatory process to promote their own growth and survival. These data suggest that blocking antibodies that have significant therapeutic efficacy in other inflammatory diseases, such as rheumatoid arthritis, may have applications in therapy for cancer.

4.2 Mast Cells

Mast cells are one of the first host tissue cells to become activated in response to injury. Mast cells originate from haematopoietic stem cells in the bone marrow, and terminally differentiate in their target tissues (Chen et al. 2005; Galli 1990). Tissue-resident mast cells are primarily found in areas of the body regularly exposed to pathogens and parasites, such as the airway, gut and skin. Activated mast cells release preformed and newly synthesised inflammatory mediators, such as vasoactive histamine, cytokines and proteases, as well as lipid mediators, which promote transient arteriolar constriction followed by dilatation of veins and capillaries, and an increase in vascular permeability (Le Bitoux and Stamenkovic 2008). Since the late nineteenth century mast cells have been recognised to infiltrate the leading edge of tumours, at the interface between normal and tumour tissue. The effects of mast cells on tumours can be divided into two general categories, direct and indirect. Direct effects of mast cells include mast cell-mediated tumour cell cytotoxicity, whereas the indirect effects include angiogenesis, tissue remodelling and immune cell recruitment. Mast cells are recognised as an early and persistent infiltration into the tumour environment, even before significant tumour growth and angiogenesis has been established. Many of the theories behind mast cell functions in tumours are based upon their known roles in wound healing and infection. It is well established that, *in vitro*, mast cells migrate towards supernatants derived from tumourigenic cell lines, but not primary or non-tumour forming lines. In addition, to tumour-specific homing, progenitor mast cells constitutively home to mucosal tissues, such as the gut and airway (Abonia et al. 2005; Gurish et al. 2001), and mast cell accumulation also occurs at sites of healing and infection. Therefore, the tissue site of tumour development and localised inflammation also likely contribute to mast cell recruitment via tumour-mediated pathways (Maltby et al. 2009).

Mast cells in close proximity to tumour tissue are frequently observed to associate with the vasculature, suggesting that they may indeed play a role in

angiogenesis via the production of pro-angiogenic factors including VEGF, TNF-α, histamine and bFGF. It has been suggested that the infiltration of mast cells into the environment of a developing tumour triggers an 'angiogenic switch'. Early in its development, a tumour is heavily reliant on mast cells to orchestrate pro-angiogenic events, whereas at later stages the tumour has become autonomous in its growth and becomes mast cell-independent (Coussens et al. 1999). In one study, mast cells localised to blood vessels during the hyperplastic stage of tumour growth in a transgenic murine model, where animals were engineered to express HPV16 genes in basal keratinocytes, promoting early tumour development. It was found that mast cell accumulation was greatest near angiogenic lesions, during periods of intense angiogenesis, but remained absent within the interior tumour microenvironment. Indeed, the absence of mast cells in the interior of these tumours may be linked to a lack of staining due to degranulation, but the preponderance of data available on mast cell localisation to vascular regions would suggest otherwise.

Mast cells are critical mediators of tissue remodelling, a process central to wound repair and the growth of tumours. They perform this function through the production of proteases, which destroy protein in the extracellular matrix. These proteases possess chymase, tryptase and carboxypeptidase activities. Mast cell-mediated destruction of the extracellular matrix is considered to aid tumour dissemination and to facilitate tumour growth via stimulation of angiogenesis; destruction of the extracellular matrix results in the release of factors such as FGF and SCF, thereby increasing endothelial cell migration and neovascularisation (Maltby et al. 2009).

Mast cells are loaded with enzyme-containing granules that promote inflammation. Activation of mast cells occurs through cell surface receptor crosslinking by an array of ligands. Mast cells also display complement fragment receptors for C3a and C5a, as well as C4a, immunological by-products of an infection, which mediate indirect mast cell activation. Mast cells also express receptors for the Fc portions of both IgE and IgG. Activation of mast cells stimulates two potential outcomes: an immediate response involving the exocytosis of preformed mediators contained within granules, or a subsequent upregulation in the production of *de novo* synthesised proinflammatory molecules. *De novo* synthesised mediators include lipid mediators, such as leukotriene (LT) C_4, LTB_4 and prostaglandin D_2, which are involved in allergic and proinflammatory responses (Bischoff 2007; Marshall 2004). While anaphylactic degranulation is responsible for the pathology of immediate type I hypersensitivity and innate immune responses, piecemeal degranulation is thought to play a role in chronic inflammatory disorders and cancers. The production of these proinflammatory cytokines also occurs in parallel to the production of chemokines. Mast cells have long been recognised to recruit and activate T and B cell populations and to recruit eosinophils and neutrophils. They produce T_h2-skewing cytokines, such as IL-4, IL-5 and IL-13, as well as T_h1-skewing cytokines, such as IFN-γ, IL-12 and IL-18. Activated mast cells produce several of the CC- and CXC-chemokines, such as CCL5 and CXCL8, which recruit immune cells to sites of infection (Marshall 2004). Mast cells have

also been shown to express MHC class II molecules and CD28, and to activate T cells *in vitro* (Nakae et al. 2006; Vincent-Schneider et al. 2001). Their location at tissue boundaries makes them one of the first immune cells to encounter invading pathogens, and as such they have been suggested to play a role in initiating immune responses. Furthermore, it has been hypothesised that mast cells may precipitate early tumour rejection by stimulating early T and NK cell responses, perhaps via their antigen presentation capacity, however, this remains both controversial and unclear.

4.3 Neutrophils

There are two pools of neutrophils in circulation; the circulating pool and the marginate pool. The circulating pool consists of neutrophils that circulate freely, while the marginated pool consists of neutrophils that are sequestered to the microvascular endothelium. The marginated pool can be mobilised to the circulating pool upon receipt of acute inflammation signals, in the form of cytokines, such as IL-6. Neutrophils are recruited to sites of inflammation and tumours from the circulating pool (Friedman 2002; Tazzyman et al. 2009). In response to tissue injury, activated neutrophils arrest on the endothelium and subsequently extravasate into the tissue. The extravasation process is often accompanied by plasma fluid and protein leakage (Sarelius et al. 2006). The associated swelling at sites of inflammation is attributable to this vascular leakage. While the leakage itself is largely considered to be due to mechanical disruption mediated by transmigration of neutrophils across the endothelial barrier, an emerging paradigm emphasises a dynamic and reversible interaction between the endothelium and vasoactive mediators released from neutrophils during adhesion and migration (Yuan et al. 2002). Vascular endothelial cells respond to neutrophil activation through a Src- and RhoA-dependent endothelial cell-cell interaction, which is characterised by VE-cadherin and β-catenin phosphorylation and disorganisation of adherens junctions. This response is coupled with MLC phosphorylation-dependent cytoskeletal contraction (Kumar et al. 2009; Tinsley et al. 2002; Yuan et al. 2002). It has also been shown that oxidants and cytokines released by neutrophils cause endothelial hyperpermeability by activating the contractile machinery and opening cell-cell junctions through associated signalling pathways. Cytokines derived from activated leucocytes, such as neutrophils, mediate the upregulation of endothelial adhesion molecules, such as ICAM-1, VCAM-1 and E-selectin. These molecules interact with their ligands on activated leucocytes to mediate rolling and arrest on the endothelial surface, followed by subsequent diapedesis. Following extravasation, the activated neutrophils chemotax towards the site of injury via interaction of chemokines, or tumour/stroma-derived CXCL8, with G-protein coupled receptors on their surface. At the wound, neutrophils engulf and break down invading pathogens.

Neutrophils have long been known to play an important role in normal physiological angiogenesis. For example, neutrophils act as the source for VEGF

during the proliferation and growth of the endometrium during the menstrual cycle. There is now an abundance of evidence showing that neutrophils may play an important role in both tumour and inflammatory angiogenesis. In an *in vivo* corneal model, neutrophil depletion significantly attenuated inflammatory angiogenesis, and reduced the protein levels of VEGF, MIP-1 alpha and MIP-2 in the cornea (Gong and Koh 2009). In a mouse model of multistage carcinogenesis, Nozawa et al. (2006) showed that MMP-9-expressing neutrophils are predominantly found inside angiogenic islet dysplasias and tumours. Transient neutrophil depletion reduced VEGF–VEGF receptor interaction, a surrogate marker of MMP-9 activity, markedly reducing the frequency of the initial angiogenic switching stages in dysplasias that occur during pancreatic islet carcinogenesis. These studies highlight the importance of neutrophils in the angiogenic process. Many different tumour types, and indeed their stromal compartments, are rich sources of CXCL8, a potent chemoattractant for neutrophils. Expression of CXCL8 can be upregulated by tumour microenvironmental conditions, such as hypoxia and acidosis (Kennedy et al. 1997; Xie 2001). Elevated levels of the CXCL1 and CXCL6 chemokines has been detected in gastrointestinal tumours, which are both chemotactic for neutrophils (Cuenca et al. 1992; Eck et al. 2003; Gijsbers et al. 2005; Proost et al. 1993). Elevated neutrophil infiltration has been observed in numerous different cancer types. In colon cancer, neutrophil numbers are higher in adenocarcinoma tumour samples compared with normal tissue biopsies. Interestingly, their density is highest in invasive and ulcerated areas (Nielsen et al. 1996). Neutrophil infiltration into tumours is associated with increased mutation frequency leading to tumorigenesis, presumably through the production of copious amounts of DNA-damaging ROS (Sandhu et al. 2000). As neutrophils interact with the vascular endothelium and extracellular matrix they release MMPs, which cleave proteins allowing for cellular migration to sites of inflammation. Disruption of the basement membrane and extracellular matrix in this way is thought to facilitate tumour metastasis. Thus, while the effects of neutrophils are largely mediated in host defence, clearly they can also be hijacked by the growing tumour.

4.4 T Cells

As one of the major elements of the adaptive immune response, T cells play an important role in inflammation and tumourigenesis. Tumour-infiltrating lymphocytes (TILs) containing various proportions of $CD3^+$ $CD4^+$ and $CD3^+$ $CD8^+$ T cells are a major component of the tumour microenvironment. TILs in medullary breast carcinomas form lymph node-like structures, suggesting that the immune response is operating in situ (Coronella et al. 2002). They also act as a source of tumor antigen-specific lymphocytes, which have been used in many studies in adoptive transfers following IL-2-mediated clonal expansion in culture (Whiteside 2008; Zhou et al. 2004). The accumulation of tumour antigen-specific T cell populations may be evidence of host-mediated tumour immunosurveillance, however, these T cells are largely ineffective in controlling tumour growth.

This may be due, in part, to regulatory T cells (T_{reg}), both naturally-occurring and antigen-specific. T_{reg} are a subset of regulatory T cells that serve to limit proliferation of other T cell subsets through either contact-dependent mechanisms or through the release of IL-10 and TGF-β. In addition, antigen-specific T cell responses may be ineffective due to an immunosuppressive environment within the established tumour.

Predominantly consisting of 'self', and lacking co-stimulatory and danger signals, tumours are generally considered as poorly immunogenic, and by failing to activate antigen-presenting cells may 'passively' anergise effector T cells. In ovarian cancer, tumour cells and TAMs attract pre-existing T_{reg} cells to the tumour site via production of CCL22 (Curiel et al. 2004). An ever increasing amount of evidence indicates that effector T cells can be converted into T_{reg} cells, *de novo*, either within the tumour microenvironment or in local draining lymph nodes by antigen-presenting cells tolerised by the tumour environment (Colombo and Piconese 2007; Saurer and Mueller 2009; Zou 2005, 2006). Many cancer types overexpress COX-2, a key enzyme involved in the biosynthesis of PGE_2 (Baratelli et al. 2005). Indeed, a positive feedback loop for tumour-mediated immunosuppression is evident with respect to COX-2. PGE_2 can upregulate indoleamine 2,3 dioxygenase, an enzyme that catalyzes the degradation of tryptophan resulting in the generation of immunosuppressive metabolites, in dendritic cells (Braun et al. 2005), as well as directly induce FOXP3 in $CD4^+$ and $CD25^-$ T cells (Baratelli et al. 2005).

The differential activation of T cell subsets has been related to the various morphological aspects of the inflammation observed in inflammatory bowel diseases (IBD), such as ulcerative colitis (UC) and Crohn's disease (CD). UC has been characterised by atypical Th_2 cells producing TGF-β and IL-5, leading to a diffuse mucosal inflammation with superficial ulcerations continuously extending from the distal rectum to various degrees of the colon. The immune response in CD is reflective of transmural inflammation, affecting virtually any part of the GI tract, mediated by a Th_1 type response with T cells producing IL-2, IL-12, IFN-γ and Th_{17} cells producing IL-17 (Fuss et al. 1996; Neurath 2007; Podolsky 2002; Waldner and Neurath 2009; Xavier and Podolsky 2007).

NFκB activation in immune cells drives the expression of numerous proinflammatory cytokines, such as TNFα, IL-1 and IL-6 (Atreya et al. 2008). IL-6 has been implicated in playing a central role in the pathogenesis of IBD, with raised serum levels of IL-6 detected in CD patients (Mitsuyama et al. 1991; Yamamoto et al. 2000). In addition, increased levels of IL-6 have also been shown in patients presenting with colorectal cancer, indicating it as a cytokine likely to be involved in inflammation-related tumorigenesis. In cancer, associated with colitis one of the potential downstream signalling targets of IL-6 produced from $CD4^+$ T cells is STAT3 (Becker et al. 2004), the constitutive activation of which is well established as being a feature of tumour promotion in many cancer types (Aggarwal et al. 2009; Devarajan and Huang 2009; Yu et al. 2009). Indeed, constitutive activation of STAT3 in some melanoma cell lines inhibits TNF-α and IL-1β production and induces tolerogenic factors, such as IL-10, which inhibits the

functional maturation of dendritic cells, and may therefore lead to the tolerisation of tumour-infiltrating lymphocytes (Drake et al. 2006; Kreis et al. 2007; Oble et al. 2009). Waldner and Neurath (2009) suggest that while $CD4^+$ T cell infiltration has been shown to support host anti-tumour responses in cases of sporadic cancers (Yu and Fu 2006), the opposite might be true of inflammation-related cancer. In support of this are the findings of Osawa et al. who showed that an anti-inflammatory Th_2 response promotes tumour growth in a mouse model of colon cancer (Osawa et al. 2006).

While most cancer data suggests the occurrence of immunosurveillance, particularly in GI cancers, the fact that GI malignancy is relatively high in incidence and mortality also suggests that the anti-tumour immune response is insufficient, which is probably true for most cancer types. Failure to eradicate the tumour is thought to be attributable to immunoselection, whereby non-immunogenic tumour cells are positively selected, and immunosubversion, a dynamic process involving tumour-mediated immunosuppression (Zou 2005). $CD8^+$ T cells play a central role in the immunosurveillance of cancer. The presentation of tumour-specific antigens displayed on dendritic cells to $CD8^+$ T cells, through MHC class I interactions and co-stimulatory signals, results in T cell activation and the subsequent release of effectors, such anti-proliferative IFN-γ and cytotoxic perforins and granzymes (A/B), which induce tumour cell apoptosis (Maher et al. 2002). T cell infiltration of tumours with either $CD4^+$ or $CD8^+$ T cells, or both, has been associated with improved prognosis in multiple cancers, including colorectal cancer (Baeten et al. 2006; Chiba et al. 2004; Pages et al. 2009) and oesophageal cancer (Hosch et al. 1997; Ma et al. 1999; Schumacher et al. 2001). While the role for $CD4^+$ cells in colorectal cancers is relatively well-defined, the role of $CD8^+$ T cells and T_{reg} is less obvious. Since both these T cell types are involved in both intestinal inflammation and immunosurveillance, they can either promote or suppress tumour proliferation, which has significant implications for the immunotherapy of inflammation-associated malignancy.

5 Genetic Influences of Inflammation and Cancer

It is well established that tumour initiating events arise as mutations in the DNA, occurring spontaneously or as a result of carcinogens (as discussed in Sects. 3 and 3.1). Most leucocytes have the capacity to produce ROS, including nitric oxide, superoxide anion, hydroxyl radicals and hydrogen peroxide. In addition, RNS, such as nitrogen oxides and peroxynitrites, are also formed and can react to generate mutagenic DNA adducts (Marnett 2000; Valko et al. 2006). NO can mediate its tumorigenic effects through a number of mechanisms, such as the induction of ssDNA or dsDNA breaks, deoxyribonucleotide or deoxyribose modifications, DNA cross-linking or damage to proteins. These alterations can result in altered gene transcription, signal transduction, genomic instability, as well as replication errors (Cooke et al. 2003). Evidence also suggests that mitochondrial DNA damage can contribute to tumour initiation and promotion, and mutations

and altered expression of mitochondrial genes encoding complexes I, III, IV and V have been demonstrated in several different cancer types (Penta et al. 2001). Mutations to the DNA can also result in the aberrant expression and function of cellular repair effectors, such as p53 (Ohshima 2003).

ROS are a natural element of the normal cellular metabolic process, but can induce significant DNA damage at the heightened levels observed during the inflammatory process. While the vast majority of the DNA-damaging effects of ROS are non-specific, many studies have demonstrated that ROS can specifically activate certain intracellular signalling pathways, contributing to tumour development. ROS can be divided into oxygen free radicals (superoxide, hydroxyl ion and NO) and non-radicals (H_2O_2). H_2O_2 can be catalysed to produce hydroxyl ions, which can induce K-*ras*. NO can induce the proto-oncogene p21 and the tumour suppressor p53. One of the major redox sensitive transcription factors is NFκB, which as previously mentioned is a key play in inflammatory mediator signalling.

The NFκB transcription factor was discovered in 1986 and has since been shown to be ubiquitously expressed in all human cell types (Sen and Baltimore 1986; Singh et al. 1986). NFκB is largely considered to be the primary inflammation-associated transcription factor, as it is activated in response to multiple proinflammatory cytokines and pathogens. NFκB exists in the cytoplasm in either a homodimeric or heterodimeric form. The NFκB family consists of 5 subunits, including p65, p50, C-Rel, RelB and p52, however, NFκB exists mainly in the form of a p50/p65 heterodimer. In the cytoplasm, NFκB is found in an inactive state bound to the inhibitor IκB. Upon appropriate stimulation, IKK (IκB kinase), a complex of two catalytic subunits IKKα and IKKβ as well as a regulatory protein called NEMO (NFκB essential modulator), is activated and subsequently phosphorylates IκB, targeting it for polyubiquitination and ultimately proteasomal degradation. Once released from IκB, the NFκB dimer translocates to the nucleus where it binds to κB-regulatory elements within gene promoter regions, initiating the transcription of >200 genes (See Table 2) (Karin 2006; Karin and Greten 2005).

NFκB is an exquisitely redox sensitive transcription factor (Mercurio and Manning 1999) (See Fig. 4). It is now well established that ROS can activate NFκB, with the level of cellular antioxidants, such as glutathione and thioredoxin, serving to regulate the NFκB signalling capacity of ROS. However, the precise mechanisms whereby ROS activate NFκB are not yet fully understood. The process by which cellular ROS are regulated is highly complex and diverse; therefore, the mechanism by which NFκB is activated by ROS may be equally complex. As an example, it has been shown that three cell-specific pathways lead to NFκB activation in response to IL-1 (Bonizzi et al. 1999).

There is a vast amount of evidence supporting the role for NFκB in the tumour initiation, promotion and progression process (Lu and Stark 2004). The genes for NFκB p65, C-Rel, RelB and p52 are located at sites of recurrent translocations and genomic rearrangements in various cancers (Bargou et al. 1997; Gilmore and Morin 1993; Mathew et al. 1993). Numerous studies have shown that NFκB is constitutively activated in a number of cancers, including breast, hepatocellular, oesophageal, colorectal cancer, acute lymphoid leukaemias, multiple myelomas,

Table 2 Genes regulated by NFκB activation. Adapted from Wu and Kral (2005)

Class of molecule	Target gene
Cytokines	IL-1, IL-2, IL-6, IL-8, IL-9, IL-11, IL-12, IL-15, IFN-γ, LT-α, LT-β, TNF-α, TNF-β
Immune response	Igε chain, Igκ chain, IL-2 receptor, MHC class I, MHC class II, T cell receptor, β2-microglobulin
Growth factors	Colony-stimulating factors (CSFs), PDGF, Thrombospondin, VEGF, IGFBP1, IGFBP2
Transcription factors	p53, c-Myc, IκB, c-Rel, IRF1, IRF2
Enzymes	Collagenase I, Lysozyme, Xanthine Oxidase, Transglutaminase
Adhesion molecules	ELAM-1, ICAM-1, VCAM-1, E-selectin
Acute phase proteins	Complement factor B, C4, CRP, angiotensinogen, urokinase-type plasminogen activator
Apoptosis-associated	IAPs, Fas, Bcl-2

prostate, colon, pancreatic, ovarian, melanoma, NSCLC, SCC, thyroid carcinoma, fibrosarcoma and gastric carcinoma (Aggarwal et al. 2006; Bharti et al. 2004; Dejardin et al. 1999; Deng et al. 2002; Greten et al. 2004; Herrmann et al. 1997; Hideshima et al. 2002; Higgins et al. 1993; Hold and El-Omar 2008; Ivarsson et al. 2000; Kordes et al. 2000; Krappmann et al. 1999; Luo et al. 2005; Mori et al. 1999; Mukhopadhyay et al. 1995, 2001; Okamoto et al. 2007; Palayoor et al. 1999; Pikarsky et al. 2004; Sanda et al. 2005; Shah et al. 2001; Shishodia and Aggarwal 2004; Tamatani et al. 2001; Visconti et al. 1997; Wang et al. 1999; Yamanaka et al. 2004; Yang and Richmond 2001), presumably through it actions as a cell survival factor. In cell lines, overexpression of activated NFκB promotes cellular proliferation, whereas suppression of NFκB blocks proliferation. In several tumour types NFκB is activated in an autocrine fashion through the release of cytokines such as TNF-α (cutaneous T cell lymphoma (Giri and Aggarwal 1998; O'Connell et al. 1995), Barrett's oesophagus (Tselepis et al. 2002), IL-1α (head and neck squamous cell carcinomas and melanomas (Kimura et al. 1998; Wolf et al. 2001)), IL-1β (pancreatic carcinoma (Arlt et al. 2002)), GMCSF or GCSF (lung cancer (Uemura et al. 2004)), BAFF and APRIL (B cell lymphomas (He et al. 2004; Kern et al. 2004)). NFκB regulates gene such as IL-1, IL-2, IL-6, IL-8, IL-12, IFN-γ and TNF-α. It also regulates cell-adhesion molecules such as ICAM-1, VCAM-1, ELAM-1 and E-selectin, regulators of the cell cycle, such as p21 and cyclin D1, enzymes, such as COX-2, iNOS and 5-Lox, anti-apoptotic molecules, such as the caspases, IAPs, survivin, Bcl-2 and Bcl-xl, as well as other transcription factors,

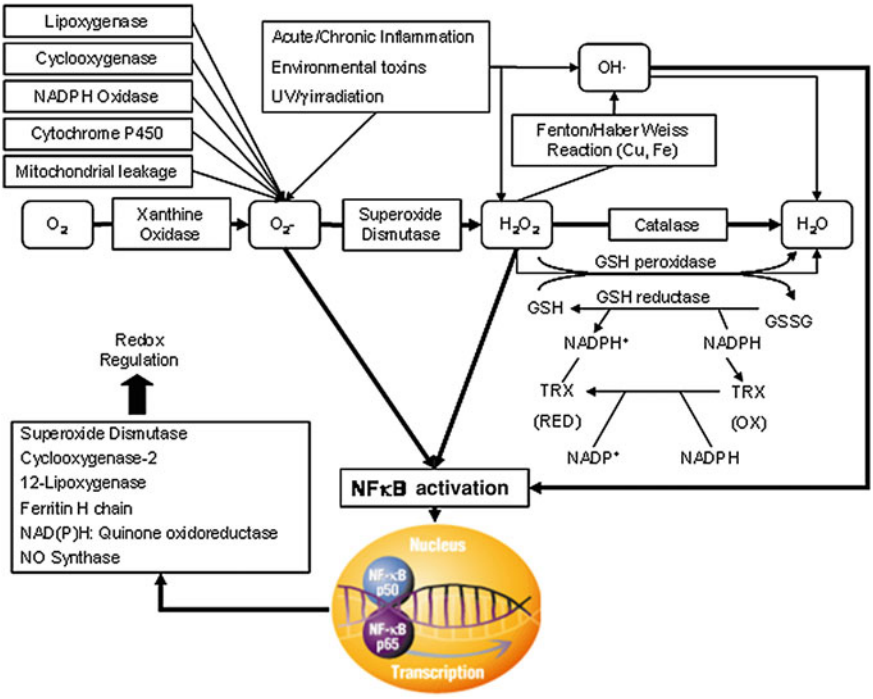

Fig. 4 Schematic representation of reactive oxygen intermediate generation systems and mechanisms of activation of NFkB. The principle reactive oxygen intermediates are the superoxide anion (O_2-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH·). The xanthine oxidase enzyme can split molecular oxygen (O_2) leading to the generation of O_2-. Other enzymes involved in the formation of O_2- include lipoxygenase, cyclooxygenase, NADPH oxidase and cytochrome P450. Mitochondrial leakage is also a source of intracellular superoxide. The superoxide anion can be converted by the enzyme superoxide dismutase to H_2O_2 and molecular oxygen. The hydroxyl radical is formed through the metal catalysed Fenton/Haber-Weiss reaction. O_2-, H_2O_2 and OH· can each activate NFkB, leading to the transcription of certain genes, including those encoding enzymes involved in redox regulation. Receptor-mediated cellular activation, as well as stress-induced (via environmental toxins, inflammation and γ- or UV-irradiation), result in the generation of O_2-, H_2O_2, and OH·. GSH = reduced glutathione; GSSG = oxidised glutathione; NADPH = nicotinamide dinucleotide phosphate; NO = nitric oxide; OX = oxidation; RED = reduction; TRX = thioredoxin. Adapted from Mercurio and Manning (1999)

including p53, c-Myc and API (Abdel-Latif et al. 2009; Wu and Kral 2005). The involvement of NFκB in the angiogenic process appears to be less clear than originally thought; while it is true that NFκB mediates the transcription of promoters of angiogenesis, such as VEGF, IL-6, MMPs and MCP-1, there is evidence demonstrating a suppressive role for NFκB in angiogenesis (Kisseleva et al. 2006; Shen and Tergaonkar 2009). Kisseleva et al. showed in an *in vivo* IκKα-mutant mouse model that tumour vascularisation was markedly increased. Furthermore, known angiostatic agents, such as the 16 kDa prolactin fragment, angiostatin and

Neovastat, are closely associated with the activity of NFκB (Gingras et al. 2004; Shen and Tergaonkar 2009; Tabruyn and Griffioen 2007, 2008; Tabruyn et al. 2007). As cancer therapeutics aim to suppress NFκB activation and inhibit angiogenesis, these data suggest a cautionary note regarding the use of potential anticancer agents that block NFκB (Shen and Tergaonkar 2009; Tabruyn and Griffioen 2007, 2008).

In addition to being constitutively activated in pre-malignant and cancerous tissues, including oesophageal (Abdel-Latif et al. 2004), NFκB is also found in an activated state in inflamed gut specimens for IBD patients, with macrophages and epithelial cells as the main sources (Neurath et al. 1996). In a study by Rogler et al. the level of activated NFκB significantly correlated with the severity of intestinal inflammation observed in IBD patients (Rogler et al. 1998). The increased expression of NFκB in macrophages from IBD specimens is accompanied by an increased capacity of these cells to produce TNF-α, IL-1 and IL-6 (Neurath et al. 1996), which may ultimately be involved in promoting the progression to cancer in these patients.

It is now well recognised that gene polymorphisms contribute significantly to disease susceptibility and severity. This is particularly true for cancer. Single nucleotide polymorphisms, or SNPs, are naturally-occurring variations in the genetic code that occur in a relatively high percentage of the population (>1%). SNP analysis is particularly useful for studies trying to identify mutations that play a role in multifactorial diseases, such as cancer, which can have complex biological as well as environmental components (Risch 2000). Following completion of the human genome project, and analysis of the relative frequencies of SNPs in the genome, it is estimated that there may be as many as 10 million SNPs in the human genome. Many of these SNPs lie in non-coding regions of the genome, and many of those that lie in the coding sequence may result in synonymous amino acids. Indeed, with the expanding research into the field of non-coding microRNAs, which regulate gene expression at the translational level (Lynam-Lennon et al. 2009), the involvement of SNPs in the non-coding regions of the genome is likely to herald a new era in SNP analysis and its association with disease. However, specific non-synonymous SNPs in the coding regions of the genome have been shown to correlate with and influence the inter-individual variation in the magnitude of inflammatory responses, obviously having ramifications for tumourigenesis.

Cytochrome P450 (CYP) represents a family of monooxygenases that coordinate phase I metabolism of many endogenous and exogenous factors, including carcinogenic agents, drugs and xenobiotics (Lewis et al. 2004). The primary CYPs that metabolise carcinogens in humans are CYP1A1, CYP2A6, CYP1B1 and CYP2E1 (Gonzalez 1993). Polymorphisms have been identified in most of these carcinogen-associated CYP genes, which result in proteins with altered expression level and/or activity (Guengerich 1994; Guengerich et al. 1991). The alterations in proteins derived from these polymorphic CYP genes likely has an impact on the cellular capacity to metabolise carcinogens, which may ultimately alter cellular DNA repair mechanisms, altering cancer susceptibility.

Given that ROS are a key element in mediating DNA damage ultimately promoting tumour initiation, promotion and progression, it is not surprising that polymorphisms in specific genes, such as those involved in the DNA repair mechanism or in antioxidant genes, should have an effect on inflammation-related tumourigenesis. Antioxidants represent a major mechanism for protecting cells from oxidative stress, particularly in the context of chronic inflammation. Many of the antioxidant genes are known to be polymorphic, including Manganese superoxide dismutase (MnSOD or SOD2), myeloperoxidase (MPO), catalase (CAT), glutathione peroxidase (GPX), glutathione S-transferase (GSTM1, GSTT1, GSTP1), epoxide hydrolase (EPHX1) and NAD(P)H quinone oxidoreductase (NQO1) (Klaunig et al. 2009). The variant allele of MnSOD is associated with an increased risk of lung (Liu et al. 2004), breast (Bewick et al. 2008), prostate (Kang et al. 2007) and ovarian cancer (Olson et al. 2004), as well as non-Hodgkin's lymphoma (Wang et al. 2006). Catalase is another antioxidant enzyme that catalyses the conversion of hydrogen peroxide to water and oxygen (Hunt et al. 1998). While there are polymorphisms in the CAT gene present with high population penetrance, no study has yet identified an association of this SNP with tumourigenesis. GPX1, a gene encoding a glutathione peroxidase enzyme, protects against oxidative damage by reducing cellular H_2O_2 levels and an array of peroxides (Arthur 2000). A P198L polymorphism in GPX1 is somewhat a population-inconsistent SNP associated with bladder (Ichimura et al. 2004), lung (Raaschou-Nielsen et al. 2007), prostate (Arsova-Sarafinovska et al. 2008) and breast cancer (Ravn-Haren et al. 2006).

One of the major and most studied forms of oxidative DNA lesions is 8-hydroxyguanine (oh^8G) (Shinmura and Yokota 2001), a lesion induced by ROS. The oh^8G lesion is repaired by a number of base repair enzymes, including OGG1, NEIL1, APE1 and MUTYH (Evans et al. 2004), with polymorphisms applicable to each of these enzymes. The OGG1 gene encodes a DNA glycosylase/AP (apurinic/apyrimidinic) lyase that can directly remove oh^8G lesions from damaged DNA (Shinmura and Yokota 2001). OGG1 is highly polymorphic within the human population. In many cancers, including lung, esophageal, gastric and prostate, OGG1 is somatically mutated and confers increased risk. Polymorphisms within this gene alter glycosylase activity and function, impairing the cellular capacity to excise and repair ROS-mediated DNA damage. Thus, OGG1 polymorphisms may alter individual susceptibility to cancer development (Shinmura and Yokota 2001).

CD is characterised by a destructive inflammation of the intestine, with an imbalanced immune response to luminal microbiological or nutritional antigens appearing to play a major causative role (Niessner and Volk 1995). It has been shown that a polymorphism in the NFκB inhibitor α (NFκBIA) gene is associated with CD in patients who lack a predisposing allele of the CARD15 gene, a gene involved in the recognition of muramyl dipeptide derived from LPS and the activation of NFκB (Klein et al. 2004). It has been shown that polymorphisms in the *IL-1β* gene increase the risk of gastric cancer by 2–3-fold in patients infected

with *H. pylori* (El-Omar et al. 2000; El-Omar et al. 2003; Klein et al. 2004). Furthermore, SNPs in the *TNF-α* and *IL-10* genes are additional risk factors for non-cardia gastric cancer (El-Omar et al. 2003).

6 Concluding Remarks

As we have learned, the physiological response to cancer has significant overlap with the response to tissue injury in the context of inflammation. While acute inflammation associated with tissue damage and wound healing is normally self-limiting and chronic inflammation within a pre-malignant environment, be this due to infection, physical or chemical trauma, favours neoplastic progression. Cancer cells can educate immune infiltrate to produce factors important for tumour growth and metastasis. Consequently, the sustained activation of immune cells within the tumour microenvironment results in a persistence of proinflammatory factors, which ultimately promote tumour growth and survival. The nature and intensity of the inflammatory infiltrate varies as the tumour progresses, a process dependent on the local milieu that is created and shaped by the tumour. The dissection of the molecular basis for inflammation-driven carcinogenesis has identified a number of key mediators in this process, such as the cytokines TNF-α and IL-1, transcription factors such as STAT3 and NFκB, and even altered susceptibility driven by host genetics. It is apparent that our comprehension of inflammation and its role in the carcinogenic process is far from complete. Only in fully understanding the relationship between inflammation and cancer will we be able to adequately manipulate the process. Ultimately the challenge ahead is to reinstate the normal inflammatory process, by increasing tumour suppressive factors and decreasing tumour promoting factors.

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Inherited Syndromes Predisposing to Inflammation and GI Cancer

Shane Duggan, David Prichard, Murat Kirca and Dermot Kelleher

Abstract

Cancers arising within the gastrointestinal (GI) tract are commonly associated with an immune component at their inception and later in their maintenance. While many of the immune factors and immune cell types surrounding these lesions have been highlighted, the underlying pre-dispositions in immunesupported carcinogenesis are not well characterised. Inherited Mendelian GI disorders such as polyposis syndromes, while classically due to germline mutations in non-immune genes, commonly demonstrate alterations in key immune and inflammatory genes. In some cases immune based therapies have been shown to provide at least some benefit in animal models of these syndromes. The advent of genome wide association studies has begun to powerfully examine the genetic nature of complex non-Mendelian GI diseases highlighting polymorphisms within immune related genes and their potential to provide the niche in which GI cancers may originate. Here in the role in which Mendelian and non-Mendelian genetics of immune related factors supporting GI malignancy will be presented and discussed.

Contents

S. Duggan · D. Prichard · M. Kirca · D. Kelleher
Department of Clinical Medicine and Institute of Molecular Medicine, Trinity College Dublin, Dublin 2, Ireland

D. Prichard · M. Kirca · D. Kelleher (✉)
Department of Gastroenterology, Institute of Molecular Medicine, Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8, Ireland
e-mail: Dermot.kelleher@tcd.ie

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1 Introduction

Chronic inflammation has long been established as an aetiological factor in the genesis of neoplasia within the gastrointestinal tract (Secher et al. 2010; Itzkowitz and Yio 2004; Grivennikov et al. 2010). Recent data suggests that even in the absence of an overt macroscopic inflammatory reaction, the activation of pathways involved in inflammation may result in mutagenic and neoplastic consequences. As the gastrointestinal tract represents the largest interface between host environment, innate immune response and immune surveillance, the potential for disturbances resulting in pro-carcinogenic inflammation within this organ is high. Both innate and adaptive immune responses may play a role and there is a substantial literature regarding the potential role for toll-like receptors (TLRs) in the pathogenesis of inflammation and cancer.

In recent years great strides have been made with respect to the molecules involved in epithelial and inflammatory cell response to bacterial infection and innate immunity. It is now understood that such cells are capable of sensing microbial products and components of the outer membrane of Gram-negative bacteria, such as lipo-polysaccharide (LPS), through specific receptors known as toll-like receptors (TLR) (Conroy et al. 2008). These receptors recognise pathogen-associated molecular patterns (PAMPs) of infectious agents such as flagellin (TLR5) (Im et al. 2009) viral RNA (TLR7) (Diebold et al. 2004) and LPS (TLR2 and 4) (Kirschning et al. 1998; Chow et al. 1999). Mutations within the genes encoding these receptors are known to affect responsiveness to infectious agents (Etokebe et al. 2010). The gastro-intestinal system is exposed to many of these agents due to the constant presence of bacteria in the intestinal microflora. It is a commonly held concept that intestinal diseases may initially result from aberrant responses to normal microflora or new bacterial infection that colonises and triggers disease onset. Furthermore, there is growing evidence for the involvement of TLRs in cancer development and in the potential use of TLR agonists in its treatment (Conroy et al. 2008).

Mendelian genetic disorders such as the polyposis syndromes are clearly associated with the pathogenesis of gastrointestinal malignancy. However, more complex genetic approaches including genome-wide association studies (GWAS) have identified a wide diversity of genes involved in the pathogenesis of

gastrointestinal diseases such as inflammatory bowel disease and coeliac disease, many of which are involved in the control of inflammation. Such genetic changes may promote inflammation and regeneration and may also affect fundamental cellular processes such as apoptosis which can impact on survival of cells with malignant potential. While there is evidence to suggest that basic inflammatory processes, including for example cyclo-oxygenase products, may be implicated in the pathogenesis of even the polyposis syndromes, there is now considerable interest in the mechanisms involved in malignant change in non-Mendelian inflammatory diseases of the gastrointestinal tract, which have a heritable component.

2 Role of Inflammation in Mendelian Gastrointestinal Disease Polyposis Syndromes

The polyposis syndromes comprise a heterogeneous group of diseases, all autosomally inherited, although with variable penetrance. Lynch syndrome (Hereditary Non-Polyposis Colon Cancer, HNPCC) is the most commonly occurring of these syndromes, accounting for approximately 3% of all colorectal cancer (CRC). Familial adenomatous polyposis (FAP) occurs with a lower frequency estimated to between 1:6850 and 1:23790. These two conditions are characterised by the adenoma to carcinoma sequence of progression. Peutz-Jeghers Syndrome (PJS) and Juvenile Polyposis Syndrome (JPS) are rarer conditions in which cancer develops through the dysplastic transformation of hamartomatous lesions. An inflammatory infiltrate is a component of the majority of adenomas and carcinomas seen in the colon, although the relative inflammatory response varies among sporadic tumours and hereditary syndromes (Michel et al. 2008).

2.1 Polyposis Syndromes: Familial Adenomatous Polyposis

Familial Adenomatous Polyposis (FAP) and its associated syndromes (Gardner's, Turcot's and Attenuated FAP) are characterised by autosomal dominant pedigrees. The phenotype is characterised by hundreds to thousands of colonic polyps which harbour malignant potential. There is also an increased risk of developing adenocarcinoma in the ampulla of Vater and stomach, as well as extra-intestinally, in the liver (hepatoblastoma), thyroid (follicular) and brain (medulloblastoma) (Lynch and de la Chapelle 2003).

Germline mutations in the adenomatous polyposis coli (APC) gene on chromosome 5 is the underlying cause of this disease, however, the penetrance of mutations in this gene vary from 20 to 100% in different pedigrees (Lynch and de la Chapelle 2003). The APC protein plays a critical tumour suppressing role by antagonising Wnt induced cell proliferation through binding and phosphorylating proteins such as beta-catenin, resulting in its destruction. Primarily, without the degradation of beta-catenin, this protein can activate TCF-4 and lef-1 transcription

factors, which upregulate the MYC oncogene and contribute to the development of CRC via aberrant progression of the cell cycle. APC has further roles in cell migration, adhesion chromosome separation, spindle assembly and apoptosis, the disruption of which may also promote tumour initiation and progression.

Both human and murine studies suggest a significant role for cyclooxygenase-2 (COX-2) in this syndrome. COX-2 is upregulated in FAP-associated cancers (Hsi et al. 1999). This inducible cyclooxygenase enzyme is involved in the creation of prostaglandins from arachidonic acids. Prostaglandins act through G-protein coupled receptors to promote and propagate localised inflammation and mitogenesis in both a paracrine and autocrine manner (Khan et al. 2001; Sinicrope et al. 1999). Prostaglandins may also transactivate a number of pathways in cancer cells such as EGFR signalling further promoting metaplastcity (Wu et al. 2010). COX-2 knockout mice with mutated Apc genes result in a significant reduction in the size and number of colonic polyps (Oshima et al. 1996). COX-2 inhibitors have been used in an attempt to prevent adenoma and carcinoma progression in both murine and human studies. Trials with the COX inhibitor Sulindac demonstrated adenoma regression initially, although effects of COX-2 inhibition were transient and appeared to result in the hyper-proliferation of colonic adenomas after prolonged regular use (46 months) (Tonelli et al. 2000). Later trials using COX-2 preferential inhibitors such as celecoxib or refecoxib demonstrated significant chemo-preventative effects (Steinbach et al. 2000; Bertagnolli et al. 2006; Baron et al. 2006) but widespread use of these drugs in this setting has been precluded due to an increased level of cardiovascular disease (Arber and Levin 2008; Solomon et al. 2005; Bresalier et al. 2005). Polymorphisms in COX-2 do not have significant effect in the FAP phenotype either in the colon or on extra-gastrointestinal manifestations (Humar et al. 2000; Peters et al. 2009). The functional COX-2 promoter polymorphism at −1195G associated with low COX-2 expression has recently been associated with FAP in a small clinical cohort but with a p-value approaching in-significance and cannot be explained with respect to COX-2 overexpression within this disease setting (Peters et al. 2009; Eisinger et al. 2006).

2.2 Polyposis Syndromes: Peutz-Jeughers Syndrome

Peutz-Jeughers Syndrome (PJS) results from a germline mutation in the LKB1 gene now more commonly referred to as serine/threonine kinase 11 (STK11). This gene codes for a serine/threonine kinase which plays a role in cell cycle, apoptosis and cellular polarity. The phenotype is characterised by melanin hyperpigmentation of the buccal membrane and the presence of multiple hamartomatous polyps throughout the intestine. Neoplastic transformation of these gastrointestinal polyps may occur through a dysplasia—neoplasia pathway. COX-2 is also upregulated in polyps and cancers associated with this disease indicative of an inflammatory component to this disease (De Leng et al. 2003; Rossi et al. 2002). Immunohistochemical studies of PJS demonstrate a statistically significant correlation between the expression of total LKB1 (wild type and mutant) and COX-2,

although the underlying molecular connection between them remains unknown (Wei et al. 2003; Shackelford et al. 2009). COX-2 inhibition in mouse models significantly reduces polyps burden (Udd et al. 2004) and similar effects have also been reported in humans (Udd et al. 2004).

2.3 Polyposis Syndromes: Juvenile Polyposis Syndrome

Multiple juvenile polyps occur in Juvenile Polyposis Syndrome (JPS) both in colon and elsewhere, predisposing to an increased risk of dysplastic and neoplastic change. 50–60% of patients with this disease have mutations in SMAD4 or BMPR1A, both of which are associated with the TGF-β signalling pathway (Geboes et al. 2007; Daniels and Montgomery 2007; Howe et al. 2004). While initially thought to be linked to PTEN mutations and alterations in Akt/PKB signalling, this has subsequently been questioned and has been shown to carry similar mutations and phenotypes to Cowden syndrome (Eng and Peacocke 1998; Lynch et al. 1997). Similar to sporadic and FAP-associated adenomas and carcinomas, the levels of COX-2 are higher in these polyps than in surrounding tissue, although the defining germ-line mutation exerts no effect on the expression of COX-2(Brazowski et al. 2005; Kurland et al. 2007; van Hattem et al. 2009).

Numerous studies have documented an inflammatory cell infiltrate, especially in the lamina propria (Weller and McColl 1966; Fenoglio-Preiser and Hutter 1985). Furthermore, Kim et al. demonstrated in mouse models that SMAD4 inactivation in lymphocytes leads to the development of cancer, whereas inactivation in epithelial tissue does not exert this effect. This finding proposes a putative mechanistic link between alterations in SMAD4 signalling within the tumour setting and the propagation of cancer through inflammatory processes.

2.4 Polyposis Syndromes: Lynch Syndrome or Hereditary Non-Polyposis Colon Cancer

Lynch Syndrome or HNPCC, an autosomal dominant disease resulting in the development of multiple gastrointestinal and non-gastrointestinal tumours (Lynch and de la Chapelle 2003), is defined by mutations in DNA mismatch repair genes (Parsons et al. 1993). Mutations lead to the development of colonic, gastric, small bowel, and pancreatico-biliary tumours and extra-intestinally to endometrial, ovarian and urological tumours (Lynch and de la Chapelle 2003). The mismatch repair system includes the proteins hMLH1, hMSH2, hPMS1, hMSH6, and hMLH3. A mutation in any of these genes results in the characteristic development of high levels of microsatellite instability (MSI-H). Germ-line mutations in HNPCC affected individuals (of which MLH1 and MSH2 account for approximately 90%) (Lynch and de la Chapelle 2003; Lynch et al. 2009) are followed by further somatic mutations through failure of mismatch repair occurring in short repeat regions (and often in other MMR genes resulting in a self-fulfilling

prophecy), primary genetic damage or hypermethylation of the promoter regions of these genes (Peltomaki 2001). Genes commonly mutated as a result of MMR loss of function include TGF-β, IGF, caspase 5, and Bax, all of which are involved in cell proliferation or apoptosis.

MSI-H tumours are associated with high level of inflammatory cell infiltrate in both pre-cancerous adenomas and the tumours. The magnitude of this infiltration is thought to be associated with the reduced metastatic potential associated with this phenotype and the more favourable clinical outcomes seen in patients with MSI-H tumours with a strong Crohn's like reaction (CLR) (Buckowitz et al. 2005; Gafa et al. 2000; Gryfe et al. 2000; Watson et al. 1998). Between 10–15% of sporadic colorectal cancers may additionally be MSI-H positive postulating that these tumours in the same fashion may respond favourably. This inflammatory component is influenced by the expression of pro-inflammatory genes and the production of frameshift mutated peptides, which act as immunogenic tumour specific antigens (Watson et al. 1998; Ripberger et al. 2003; Schwitalle et al. 2004; Linnebacher et al. 2001). This may be in contrast to the general assumption that inflammation directly promotes carcinogenesis. However a direct immunogenic response to tumour antigens may be classified as a secondary response subsequent to the development of a tumour containing tumour-resident macrophages, tumor-infiltrating cytotoxic (CD8+) lymphocytes and natural killer cells.

Array studies of MSI-H tumours have shown that MSI-H tumour cells express mRNA of genes associated with inflammation and apoptosis at levels higher than those seen in microsatellite stable (MSS) tumours. TGF-β, IGF, and MLH1 were all down regulated in the MSI-H tumours confirming their denoted status. Validation of the array data confirmed the greater expression of inflammatory genes such as β-catenin, Interleukin 8, Toll-like receptor 2, Interleukin 1β, and Interleukin 18 as well as the apoptotic related genes granulysin, caspase 2, granzyme A, survivin, and Human Natural killer Cell enhancing factor (NKEF) (Banerjea et al. 2009). Enhanced expression of these genes is a reflection of the observed immunogenic response and presence of infiltrating cytotoxic T-lymphocytes. The mechanism through which this form of tumour immunity differs from that observed in other diseases and provides for a better prognosis in MSI tumours is unclear.

Further enhancing the immune response to MSI-H tumours is the relatively low level of T-regulatory cells compared to sporadic and FAP-associated CRC independent of APC mutation status (Sinicrope et al. 1999; Saurer and Mueller 2009; Drescher et al. 2009). T-regulatory cells play an important role in limiting the activation of the inflammatory system, maintaining immune homeostasis and tolerance to self antigens. Their presence is thought to be associated with a poorer prognosis, although this hypothesis is currently being questioned (Mougiakakos et al. 2010; Wang 2008; Franke et al. 2008). Immunohistochemistry and PCR techniques have demonstrated that the intra-tumoral lymphocytes in MSI-H tumours are predominantly CD3+ CD8+ Granzyme B secreting cells, indicating a cytotoxic T-cell phenotype further supported by good correlation between the CD8+ and IL-2Rα+ cells (Saurer and Mueller 2009; Phillips et al. 2004).

Reinforcing this hypothesis is the higher perforin/CD3 mRNA ratio seen in MSI-H tumours when compared to MSS tumours (Le Gouvello et al. 2008).

3 Non-Mendelian Association of Inflammation and Cancer

3.1 Oesophageal Cancer

The incidence of adenocarcinoma of the distal oesophagus has increased in Western countries over the past three decades, whereas the incidence of squamous-cell carcinoma (SCC) has decreased slightly (Blot and McLaughlin 1999). Obesity, gastro-oesophageal reflux of acid and bile; and Barrett's oesophagus may be contributory factors (Chak et al. 2009; Lagergren et al. 1999; Theisen et al. 2000; Nehra et al. 1999; Sikkema et al. 2010; Hage et al. 2004). The risk of SCC of the oesophagus and the head and neck has been linked to smoking and alcohol consumption (Lee et al. 2007). The familial aggregation of Barrett's oesophagus (BO) may represent a complex genetic trait. Many studies report a strong family link in patients with Barrett's oesophagus and oesophageal adenocarcinoma (OAC). A strong familial expression of BO was seen, spanning one to four generations with two to seven affected members per family. Subsequent, reports of 24 members of 3 generations in a Spanish family showed 6 cases of OAC and 4 cases of BO (Fahmy and King 1993; Munitiz et al. 2008). Further studies have confirmed that individuals with BO and OAC are more likely to have a positive family history (Chak et al. 2002). Furthermore, Mendelian inheritance of familial BO may be more common than previously thought, inherited in an autosomal dominant fashion with incomplete penetrance (Sappati Biyyani et al. 2007).

It is hypothesised that malignancy of the lower oesophagus is supported and propagated through the maintenance of chronic inflammation and dysregulated cytokine expression. A histopathological inflammatory gradient has been observed within BO lesions (Jankowski et al. 2000; Fitzgerald et al. 2002; Tselepis et al. 2002). Cytokines such as IL-6 and IL-1B have been demonstrated to affect lower oesophageal sphincter function, implicating inflammatory mediators in the cyclical increase and propagation of oesophageal disease (Cao et al. 2006). The proinflammatory cytokines IL-8 and IL-1α are elevated throughout the oesophageal cancer sequence (metaplasia-dysplasia-adenocarcinoma) in conjunction with increased NF-kappaB activation and resistance to chemotherapeutic treatment strategies (O'Riordan et al. 2005). The exact contributions of underlying genetic pre-dispositions or local genetic abnormalities that play in the development of oesophageal malignancy remains an elusive paradigm.

A number of multicentre studies have begun to examine genetic predispositions associated with risk of oesophageal complications, assessing gene associated SNPs with roles in regulation of inflammation and redox response, both important in initial disease onset. The IL-12 (A + 1188C) genotype, associated with increased expression of IL-12p70, has been linked with increased risk of BO development from within a population with gastro-oesophageal reflux disease. However this study did

not report any association between SNPs within IL-2, IL-6, or IL-8 and development of BO (Moons et al. 2008). Interestingly, observations regarding polymorphisms associated with COX-2 have differed between Barrett's associated adenocarcinoma and oesophageal squamous-cell carcinoma (OSCC). Case–control analysis showed a 1.72-fold and 2.24-fold excess risk of developing oesophageal squamous-cell cancer for the COX-2 –1195AA or –765CC genotype carriers compared with noncarriers of OSCC (Zhang et al. 2005). To date few COX-2 polymorphisms have been associated with development of BO or Barrett's associated adenocarcinoma, however (Kristinsson et al. 2009; Ferguson et al. 2008). Future studies utilising more comprehensive GWAS approaches may shed light on underlying polymorphisms in Barrett's associated OAC, rather than the current candidate gene approaches which have largely been negative in their associations. However, a growing number of positive associations have been observed between cytokine polymorphisms, for example in IL-1B and TNFα with patient survival and/or treatment in those suffering from OAC (Deans et al. 2009; Deans et al. 2007; Azim et al. 2007). A large multicenter case–control study conducted in 14 centres examined genetic associations of 115 polymorphisms with cancers of the upper aerodigestive tract (UADT) (Canova et al. 2009). SNPs within CYP2A6, MDM2, TNFα, and GASC1 were of notable significance with strong associations to UADT cancers in particular oesophageal cancers. GASC1 variants were strongly associated with increased risk of oesophageal squamous-cell carcinoma (P trend = 0.008). The rare variant of CYP2A6 –47A > C (rs28399433) and of two SNPs in the TNFα gene were associated with a decreased UDAT cancer risk (Canova et al. 2009). Taken together, candidate gene approaches to the association of inherited polymorphisms in inflammatory mediators with oesophageal diseases have had limited success. While inflammation plays a vital role in the inception of BO and the propagation of OAC, the underlying genetic predispositions within the inflammatory context remain to be identified. There has recently been two SNPs identified which predispose to Barrett's metaplasia. These are located on autosomal chromosomes (J. Jankowski, personnel correspondence). These are significant to genome wide significance level.

3.2 Coeliac Disease Inflammation and Cancer Sequence

Coeliac disease is a common intestinal inflammatory disorder, characterised by intolerance to dietary gluten and related proteins from barely and rye. Traditionally this disease is thought to be a Th1-mediated disease utilising HLA-DQ2 and DQ8 in antigen presentation (e.g., gluten) to $CD4^{+}$T cells in the intestine. These wheat peptides are deamidated by tissue transglutaminase (TTG) leading to higher affinity for DQ2. Coeliac disease is classically associated with an increased risk (50-fold) of enteropathy associated T-cell lymphoma (Verbeek et al. 2008; Catassi et al. 2005; Gao et al. 2009) and other types of non-Hodgkins lymphomas (NHL), specifically B-cell NHLs (Gao et al. 2009; Smedby et al. 2005). Coeliac disease is also associated with the development of adenocarcinoma of the duodenum and

cancer of the oesophagus (Diosdado et al, 2010). Recent GWAS studies have identified numerous novel non-HLA loci in addition to classical HLA loci upon replication of the top 1020 most strongly associated SNPs (Hunt et al. 2008; Dubois et al. 2010). The HLA-DQ2A1/B1 locus showed the highest level of coeliac association ($P < 10^{-50}$). The majority of the newly identified loci were of an immune-related origin (IL2/IL21, IL18RAP, IL12A, CCR1/CCR3 cluster locus (Hunt et al. 2008); and regions containing BACH2, CCR4, CD80, CIITA-SOCS1-CLEC16A, ETS1, ICOSLG, RUNX3, THEMIS, TNFRSF14, and ZMIZ1 (Dubois et al. 2010)) and a number of these are shared by other autoimmune and inflammatory disorders (IL2-IL21, IL18RAP, CCR3, and SH2B3) (Hunt et al. 2008). Follow-up replication studies of celiac GWAS data specifically focused on SNPs within immune pathways and SNPs overlapping with other autoimmune disorders such as T1D, RA and CD in Welcome Trust Case Control Consortium (WTCCC) GWAS data. These studies identified 2 further novel genome-wide coeliac disease association loci on chromosome 6q23.3 and 2p16.1 (Trynka et al. 2009). The strongest association between celiac disease and other autoimmune disorders was observed in 60 kb region of strong linkage disequilibrium proximal to *tumour necrosis factor inducible protein A*20 (TNFAIP3) on chromosome 6q23.3. Intriguingly this gene has also been strongly implicated in the pathogenesis of Hodgkin's disease and other B-cell lymphomatas and further studies are required to determine whether this gene plays a role in celiac-associated lymphomata (Kato et al. 2009; Schmitz et al. 2009). The second novel coeliac disease association loci on chromosome 2p16.1 is located within the second intron *of v-rel reticuloendotheliosis viral oncogene homolog* (REL). The REL protein in conjunction with other members of the family (RELA (p65), RELB NFKB1, and NFKB2) form the core transcription factors in the mediation of NF-kB inflammatory pathway signalling and in anti-apoptotic signalling.

The discovery of coeliac-associated SNPs proximal to the gene RGS1 may have particular interest with respect to coeliac-associated lymphoma development (Piovan et al. 2007; Han et al. 2006). Chemokine receptor signalling and B-cell activation and proliferation are known to be regulated by RGS1 (Piovan et al. 2007; Han et al. 2006). Indeed, mouse model studies have demonstrated the involvement of RGS1 in B-cell movement to and from lymph nodes (Han et al. 2006; Moratz et al. 2004). It can be postulated that mutations affecting RGS1 expression and localisation may impact upon lymphoma development in celiac patients. Further work is necessary to demonstrate the mechanisms involved in the pathogenesis of both celiac disease and celiac disease-associated malignancy.

3.3 Inflammatory Bowel Disease and Cancer

Studies indicate that 5–20% of inflammatory bowel disease (IBD) is hereditable with genetic factors having a greater impact in Crohn's Disease (CD) than in Ulcerative colitis (UC) (Spehlmann et al. 2008; Halfvarson et al. 2003; Binder 1998). The hereditable genetics of IBD appear to have little direct impact on the risk of

carcinogenesis (Triantafillidis et al. 2009). Colon cancer typically occurs in ulcerative colitis in patients who have a long history of inflammation and is typically associated with a pan-colitis. The primary risk factor for CRC in IBD is thought to be chronic inflammation with the risk increasing with the duration, extent and severity of colitis and the presence of Primary Sclerosing Cholangitis (PSC) (Itzkowitz and Yio 2004; Triantafillidis et al. 2009). Candidate gene approaches have highlighted the polymorphic association of inflammatory molecules such as TNFα, IL10 and IL2/21 axis, with UC and colorectal cancer (Franke et al. 2008; Garrity-Park et al. 2008; Festen et al. 2009). Genome-wide Association Studies have highlighted SNPs associated with cell death, immunity and inflammation specific for both Crohn's Disease and Ulcerative Colitis, or general to both (Cho 2008; Anderson et al. 2009; Fisher et al. 2008). Shared disease susceptibility loci discovered for UC and CD included many genes with known immune functions (IL23R, IL12B, HLA, NKX2-3 and MST1 (Fisher et al. 2008); IL18RAP, IL12B, JAK2, STAT3, HLA-DRB1 and HLA-DQA (Anderson et al. 2009) in addition to further disease specific immune-related loci. Emerging roles for the involvement of the T helper-(Th)17 differentiation pathway and innate immunity have been suggested from the susceptibility loci identified through these GWAS studies (Anderson et al. 2009; Fisher et al. 2008; Raelson et al. 2007; Rioux et al. 2007; Duerr et al. 2006). Proto-oncogenes such as MYC and Sp1 have been shown to be induced in UC through inflammatory mechanisms, implicating chronic inflammation in disease onset (Macpherson et al. 1992; Maggio-Price et al. 2005; Brentnall et al. 2009). At this point, further studies are required to delineate between the roles of genetic and environmental factors in the pathogenesis of IBD-associated malignancy.

3.4 Gastric Cancer and Inflammatory Genes

Gastric cancer (GC) typically occurs on a background of life-long *H. pylori* infection associated with chronic inflammation, gastric atrophy, and achlorhydria (Argent et al. 2008; Niwa et al. 2010; Polk and Peek 2010; Waghray et al. 2010). Interleukin 1 is probably the most potent suppressant of gastric acid secretion known and is directly associated with the development of achlorhydria (Furuta et al. 2002; Uehara et al. 1989; Saperas et al. 1992; Kondo et al. 1994). Polymorphisms in the ILI-1 gene cluster known to be associated with enhanced IL-1 activity have been associated strongly with the development of gastric cancer (Waghray et al. 2010; El-Omar et al. 2001). Polymorphisms in TLR9 have also been linked with the development of gastric atrophy and hypochlorhydria in H.pylori infected patients (Ng et al. 2010). Patients carrying TLR4 + 896G polymorphism display a significantly increased odds ratio (11-fold; 95% CI = 2.5–48) of developing GC (Hold et al. 2007). However this SNP in conjunction with TLR4 + 1196T, which is also GC associated, have distinct ethnic dependencies. Hence inflammatory activity can be directly linked to the pathogenesis of gastric cancer through its role in the suppression of gastric acid, a scenario which permits the generation of potentially oncogenic compounds such as nitrites within the gastric mucosa.

4 Conclusion

The involvement of inflammatory genes in both pathogenesis and outcome is a subject of emerging detail with respect to gastro-intestinal carcinogenesis. It is understood that chronic GI inflammatory conditions with an inherited component predispose to cancer development but the exact mechanisms through which this occurs may only be postulated. Further detailed genetic studies coupled to functional analysis of identified genes and the analysis of external environmental influences may permit a more detailed dissection of the pathways involved in inflammation-cancer sequences in the gastrointestinal tract.

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Stem Cells and Inflammation in the Intestine

Adam Humphries, Trevor A. Graham and Stuart A. C. McDonald

Abstract

Knowledge of stem cell biology in the intestine is increasing exponentially and it is one of the current hot topics 'of the day'. Yet it is only recently that molecules such as Lgr5 and Bmi1 have been shown to reliably mark stem cells and have revealed the stem cell location throughout the murine gastrointestinal tract. However, there is a scarcity of meaningful work within their human counterpart. Nevertheless, recent studies have demonstrated the processes of niche succession, where one stem cell takes over the entire population of stem cells within a crypt; and monoclonal conversion, whereby the entire crypt becomes a clonal population of cells, are present in the human crypt. This work has also shown how crypts themselves divide and expand in the human colon.

Contents

A. Humphries · T. A. Graham
Histopathology Unit, London Research Institute, Cancer Research UK, London, UK

S. A. C. McDonald (✉)
Centre for Digestive Diseases, Blizard Institute of Cell and Molecular Science, Barts and the London School of Medicine and Dentistry, 2 Newark Street, Whitechapel, London, E1 2AT, UK
e-mail: stuart.mcdonald@cancer.org.uk

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Chronic inflammation in the intestine has been shown to be a significant risk factor for carcinogenesis (Saleh and Trinchieri 2011). If cancer is seen as a disease of stem cells, exposure of stem cells to inflammation must have a profound contributing effect on this. Increased cellular turnover, exposure to inflammatory mediators and cycles of damage and repair all contribute to a state of stem cell instability increasing the likelihood of a carcinogenic event.

In this chapter, we review the structure of the stem cell compartment within the intestine and discuss stem cell biology within normal and inflamed conditions. We discuss how the intestine adapts to inflammatory damage and the mechanisms of epithelial repair. Furthermore, we examine how field cancerisation (a process whereby an area of epithelium is conditioned to be predisposed to tumourigenesis) is affected by chronic inflammation. Finally, we ask if stem cell therapies have been effective in treating inflammatory disorders such as Crohn's disease (CD) and ulcerative colitis (UC).

1 The Colonic Crypt

The human colonic epithelium consists of a single layer of columnar cells that form the functional unit of the gut—*the crypt*—via finger-like projections into the underlying connective tissue, with a population of approximately 2,000 cells per crypt. Unlike the small bowel, there are no villi to be found and the main physiological function of the colon is salt and water absorption. Hence, the predominant terminally differentiated epithelial cells are columnar absorptive cells known as *colonocytes*, and mucous-secreting *goblet* cells. *Endocrine* cells, also known as *neuroendocrine* or *enteroendocrine* cells, that secrete a variety of peptide hormones in an endocrine or paracrine manner, are the other main cell type, although these are seen less frequently. *Paneth* cells are occasionally found in the colonic epithelium, the true function of these granular epithelial cells is still unclear although they are thought to have an important role in innate immunity and secrete a number of defensins and anti-microbial peptides (Salzman et al. 2007). There is emerging evidence that they can be very long-lived and are found in abundance at the base of the small intestinal crypt, although in the large bowel are only occasional found in the ascending colon and some disease states.

The concept that these differentiated cells arise from a single multipotential stem cell through a number of *committed progenitors* was first formulated by Cheng and Leblond (1974) as the *Unitarian Hypothesis* in 1974, and is well founded in animals (Ponder et al. 1985; de Dombal et al. 1990). The first few generations of stem cell divisions are known as *transit-amplifying* cells, these divide rapidly to significantly increase the number of progeny that result from a

single stem cell division, as cells then move up the crypt from the base towards the luminal surface where they become committed to a certain lineage—*committed progenitor* cells, as further migration up the crypt axis occurs. Cells are increasingly differentiated until when they reach the luminal surface terminally differentiation has occurred. Once at the luminal surface, differentiated cells are shed 5–7 days after their initial conception in the lower crypt.

1.1 Multi-Potential Stem Cells are Housed Within a Basal Niche: The Crypt Hierarchy

All the experimental evidence to date supports the theory, originally proposed by Williams et al. (1992), that, within the colonic crypt, adult tissue-specific multipotential stem cells are positioned at the base of the colonic crypt within a *stem cell niche*. The *niche* is a micro-environment that consists of the stem cells themselves and a sheath of surrounding mesenchymal cells, of the myofibroblast lineage—*pericyrptal myofibroblasts*, separated by the basal lamina. Interactions between the pericryptal myofibroblasts and niche stem cells via paracrine secretion of cytokines and growth factors are important for maintaining the various cell phenotypes. Indeed, it is likely that the niche micro-environment created by the surrounding stromal/mesenchymal cells plays a more important role in maintaining basal crypt cells in the undifferentiated or 'stem-like' state than the intrinsic genetic programming within the stem cells themselves. An interesting recent study by Mathur et al. (2010) has demonstrated that in *Drosophila*, the niche may initially be made of progenitor cells which, through BMP 2/4 homologue signalling from peripheral cells, maintain themselves in an undifferentiated state. When peripheral cells are lost, this allows the progenitors cells to respond to notch and begin to differentiate: yet one progenitor remains to form an intestinal stem cell. This is interesting as the current dogma is that stem cells move away from the niche to differentiate whereas this system shows a niche physically breaking down to initiate differentiation, suggesting that niches are transient.

1.2 Stem Cell Number

Most of the evidence for the structure of the colonic crypt comes from murine labelling experiments using somatic mutagens or chimeric mice, where one strain carries a demonstrable marker. Much of the work has been done on the small intestine, although the principles have been applied to the colon as many similarities exist. Debate continues to exist over the number of stem cells housed within the niche and the properties of niche dynamics.

Stem cells within a niche can either expand asymmetrically or symmetrically: (i) an asymmetric stem cell division yields two daughter cells, one of which remains within the niche as a stem cell and the other leaving the niche being

destined for differentiation, these are also known as *q* divisions (Meineke et al. 2001), (ii) symmetrical stem cell divisions either produce two stem cells that remain within the niche—*p* divisions, or two daughter cells that exit the niche and differentiate—*r* divisions, with the resulting extinction of that stem cell line (Loeffler et al. 1993; Kim and Shibata 2002). It is by this process of symmetrical *r* divisions, in addition to random apoptosis of stem cells, that one stem cell line can, stochastically or by a selective advantage incurred as a result of a mutation, come to dominate a niche, a process known as *niche* or *clonal succession*. The progeny of this stem cell will then go on to occupy the whole crypt—termed *monoclonal conversion*.

The theory that the intestinal crypt is supported by a number of stem cells residing within a niche, rather than a single stem cell, initially came from a mouse study by Wlliams et al. (1992). They marked cells via mutagen-induced loss of the X-linked enzyme glucose-6-phosphate dehydrogenase (G6PD) in crypt cells and compared the time-course of mutated phenotypes in both the small and large intestine. A short time after administration of the mutagen a high frequency of partially-mutated crypts were observed, over time a rapid increase in wholly-mutated crypts was observed that reached a plateau at the same time that partially-mutated crypts disappeared. The time taken for the appearance of wholly-mutated crypts in the large intestine was approximately 4 weeks, but 21 weeks in the small intestine, the authors suggested their results were consistent with several stem cells supporting a crypt niche and that the number of stem cells may differ between the small and large colon, with a greater number of niche stem cells in the small intestine to explain the time differences observed. The theory that the intestinal crypt is maintained by a stem cell niche housing multiple stem cells has since been supported by many further studies in both the mouse and human.

Evidence from the mouse points to 4–6 basal stem cells in the colonic crypt (Bjerknes and Cheng 1999; Cai et al. 1997; Potten et al. 1997), and a hierarchical structure to the stem cell compartment has been proposed (Bjerknes and Cheng 1999; Cai et al. 1997; Potten et al. 1997). Cai et al. (1997) utilised the sensitivity of stem cells to very small dose of radiation in order to reduce crypt stem cell numbers to different extents and then measured the regenerative ability of the crypt cells to estimate the numbers of clonogenic cells. The authors concluded that, in the mouse, each colonic crypt consists of approximately 300–450 cells with 4–6 ultimate–lineage ancestor stem cells at the base, a further two tiers of cells above this retain clonogenic stem cell properties and have the ability to repopulate the crypt epithelium and regenerate the lower tier stem cells if the lower cells are damaged.

It is not possible to morphologically differentiate stem cells from other cells within the crypt and, although a number of stem cell markers have been proposed for the intestine none have been proven to specifically mark stem cells. Recent approaches in the mouse model have utilised the ability of stem cells, once clonally marked, to re populate the crypt with clonal progeny of all cell lineages—*clonal replacement* or *monoclonal conversion*—as a marker of multipotentiality. Barker et al. (2007) demonstrated that *Lgr5*

(leucine-rich-repeat-containing G-protein-coupled receptor 5), an intestinal *Wnt* target gene, was exclusively expressed at the colonic crypt base in mice, and that these cells were capable of producing all differentiated cell lineages and were thus multipotential i.e., stem cells.

Further insights supporting the existence of a stem cell niche in the human colon have been provided by studying methylation patterns at CpG islands in individual crypts, a random process known to increase with age (Ahuja et al. 1998; Issa 2000). Quantitative analysis of these patterns in non-expressed genes in the human colon supported the theory that colonic crypts are maintained by stem cell niches containing multiple cells—possibly greater than eight stem cells, rather than a single immortal stem cell, and that successive niche succession cycles take place in colonic crypts which is a natural consequence of the existence of symmetric cell divisions (Kim and Shibata 2002; Yatabe et al. 2001; Nicolas et al. 2007). Modelling studies based on the CpG island methylation patterns estimated that the niche succession time, i.e., the time for a single stem cell line to come to dominate the niche, to be 220 days (95% interquantile range of 2–1,900 days) (Yatabe et al. 2001). Thus, clonal succession is a characteristic of a niche.

1.3 Lineage Progenitors

Little is currently known about the intermediate progenitor cell types in the crypt, Bjerknes and Cheng (1999) used chemical mutagenesis to randomly mark crypt cells by somatic mutation of the *Dib-1* locus in SWR and F1 mice, thus they were able to identify and study the dynamics of mutant clones containing only mucus cells, only columnar cells and clones that contained a mixture of cell types. The authors concluded that both short and long-lived mucus and columnar cell progenitors exist in the crypt, with long-lived progenitors located in the lower-third of the crypt and short-lived progenitors seen higher-up the crypt axis. In this study, over 90% of persisting mutant clones containing a mixture of cell types were seen to arise from a crypt base columnar cell—supporting this as the location for the stem cell.

Therefore, within the human colonic crypt, multiple stem cells reside within a niche—possibly >8 in number, stem cells may undergo symmetric or asymmetric divisions to produce daughter cells that leave the crypt and form the population of transit-amplifying cells. Cells then become committed to a certain lineage, and in the lower part of the crypt a population of long-lived committed precursors exist, with their progeny subsequently undergoing further differentiation and migrating to the luminal surface. The exact life-span of committed progenitor cells in the human crypt is unknown, although it is thought this could be many years. Within the niche, the processes of niche succession and monoclonal conversion enable a single stem cell line and its progeny to completely populate the crypt, with successive niche succession cycles taking place over the life-time of a crypt.

1.4 Determinants of Cell Fate

There are a number of key regulatory signal pathways that are thought to be important in organising the architecture of the crypt, maintaining the stem cell phenotype and controlling cell migration and differentiation. Although most of the work done on evaluating these pathways has been done on the small intestine, similar control mechanisms are thought to be in operation in the colon.

1.4.1 Cellular Proliferation

High levels of *Wnt* signalling are found at the crypt base, the location of the stem cell niche, and this is thought to be crucial for maintaining cells in the proliferative and undifferentiated state (Crosnier et al. 2006). Mutations in adenomatous polyposis coli (*APC*) result in overactivation of *Wnt* signalling and formation of multiple polyps that consist of huge numbers of giant crypts that grow without limit (Morin et al. 1997). *Notch* genes encode trans-membrane receptors that are also important in regulating communication between cells, inhibition of *Notch* results in a large loss of proliferative crypt cells (van Es and Clevers 2005) and thus both *Wnt* and *Notch* pathway activation appears to be required in combination in order to maintain the stem cell phenotype at the crypt base.

1.4.2 Cellular Segregation

Wnt signalling has been shown to control the expression of the *EphB* receptors and *Ephrin* ligands—*Ephrin B1* and *Ephrin B2* that are involved in maintaining cellular boundaries. *Wnt* switches on expression of *EphB2* and *EphB3* receptors and inhibits *Ephrin B1* production, this restricts these cells to the crypt base; as one moves up the crypt axis there is increased *EphrinB1* ligand expression and loss of *Wnt* signalling/*EpHB* receptor expression and so this serves to prevent downward migration of cells due to repulsion between *EpHB* and *EphrinB1*.

1.4.3 Cellular Differentiation

Wnt signalling is thought to have a variety of important roles, being not just essential for cellular proliferation but also having a role in determining cell fate. Blocking of *Wnt* signalling results in the loss of secretory cell lineages, but enterocyte differentiation appears to be unaffected (Pinto et al. 2003); in cases of excessive *Wnt* activation then increased numbers of Paneth cell committed precursors are observed (Andreu et al. 2005). *Notch* signalling also has an inhibitory effect on secretory differentiation and it is thought that cells escaping *Notch* inhibition are able to commit to a secretory lineage and production of Notch *Delta/Jagged* ligands by these cells also results in lateral inhibition of neighbouring cells from following the same lineage pathway (Crosnier et al. 2006). *Math-1* was first identified as a transcription factor important for the differentiation of neuronal cells (Akazawa et al. 1995), and has since been shown to be expressed in the gut epithelium with *Math-1* protein being required for secretory cell differentiation (Yang et al. 2001). *Hes-1* is a Notch signalling component and a negative regulator of secretory cell differentiation—deletion of *Hes-1* results in increased numbers of

secretory cell types and elevated *Math-1* expression (Jensen et al. 2000). Thus, in summary, *Wnt* signalling drives the *Notch* pathway, those cells that become committed to the secretory lineage escape *Notch* activation via expressing the *Delta/Jagged* ligands with subsequent inhibition of *Hes-1* and increased *Math-1* expression; *Notch*-activated cells are under lateral inhibition from committed secretory progenitors, activated notch signalling up-regulates *Hes-1* with resulting suppression of *Math-1* and commitment to an absorptive fate.

1.5 Stem Cell Dynamics: Colonic Crypts are Clonal Populations

There is a large body of evidence to support the theory that adult individual crypts are monoclonal, containing cell populations ultimately derived from a single multipotential stem cell, and we have already discussed how *niche succession* with subsequent *monoclonal conversion* is inherent to the dynamics of a stem cell niche.

Studies utilising mouse chimeras have given valuable insights into the clonal evolution of intestinal crypts. Utilising differential binding of the lectin *Dolichos biflorus* agglutinin (DBA) to B6-derived and not SWR-derived cells, Scmidt et al. (1988) created C57BL/6 J Lac (B6) ↔ SWR mouse aggregation chimeras in order to assess crypt clonality in the developing mouse small intestine. Mixed crypts containing cells of both genotypes were seen in the neonatal period, but by day 14 these had all disappeared and only crypts containing cells of a single genotype were observed. Thus, intestinal crypts are polyclonal at birth but by day 14 all crypts are monoclonal, with crypts undergoing an apparent purification during the rapid growth and high rates of crypt fission occurring at this time.

In the human, the chance finding of an individual who had undergone a prophylactic colectomy for Familial Adenomatous Polyposis Coli (FAP), and was also an XO/XY mosaic, enabled non-isotopic in situ hybridisation studies to show that colonic crypts were comprised exclusively of either XO or XY cells (Novelli et al. 1996). Thus, crypts are clonal and derived from a multipotential stem cell. Additional studies on human tissue in individuals heterozygous for the naturally occurring polymorphisms for the G6PD Meditteranean mutation (563 C > T) and the gene encoding for the enzyme O-acetyl transferase (OAT) as clonal markers have confirmed the conclusion that human intestinal crypts are clonal populations (Novelli et al. 2003; Campbell et al. 1996). Recently it has been shown that colonic stem cells and their progeny contain non-pathogenic mutations in their mitochondrial DNA, specifically mutations in the cytochrome *c* oxidase (COX) gene—a component of complex IV of the respiratory chain, that are relatively common (Taylor et al. 2003). The mitochondrial genome is prone to mutation due to a lack of protective histones and poor DNA repair mechanisms. Mutations can expand stochastically within a cell and over time cells will become either *homoplasmic*—all the mitochondria in the cell are mutated, or *heteroplasmic*—the cell contains a mixture of mutated and wild-type mitochondria. This stochastic

expansion is a lengthy process, and for a mutated cellular phenotype to be observed homoplasmy or a high degree of heteroplasmy must be present, thus stem cells are the only cells that have a sufficient life-span to accumulate these mitochondrial mutations to a level that results in a biochemical deficiency. Two-colour enzyme histochemistry can be used to simultaneously detect the mtDNA-encoded COX and nuclear DNA-encoded succinate dehydrogenase (SDH), a component of complex II of the respiratory chain. Three different types of crypts are observed: wild-type brown crypts, wholly-mutated crypts and partially-mutated crypts containing both mutant and wild-type cells. Laser micro-dissection on wholly-mutated crypts followed by PCR coupled with mtDNA sequencing confirm the presence of conserved clonal mitochondrial mutations within crypts (Taylor et al. 2003; Greaves et al. 2006), confirming that crypts are clonal populations as here a crypt has been repopulated from a single mutated stem cell via *niche succession* and *monoclonal conversion*. The existence of crypts containing two cell populations, one wild-type and one mutated, demonstrates that human intestinal crypts contain at least two stem cells and has enabled the dynamic analysis of clonal expansion and identification of the stem cell niche in human colonic tissue. Thus we now have evidence in human tissue of niche succession and monoclonal conversion 'in action', and that multiple stem cells maintain a crypt.

The time taken for the progeny of a mutated stem cell to colonise the crypt is known as the *clonal stabilisation time* (Campbell et al. 1996). We have already discussed that this has been shown to differ between the small and large intestine in the mouse, being only 28 days in the colon as opposed to 12 weeks in the small bowel (Williams et al. 1992; Park et al. 1995). Campbell et al. (1996), by using loss of O-acetylation of sialomucins after pelvic irradiation in patients heterozygous for O-acetyltransferase gene activity as a clonal marker, estimated the clonal stabilisation time in humans to be approximately one year; this was supported by modelling studies using methylation patterns of CpG islands that gave an estimated niche succession time of 220 days (Yatabe et al. 2001) in human crypts.

1.6 Mechanisms of Fixation

The processes of niche succession and monoclonal conversion allow mutations to be fixed in a number of ways:

1.6.1 Genetic Drift

Due to the small number of stem cells within the colonic niche, they are prone to genetic drift, therefore a single stem cell clone can come to dominate the niche as a result of stochastic non-selective events. As a result neutral mutations that do not affect the fitness if a cell can, over time, become fixed within the population (Kimura 1968).

1.6.2 Natural Selection

Occasionally mutations in a stem cell, especially those involving a tumour suppressor gene such as *APC*, bestow a selective growth advantage to the recipient cell. This allows the processes of niche succession and monoclonal conversion to occur rapidly so that the mutated stem cell progeny occupy the whole crypt. These mutations often result in what is termed a *selective sweep* where natural selection rapidly drives the advantageous allele to fixation (Nowell 1976; Cairns 1975).

1.6.3 Hitchiking

Although tumourigenesis involves the accumulation of mutations that result in a growth advantage to the cell, not all confer an immediate selective advantage in isolation but in combination with others they contribute to a malignant phenotype. These mutations may become established merely by being linked to or *hitchhiking* with an allele that does provide a selective advantage (Maley et al. 2004; Smith and Haigh 1974).

2 The Effects of Inflammation on Stem Cells and their Niche

Inflammatory bowel diseases (IBD) such as CD or UC are characterised by inflammatory infiltrates into the mucosa driven and are perpetuated by bacteria (autologous or otherwise) or autoimmune factors, respectively. Infiltrating mucosal T cells (Hursting et al. 1997) are well known to produce factors such as proinflammatory cytokines such interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α) as well as a plethora of others. The effects of these cytokines upon the crypt epithelium are profound; the mucosa thickens and the crypts elongate and become hyperplastic and there can be an eventual loss of crypts replaced by infiltrate if the inflammation is severe enough.

It has been reported that chronic inflammation can induce molecules such as P-cadherin which in turn cause crypt bifurcation perhaps through symmetric stem cell division (Milicic et al. 2008). Differentiated epithelial cells themselves react to proinflammatory factors. Enterocyte tight junction proteins are particularly sensitive to IFN-γ that induces a loss in barrier function or permeability (Beaurepaire et al. 2009). Goblet cells or mucodepletion is a common characteristic of UC, moreover, mice expressing mutations in MUC2 invariably develop a UC-like inflammatory bowel disease (van der Sluis et al. 2008). Paneth cells in the small intestine contain large concentrations of anti-microbial peptides called defensins, regulated by MyD88 activation of toll-like receptors on their surface. These are dramatically reduced during chronic inflammation, indeed, CD patients expressing the NOD2 mutation have almost absent defensin expression (Bevins et al. 2009).

There is a dearth of information on the direct effects of inflammation on crypt stem cells. It is unknown whether crypt hyperplasia is caused by an increase in stem cell number or an increase in the number of transit-amplifying cells

proliferating. Furthermore, it is unknown if the apparent loss of crypts in severe IBD is due to stem cell death. Assessing stem cell number in the normal human intestine has proved a trick too far for epithelial cell biologists, and therefore so has calculating stem cell numbers in chronic inflammation.

However, there is relatively more known about the effects of inflammation on the stem cell niche, which in turn would have effects on the stem cells themselves.

As previously stated the gastrointestinal stem cell niche is comprised of intestinal sub-epithelial myofibroblasts (ISEMFs), the stem cells themselves and surrounding differentiated epithelial cells such as Paneth cells (Sato et al. 2011). ISEMFs are important producers of matrix metalloproteinases (MMPs) which can digest extracellular matrix components and the basement membrane (Pender and MacDonald 2004). While no specific effect on intestinal stem cells has been documented, mice that are deficient in the matrilysin have been shown to be resistant to tumour development (Wilson et al. 1997).

ISEMFs are also potent effectors of inflammation. They can express proinflammatory cytokines such as TNF-α, interleukin- (IL)-1 and can also proliferate in response to such cytokines, particularly in IBD (Andoh et al. 2002). Interestingly, IL-23 produced by ISEMFs has been shown to be important in the production of IL-17-producing cells (the so-called Th17 memory cell). Furthermore, IL-23 can induce IL-1β and TNF-α production from surrounding macrophages in a synergistic fashion (Zhang et al. 2005). While there is a clear relationship between IL-17/IL-23 and IBD (Sarra et al. 2010), and they are potent inducers of intestinal pathology (Buonocore et al. 2010), there is no clear evidence as to the specific effect of such inflammatory mediators on the mammalian intestinal crypt stem cell.

Drosophila melanogaster has, however, yielded some interesting evidence on the control of stem cell proliferation by inflammatory mediators. Ren et al. (2010) in a recent paper have shown that a direct connection between intestinal stem cells and the basement membrane results in the activation of the Hippo (Hpo) pathway, which inhibits the transcriptional co-activator Yorkie (Yki). Dextan-sulphate sodium (a commonly used initiator of mucosal inflammation in mice) administration breaks this contact allowing activation of Yki and stem cell proliferation. Furthermore, the same group have shown that Yki can up-regulate expression of inflammatory cytokines such as the unpaired family (Upd1-3) which can also induce ISC proliferation through the JAK-STAT pathway (Beebe and Huttenlocher 2010). This pathway can also be negatively regulated by Notch inhibition of Upd cytokines (Liu et al. 2010). The balance between Notch and Hippo-mediated control of ISC proliferation is not yet fully understood. However, the JAK-STAT pathway is frequently employed in mammalian inflammatory cytokine signalling and it is logical to assume that mammalian ISC is also regulated by similar mechanisms. Indeed Andoh et al. (2009) have shown that the novel cytokine IL-24, an activator of the JAK1/STAT3 cascade is enhanced by proinflammatory cytokines in IBD.

3 Concluding Remarks

If there is one area of stem cell biology that has been under-researched it is that of the effects of inflammation on stem cells in the intestine. We assume that chronic inflammation is a potent carcinogen, and if we subscribe to the cancer stem cell theory of tumour development then our knowledge of the effects of inflammation on stem cells must improve. Recent developments in identifying stem cells within the crypt should allow thorough investigations of the effects of inflammatory mediators and potentially allow drug targets to be developed that specifically protect or control stem cell biology in these conditions.

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Acid Reflux and Oesophageal Cancer

Anna Nicholson and Janusz Jankowski

Abstract

Barrett's metaplasia is one of the commonest premalignant lesions in the western world following colorectal adenomas. One in 50 of the adult population develops Barrett's as a consequence of chronic gastro-oesophageal reflux. The mucosal inflammation seen within patients with gastro-oesophageal reflux seems likely to drive the growth of the metaplastic mucosa and also help direct further oncological change, yet the molecular events that characterize the pathway from inflammation to metaplasia to dysplasia and adenocarcinoma are poorly understood. There is hope that understanding the role of oesophageal inflammation will provide important insight into the development of Barrett's metaplasia and oesophageal cancer. This chapter will discuss the inflammation seen within context of Barrett's oesophagus and also clinical trials which hope to address this common premalignant disease. There are several ongoing clinical trials which are aiming to provide data using anti-inflammatory therapies to tackle this important premalignant condition. There is new data presented which suggests that data from the aspirin esomeprazole chemoprevention trial (AspECT) may hold the clue to disease treatment and that the cytokine TNF-α seems to be a key signalling molecule in the metaplasia–dysplasia–carcinoma sequence. Specifically it appears that both epigenetic and inherited genetics cooperate to modulate the prognosis.

A. Nicholson (✉) · J. Jankowski
Centre for Digestive Disease, Blizard Institute, Queen Mary University of London, London, UK
e-mail: a.nicholson@qmul.ac.uk

J. Jankowski
University of Oxford, Oxford, UK

J. Jankowski
Royal Infirmary, Leicester, UK

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Barrett's metaplasia · Oesophageal adenocarcinoma · Inflammation · Non-steroid anti-inflammatories · Proton pump inhibitors

Contents

Oesophageal Adenocarcinoma (OA) is a fatal cancer and is increasing in incidence in the western world. OA is currently fifth in the most common of fatal cancers with annual incidence and death rates increasing by 3% per year (Jankowski and Hawk 2006).

OA has a poor prognosis mainly because patients usually present at a late stage of the disease and the 5 year survival rates for the disease are about 16% (Jemal et al. 2008). Oesophageal Adenocarcinoma has been shown to be commonly associated with a premalignant condition called Barrett's Metaplasia (BM) in which the stratified squamous epithelium of the oesophagus is replaced by a metaplastic columnar epithelium (Lagergren 2005; Nandurkar and Talley 1999). BM is thought to develop as a result of gastro-oesophageal reflux disease in which acid and bile reflux into the oesophagus (Eisen et al. 1997; Zhang et al. 2009). It follows that OA is thought to be a microcosm of evolution, developing sequentially along the metaplasia-dysplasia-adenocarcinoma sequence (Jankowski et al. 2000). Progression is attributed to a series of genetic and epigenetic events that ultimately allow for clonal selection of Barrett's cells via subversion of intrinsic control mechanisms

Table 1 Examples of malignancies strongly associated with chronic inflammatory conditions

Malignancy	Associated chronic inflammatory condition
Nasopharyngeal carcinoma	Epstein-Barr virus
Oesophageal (*Barrett's*) adenocarcinoma	Duodeno-gastro-oesophageal reflux disease
Gastric cancer	*Helicobacter pylori* (*H. pylori*) and intestinal metaplasia of stomach
Hepatocellular carcinoma	Hepatitis C
Cholangiocarcinoma	Chinese liver fluke infestation
Pancreatic carcinoma	Chronic pancreatitis
Gallbladder cancer	Chronic cholecystitis
Small intestine and colon cancer	Chronic non-specific ulcerative colitis and Crohn's disease
Anogenital cancer	Human papilloma virus

regulating cellular proliferation and/or apoptosis (Jankowski et al. 1999). This section will look at the implications and associations of inflammation in this progression and how we can target inflammation to help treat this fatal disease.

1 Inflammation in GI Cancer

The link between inflammation and cancer was first suggested by Rudolph Virchow in 1863 and now the epidemiological data shows a clear association between the two (Balkwill and Mantovani 2001; Coussens and Werb 2002). In the GI tract, many inflammatory tumours are seen and the cause of the inflammation can be infective; such as a virus or caused by a non-infective irritant; such as a chemical. A list of the associated GI cancers can be seen in Table 1 (Balkwill and Mantovani 2001).

Inflammation as a response to tissue damage is seen commonly in GI cancers (Fig. 1). Inflammation is part of the body's immune system; the immune system is comprised of the innate and adaptive systems. The innate system is the body's first line of defense against pathogens and injury; it is a non-specific line of attack, comprised of inflammation, complement system and leukocytes and cells of the immune system. A more sustained and specific line of defense for the body is the adaptive immune system which comprises specific leukocytes called lymphocytes and antibody production. The adaptive immune system allows the immune system to recognise and remember specific pathogens and to mount a stronger attack next time the body comes into connect with it.

Inflammation is the very first response to damage or pathogens and is stimulated by chemical factors released by injured cells and serves to establish a physical barrier against the spread of infection. Also it promotes healing of any damaged tissue. Inflammation is normally self limiting; however, dysregulation of

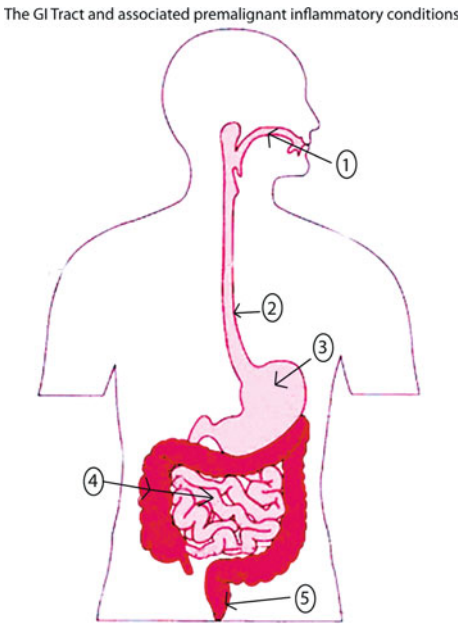

Fig. 1 A diagram to show the structures of the gastrointestinal tract and areas where inflammatory conditions can lead to malignancy. *1* Nasopharyngeal carcinoma *2* Oesophageal carcinoma *3* Gastric cancer *4* Small bowel and colon cancer *5* Anogenital cancer

any of the converging factors can lead to abnormality and pathogenesis. During tissue injury associated with wounding, cell proliferation is enhanced while the tissue regenerates; proliferation and inflammation subside after the assaulting agent is removed or the repaired completed. In contrast, proliferating cells that sustain DNA damage and/or mutagenic assault continue to proliferate in micro-environments rich in inflammatory cells and growth/survival factors that support their growth. In a sense, tumours act as wounds that fail to heal (Dvorak 1986).

2 Gastro-Oesophageal Reflux Disease

Gastro-oesophageal reflux disease (GORD) is a common problem in western countries; it is thought that it is the most common complaint made to general physicians (Cameron et al. 1990). It is considered that around 50% of the population experience symptoms of heartburn or substantial burning sensation in the chest at least monthly and about 20% experience symptoms weekly (Dent et al. 2005). We know that obesity (El-Serag 2008), smoking and drinking alcohol are all considered risk factors associated with GORD (Friedenberg et al. 2008) and that any individual can make lifestyle choices such as healthy eating to help reduce their risk and improve their quality of life.

The causes of reflux disease can include one or more of the following;

- Transient relaxation of the lower oesophageal sphincter
- Decreased resting tone of the lower oesophageal sphincter
- Impaired oesophageal clearance
- Decreased salivation
- Bile/acid regurgitation from the duodenum into the stomach
- Delayed gastric emptying

More often than not patients also present with a hiatus or hiatal hernia, which prevents the efficient closing of the lower oesophageal sphincter, and thus leads to an increase in reflux of acid and bile salts into the oesophagus (Koek et al. 2008).

3 Inflammation and Gastro-Oesophageal Reflux Disease

As previously described reflux of caustic gastric contents into the oesophagus can cause symptoms such as heartburn and nausea, but these factors can also result in local tissue injury leading to erosive oesophagitis and stricture formation (Savarino and Dulbecco 2004). Upon injury, the damaged oesophageal cells start to release inflammatory mediators such as cytokines and chemokines, a list of the implicated substances are shown in Table 2. This leads to migration of inflammatory cells to the site of damaged tissue. Cells are recruited including T-lymphocytes, neutrophils and also there is the activation of inflammatory mediator nuclear factor kappa β (NF-$\kappa\beta$) (Balkwill and Mantovani 2001). The intended result of the inflammatory response is clearance of the causative agent which is subsequently followed by stem cell proliferation, mucosal remodelling and eventually healing (Jankowski et al. 2000). But if the causative agent is not removed and there is deregulation of these processes, it can lead to chronic inflammation and in around 10% of cases initiation of metaplasia (Jankowski et al. 2000). But why all patients experiencing GORD do not go onto develop metaplasia is still unclear, it is possible that the duration and severity of symptoms are important risk factors. The components of the inflammatory infiltrate have implications in deregulation of cellular proliferation as well as impacting on cellular adhesion and angiogenesis. The cytokine profile of Barrett's oesophagus differs from oesophagitis, with an anti-inflammatory response characterised by increased levels of T Helper 2 cytokines and a reduction in signalling through the transforming growth factor b (TGF-b) pathway (Fitzgerald et al. 2002; Fitzgerald et al. 2002). In addition, it has been shown that Barrett's oesophagus has a higher proportion of Th2 effector cells than Th1 effector cells when compared with reflux oesophagitis, demonstrating a shift to a humoral inflammatory response rather than the pro-inflammatory state that characterises oesophagitis (Moons et al. 2005). The importance of these changes is not fully understood. Additionally the inflammatory infiltrate may induce increased expression of FAS ligands on cells, which may protect them from immune surveillance and render them resistant to apoptosis (Younes et al. 2000).

Table 2 A Table to show cytokines and chemokines implicated in the metaplasia–dysplasia–adenocarcinoma sequence

Cytokine	Stage of disease progression implicated	Change in expression	Outcome
TNF-α	Non-dysplastic Barrett's	Increase in expression from oesophagitis to Barrett's	Shown to be expressed in base of Barrett's glands and may have effects on stem cells, also shown to effect oncogene transcription
COX-2	Early Barrett's	Increase in expression	Shown to be up-regulated by various stimuli including cytokines
NF-$\kappa\beta$	Barrett's and Adenocarcinoma	Activation of pathway	Acid has been shown to induce pathway, pathway has been linked to many cancers
Interleukin 1β	Oesophagitis and Barrett's	Increase at new squamo-columnar junction	Inflammation is maximal at new developing tissue
Interleukin 4	Barrett's	Increase in protein and 100-fold increase in receptor compared to squamous	Directly modulates epithelial function in the intestine and increased in progression to adenocarcinoma
Interleukin 8	Oesophagitis and Barrett's	Increase at new squamocolumner junction	Inflammation is maximal at new developing tissue
Interleukin 10	Barrett's	Increase at distal end of Barrett's	Increase seen in non-inflammed columner epithelium
IFN-γ	Adenocarcinoma	Increase positively correlated with tumour progression	IFN regulatory factor 2 (IRF-2) can suppress IFNGR1 transcription in cancer cells by binding IFNGR1 promoter, lowering the sensitivity of cancer cells to IFN-gamma (Wang et al. 2008)

4 Inflammation and Barrett's Metaplasia

In Barrett's oesophagus, the native non-stratified squamous epithelium is replaced by a mucin-secreting columnar-lined intestinal-type epithelium, which is thought to be more resistant to continued duodeno-gastro-oesophageal reflux (Eksteen et al. 2001; Shaheen and Richter 2009). This change in cell type is hypothesised to be in response to a phenotypic change at the level of the pluripotent stem cells in the glands, native squamous oesophagus and oesophageal glands (Jankowski et al. 2000; Jankowski et al. 1999) and in response to stimulation by inflammatory cytokines and growth factors, some of which are detailed below. The stromal compartment of Barrett's oesophagus is increasingly being recognised to play a role in oesophageal carcinogenesis. A recent study by Saadi et al. (2009) showed

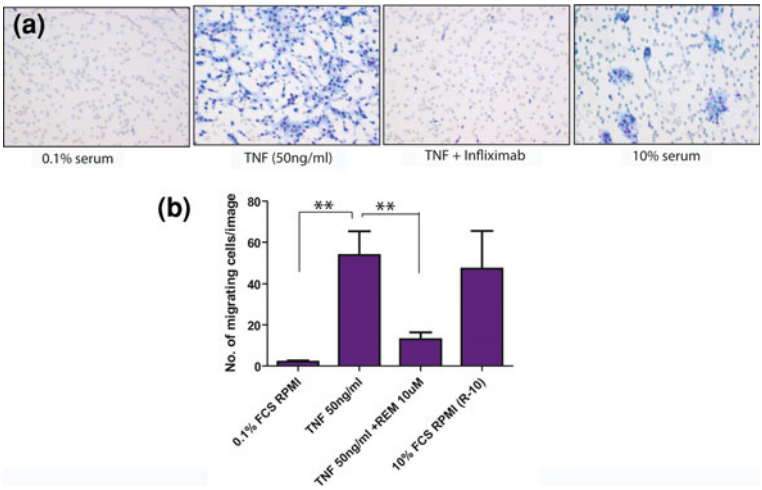

Fig. 2 TNF-α causes migration in Barretts cell lines (Qh-TERT), the effect is abrogated by the TNF- α inhibitor Infliximab. (**a**) Nitrocellulose membranes showing migrated cells. Cells cultured with low serum do not migrate through the membrane. Cells cultured in low serum with added TNF-α do migrate, an effect which is removed by the addition of the TNF-α inhibitor Infliximab, as a control cells cultured in 10% serum also migrate through the membrane. (**b**) Graphical representation of 4 experiments testing the migration of Barrett's cells cultured with TNF-α. TNF-α causes a significant increase in migration in Barretts cells ($p < 0.001$) and this effect is removed by Infliximab ($p < 0.001$)

that increased protein levels of inflammatory-related genes were significantly up-regulated in oesophageal adenocarcinoma.

4.1 TNF-α

Of the mediators of the NF-kB pathway TNF-α, in particular, has been shown to increase along the metaplasia-dysplasia-carcinoma sequence leading to an increase in the proto-oncogene c-myc via the β-catenin mediated pathway (Tselepis et al. 2002). TNF-α has been shown also to be expressed in the base of the Barrett's glands, the proposed location of the stem cells (Tselepis et al. 2002). As well as having implications with increasing proliferation TNF-α and other pro-inflammatory cytokines are able to modulate the levels of both pro and anti-apoptotic proteins Bcl-2 and Bax. A study by the author (unpublished) has shown that TNF-α can cause migration in Barrett's cells (Fig. 2).

4.2 COX-2

Cyclooxygenase-2 (COX-2) is another inducible agent with carcinogenic properties. Activation of NF-kβ possibly via TNF-α signalling can also lead to an increase in COX-2. Expression of COX-2 has been shown to be significantly

increased in Barrett's oesophagus before dyplasia development, suggesting a role early on in the Barrett's malignancy (Wilson et al. 1998). COX-2 and derived prostaglandins including prostaglandin E2 (PGE2) can contribute to malignancy by inhibiting apoptosis, increasing proliferation and angiogenesis and inducing the production of matrix metalloproteinases (MMPs). Also COX-2 can be induced by other cytokines including IL-1α and IL-1β.

4.3 NF-κB

The transcription factor NF-κB is largely implicated in oesophageal carcinogenesis. Prior to activation most NF-κB molecules are retained in the cytoplasm, bound to one of the IκB (inhibitor of NF-κB) proteins. Upon stimulation, the IKK (IκB kinase) complex is activated which phosphorylates NF-κB-bound IκB and targets them for polyubiquitination and ultimately degradation. This allows NF-κB to enter the nucleus and bind to κB-regulatory elements and co-ordinate the transcriptional activation of many immune response genes (Baron et al. 2008; Mathers et al. 2003; Logan et al. 2008). This is known as the classical NF-κB signalling pathway and is triggered in response to pro-inflammatory cytokines and micro-organisms. Once activated, NF-κB regulates expression of over 200 genes, including genes encoding cell adhesion molecules and immune response genes including cytokines, and cell proliferation (Fitzgerald et al. 2002; O'Riordan et al. 2005). Barrett's cells have shown to activate the NF-κB pathway as a way of avoiding apoptosis when they sustain damage, whereas normal squamous cells of the oesophagus undergo apoptosis when damaged by mutagens (Hormi-Carver et al. 2009). Most recently a study by Duggan et al. highlighted pseudokinase tribbles homolog 3 (TRB3) as a novel gene involved in the regulation of inflammation through modulation of NF-kB function (Duggan et al. 2010).

4.4 Interleukins

This group of cytokines which are produced by a variety of the body cells, function to promote the development and differentiation of T, B, and haematopoietic cells. Within the development of Barrett's, interleukins 1b, 4, 8, and 10 have all been implicated in the disease progression. IL-1β is a pro-inflammatory cytokine involved in immune defense against infection. Studies of the inflammatory gradient in Barrett's oesophagus show the levels of IL-1β are significantly increased at the proximal end of the segment (closest to the new squamocolumner junction) and were actually higher in the inflamed squamous than the distal Barrett's (Fitzgerald et al. 2002). IL-8, a chemokine produced by macrophages, was also up-regulated in the proximal Barrett's and has also been seen to be up-regulated in dysplasia and adenocarcinoma, however anti-reflux surgery can modulate its expression (Oh et al. 2007). IL-10, an anti-inflammatory cytokine, has been shown to be increased distally to the leading edge of the Barrett's, and its expression

up-regulated in association with adenocarcinoma (Fitzgerald et al. 2002). Finally alongside IL-10, IL-4 has been shown to be expressed specifically in Barrett's and characterised by a distinct Th2 predominant cytokine profile not seen in oesophagitis. In a study by Nguyen et al., several interleukins were each associated with poor prognosis in oesophageal adenocarcinoma and profiling these genes in tissues may have clinical utility as predictors of prognosis (Nguyen et al. 2010).

It is thought that to become cancerous a cell must undergo approximately four to seven genetic alterations in either up-regulation of oncogenes or down regulation of tumour suppressor genes. Although acid and bile have a role to play in remodelling the mucosa, such as inducing DNA damage or affecting tissue differentiation, evidence showing that once reflux disease is corrected by acid suppression drugs, Barrett's never totally regresses points to maintainance of the metaplasia and promotion to adenocarcinoma by the mild chronic inflammatory infiltrate (Fukata and Abreu 2008).

4.5 p53

The inflammatory response and reactive oxygen species have been shown to cause mutations in genes which play an important role in carcinogenesis, one very important tumour suppressor gene involved in Barrett's carcinogenesis is p53. p53 mutations have been shown in Barrett's oesophagus and in low grade dysplasia, although these mutations are thought not to be the ones seen to clonally expand to progress to carcinoma. Although in High-grade dysplasia and cancer the frequency of mutations increases dramatically, with an even high rate of allelic loss of the p53 locus. A few prospective studies of p53 immunohistochemistry have shown that patients with p53 over-expression in low-grade dysplasia have an increased risk of progressing to high-grade dysplasia and cancer (Kim et al. 1997; Weston et al. 2001; Younes et al. 1997).

Although p53 is mutated early in Barrett's there is an increase in expression as tissue becomes dysplastic. p53 is a tumour suppressor protein which has shown to be inactivated by a two-hit mechanism involving loss of heterozygosity of one allele and mutation or methylation of the second (Chao et al. 2008; Wong et al. 1997). It is also thought that it is the mutational changes occurring late in the dysplastic sequence which clonally expand throughout the region and drive the dysplasia to carcinoma.

4.6 CDX1 and CDX2

The embryonic gut is glandular throughout and CDX1 and CDX2 are homeobox proteins which play major roles in the development of the intestine, CDX2 expression arises in the proximal intestine and declines distally, whereas CDX1 expression arises in the distal intestine with overlap of both in the mid-gut. It is possible that in humans, injurious agents present in GORD activate ectopic

expression of CDX1 through NF-κ signalling which in turn initiates the development of the intestinal phenotype seen with Barrett's oesophagus. Wong et al. (2005) have found CDX1 mRNA and protein expression in all samples of Barrett's metaplasia tested but not in normal oesophageal squamous or gastric body epithelia. Conjugated bile salts and inflammatory cytokines tumour necrosis factor alpha(TNF-alpha) and interleukin 1b (IL-1b) were found to increase CDX2 mRNA expression in vitro through NF-κB signalling. CDX2 may cooperate with CDX1 in inducing and maintaining a complete intestinal phenotype.

4.7 p16

p16 (CDKN2a/INK4a) is a cyclin dependant kinase inhibitor that regulates the cell cycle at G1/S control. Germ line mutations in p16 have been shown in melanomas but also somatic mutations in the gene have been implicated in many cancers including oesophageal adenocarcinoma. Alterations in the gene can occur as mutations, loss of heterozygosity and promoter hypermethylation (Barrett et al. 1999). p16 alterations occur early in the MDA sequence and mutated lesions have the ability to undergo clonal expansion, creating a field in which other abnormalities can arise that can lead to adenocarcinoma (Wong et al. 2001). In detail, growth advantages result in preferential expansion of a mutated clone and a mutation is said to have "gone to fixation" when it expands throughout an entire field, extinguishing all competing clones. A "selective sweep" is the process of natural selection driving a mutation to fixation. It has been suggested that loss of each of the two p16 alleles predisposes to a selective sweep, and that p16 mutation fixation occurs early in the progression of Barrett's oesophagus (Maley et al. 2004).

4.8 Reactive Oxygen Species

The inflammatory cells including neutrophils produce reactive oxygen species (ROS) whose primary role is to remove the damaged cells but they can also induce genetic mutations, which can contribute to DNA damage (Clemons et al. 2007; Jaiswal et al. 2001). While most of these changes will lead to cell death, others may confer a survival advantage and lead to a clonal expansion of the premalignant Barrett's cell type (Atherfold and Jankowski 2006). High levels of ROS have been identified in ulcerated gastro-oesophageal mucosa. The production of ROS can also lead to the further increase in NF-kB activity, thus enhancing the overall inflammatory response (Fig. 3).

4.9 Polymorphisms in Inflammatory Agents

There has been little work into finding polymorphisms implicated in the oesophagus but some have been identified to affect cancer risk in other GI diseases. In *H. pylori* induced gastric cancer individuals with the IL-1B-31*C or -511*T and

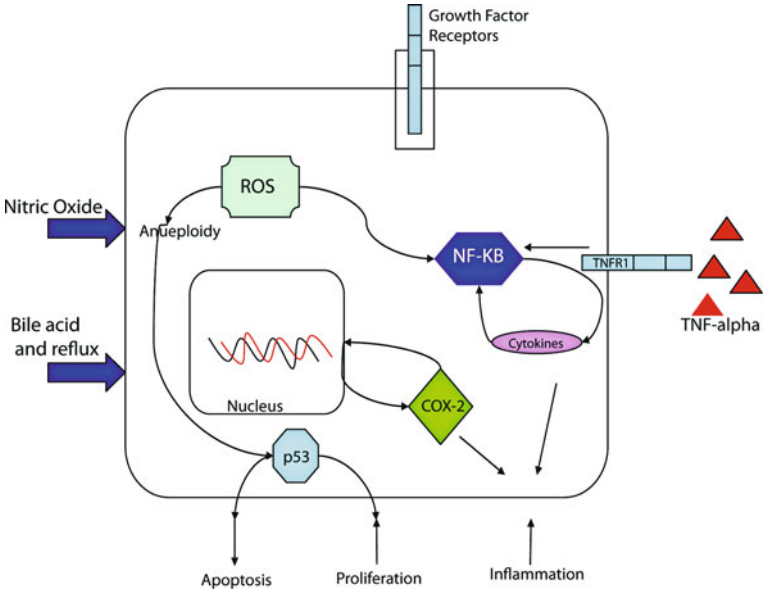

Fig. 3 The cell, an overview of mechanisms of inflammation associated carcinogenesis arising from Barrett's oesophagus. ROS—reactive oxygen species, NF-κB—nuclear factor kappa-light-chain-enhancer of activated B cells, COX-2—Cyclooxygenase 2

IL-1RN*2/*2 genotypes are at increased risk of developing hypochlorhydria and gastric atrophy in response to *H. pylori* infection. In addition to IL-1 gene cluster polymorphisms, pro-inflammatory genotypes of TNF-α and IL-10 have also been identified as risk factors for gastric cancer (El-Omar et al. 2000; Figueiredo et al. 2002).

With these genetic changes and new microenvironment produced by the infiltrating inflammatory cells, the pluripotent stem cells in Barrett's glands/native oesophagus/oesophageal glands are able to acquire the mutations they need to result in the observed metaplastic change while avoiding cell apoptosis or atrophy. Although all these changes occur when the normal squamous oesophagus changes into Barrett's oesophagus, only a small percentage (0.5–2%) of patients with Barrett's will progress to adenocarcinoma there is a need to identify biomarkers to identify these patients at risk (Tischoff and Tannapfel 2008; Fitzgerald 2005). There are a few biomarkers which have been investigated but none have yet to be taken into the clinic (Kerkhof et al. 2007). Most recently a large scale genome wide association study is being undertaken in patients with Barrett's oesophagus, this work is being carried out by the Eagle consortium as part of the WTCCC2. It is hoped that this study will identify common variants in alleles which predispose people to developing Barrett's oesophagus.

5 Anti-Inflammatory Therapies and Clinical Management of Oesophageal Adenocarcinoma

5.1 Proton Pump Inhibitors

Reflux associated diseases including Barrett's are treated with proton pump inhibitors (PPI's) to help reduce the reflux and prevent neoplastic change associated with the infiltrate, although it has been shown that there are patients who do not respond to PPI's (Fass and Sifrim 2009). PPI's have numerous beneficial effects including symptoms control, reduction of inflammation and promotion of the development of squamous islands (Shaheen 2005). However even with these benefits PPI therapy has been shown to cause hypergastrinemia and has not prevented recent increase in the incidence of oesophageal cancer.

5.2 Non-Steroidal Anti-Inflammatories

Aspirin and other non-steroidal anti-inflammatory drugs are increasingly used in the treatment of the disease and are thought to be implicated early in the disease progression. The use of non-steroidal anti-inflammatory drugs (NSAIDs) has been shown to reduce the incidence of several cancers associated with chronic inflammation. The principle target of NSAIDs is cyclo-oxygenase (COX).

5.2.1 COX

COX converts arachidonic acid to prostaglandins and exists as two isoforms, COX-1 is expressed on many tissues and maintains physiological funcions, whereas COX-2 is expressed at low levels in normal tissues and is up-regulated by pro-inflammatory cytokines, growth factors and tumour promoters. COX-2 has been shown to be over-expressed in patients with Barrett's metaplasia and oesophageal adenocarcinoma (Fullard et al. 2006) and also tumours with high expression have been shown to have a more aggressive phenotype (Buskens et al. 2002). Recent advances in the understanding of the cellular and molecular mechanisms of the anti-cancer effects of NSAIDs and COX-2 inhibitors have demonstrated that these drugs target both tumour cells and the tumour vasculature.

5.2.2 Studies into Using NSAID's

Aspirin is commonly used as an NSAID in chemoprevention (Bosetti et al. 2009), it has multiple effects on the inflammatory process, and several studies have tried to assess whether users of aspirin or other NSAIDs have an associated reduced risk of oesophageal cancers. Most importantly, a recent meta-analysis performed by Rothwell and colleagues showed that daily aspirin can reduce the risk of death in several common cancers (Rothwell et al. 2011).

A large study by Thun et al. (Jemal et al. 2008) showed a reduced risk reduction by 40% for oesophageal cancer in recurrent users of aspirin. From a meta-analysis

undertaken by Corley et al. (Corley et al. 2003) it was observed that the overall protective effect of aspirin is 50% compared to 25% for other NSAIDs, but the use of these drugs has to be frequent to achieve this association. There are thoughts that the patients who take aspirin frequently will also be undertaking other lifestyle choices such as vitamins and that this may be having additional effects, also there are concerns over the toxicity of NSAIDs, which are associated with GI bleeds. This shows that evidence from prospective studies such as the one by Thun and Rothwell can give limited data and what is really required is a large scale clinical trial (Moayyedi et al. 2010).

6 Clinical Trials and Future Treatment of Acid Reflux and Oesophageal Cancer

6.1 Current Treatment for Oesophageal Adenocarcinoma

The main treatment options for patients presenting with high-grade dysplasia or Oesophageal adenocarcinoma is currently surgery but this has a low 5 year survival rate.

Observational studies have shown that NSAID use reduces the risk of disease progression in Barrett's Oesophagus (Vaughan et al. 2005). There are still concerns over the use of anti-inflammatories after the Victor trial was ended after a significant increase in the cardiovascular adverse events (Kerr et al. 2007). Although aspirin has been shown to be effective in colorectal adenomas in patients with a history of lesions (Cole et al. 2009).

6.2 Anti-TNF Therapy

Previously in this report TNF-α has been shown to be implicated in Oesophageal adenocarcinoma by upregulating oncogene transcription, TNF-α is shown to be a possible therapeutic target for cancer and in 2004 the first clinical trial using TNF-anatagonists in cancer treatment were undertaken in Breast cancer (Madhusudan et al. 2004), the trial showed safety and biological activity of the treatment and further trials in other advanced cancers have confirmed this (Harrison et al. 2007; Brown et al. 2008). There is now a potential to investigate the use of anti-TNF therapy in the treatment of Barrett's metaplasia/barrett's adenocarcinoma.

6.3 Aspirin and PPI's

The AspECT trial got underway in 2005 with recruitment reaching its target of 2500 patients in February 2009. It is a national, multi-centred, phase III clinical trial recruiting Barrett's patients to one of four arms, consisting of low or high dose PPI's and aspirin or no aspirin long term. The trial aims to follow-up patients for 10 years and will report on the incidence of end points including high-grade

Table 3 A table to show the clinical trials underway to investigate the effects of anti-inflammatory therapy on GI cancers

Trial	Years of recruitment	GI disease	Years of follow-up	Patients recruited	Drugs studied
AspECT (Jankowski and Barr 2006)	Aug 2005	Barrett's oesophagus	10 years	2513	Aspirin and PPI's
Victor (Pendlebury et al. 2003)	April 2002	Colorectal cancer	7 years	2327	Rofecoxib and placebo
Fischbach et al. (Fischbach et al. 2001)	1993	Gastritis	16 weeks	374	Metronidazole, amoxicillin bismuth subsalicylate
CALGB 9270	1993	Previous colorectal cancer	7 years	635	Aspirin and placebo
APPROVe (Baron et al. 2008)	2000	Colorectal adenomas	4 years	2587	Rofecoxib and placebo
(CAPP 1) (Mathers et al. 2003)	Jan 1993	Familial adenomatous polyposis	8 years	40	Aspirin and corn-starch
CAPP 2	Jan 1999	Hereditary nonpolyposis colorectal cancer	4 years	400	Aspirin and corn-starch
ukCAP (Logan et al. 2008)	Dec 1997	Colorectal adenomas	4 years	939	Aspirin, folic acid and placebo
AFPPS	1994	Colorectal adenomas	4 years	1121	Aspirin, folic acid and placebo
APACC (Benamouzig et al. 2001, 2003)	1997	Colorectal adenocarcinomas	4 years	272	Aspirin or placebo
CBET (Heath et al. 2003, 2007)	April 2000	Barrett's oesophagus and dysplasia	2 years	222	Celecoxib or placebo

dysplasia or adenocarcinoma. After 3 years more than 85% of patients tolerated their initial dose of medicine and the drop out rate has been 7%, an interim analysis is expected in 2011 (Das et al. 2009).

6.4 Surveillance of Barrett's Patients

There are questions being asked about the surveillance of Barrett's patients and how we can better manage this condition (Armstrong 2008; Barritt and Shaheen 2008).

Since only 0–2% of Barrett's patients go onto develop adenocarcinoma there is a question over the need for constant endoscopy, better data are required to determine whether patients with mild gastro-oesophageal reflux disease would benefit from increased surveillance (Fullard et al. 2006) and linked to the AspECT trial, the BOSS study will challenge this question in Barrett's patients (Jankowski and Barr 2006).

6.5 Other Studies

There are several other clinical trials which are investigating the effects of anti-inflammatories in GI cancer (Jankowski and Hawk 2006) (Table 3). As well as Victor there were other studies which showed an increase in cardiovascular events after taking anti-inflammatories but none have been seen in the AspECT trial. Hopefully a combined therapy of aspirin and PPI's can be shown to have a significant effect on the development of adenocarcinoma and therefore we can be able to control the increase in cases of these cancers being seen in the west.

In this regard it is possible that PPI therapy may abrogate the GI ulcer complications seen with low dose aspirin therapy (Cuzick et al. 2009; Jankowski and Hunt 2008). Recently an expert independent review has nominated aspirin as the first choice chemoprevention agent for testing in the clinic (Cuzick et al. 2009).

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Helicobacter pylori and Gastric Cancer

Michael Rathbone and Barrie Rathbone

Abstract

Gastric cancer remains a major cause of cancer death worldwide. The discovery of *Helicobacter pylori* and its association with gastric cancer has opened up new insights into its pathogenesis. Gastric cancer pathogenesis is the result of a complex interplay between bacterial, host and environmental factors resulting in a step wise histological progression to neoplasia. *H. pylori* is a major factor in the early stages of cancer development and the mechanism of action of its virulence factors are being steadily unravelled. It is also now recognised that host genetic polymorphisms also play a complex role interacting synergistically with the bacterial virulence factors. The role of *H. pylori* in the causation of gastric cancer also raises the possibility of cancer prevention through screening and eradication, actions which may improve outcomes in high risk populations but which may not be cost-effective in areas of low risk. Ultimately, despite the vast improvements in knowledge, as yet there has not been a corresponding improvement in terms of gastric cancer survival rates.

Contents

M. Rathbone
St George's, University of London, London, UK

B. Rathbone (✉)
University Hospitals of Leicester, Leicester, UK
e-mail: barrie.rathbone@uhl-tr.nhs.uk

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1 Introduction

Although gastric cancer rates appear to be declining worldwide, it is still a huge problem. It was estimated as the fourth most common cancer in 2002 and the second most common cause of cancer-related mortality (Parkin et al. 2005). There is considerable geographical variation in rates of gastric cancer, demonstrating higher rates in East Asia, Eastern Europe and parts of Central and South America. The lower risk areas are South Asia, North and East Africa, North America, Australia and New Zealand (Parkin et al. 2005).

The vast majority of stomach tumours are gastric adenocarcinomas (90%) which can be classified according to position (cardia or more distal tumours) and according to histological appearance (diffuse or intestinal). Tumours of the cardia are more likely to be diffuse type tumours with more distal tumours being intestinal type.

The bacterium *Helicobacter pylori* (*H. pylori*) is now known to be the predominant cause of chronic gastritis. Colonisation of the gastric epithelium by 'unidentified curved bacilli' and the association with chronic inflammation was first brought to the attention of the medical world by (Marshall 1983; Warren 1983) who also isolated the organism. Their achievement earned them the Nobel Prize in Physiology or Medicine in 2005. In addition to its role in chronic gastritis and peptic ulceration, *H. pylori* was recognised as a carcinogenic agent for gastric cancer by the International Agency for Research on Cancer (IARC 1994).

The bacterium itself is a spiral or slightly curved gram-negative rod, 2.4–4.0 μm in length, with 2–5 unipolar flagella which play a role in motility.

This microaerophilic organism colonises the gastric mucus gel and adheres to the epithelium. While generally thought to be non-invasive there is evidence that intra- and inter-cellular invasion occurs (Necchi et al. 2007). It has exceptional urease activity helping it survive in an acidic environment. The organism is mainly contracted in childhood predominantly by the gastric-oral route. The faecal–oral or the oral–oral route together with indirect transmission through contaminated food or water may be possible (Go 2002). A large study from the north of England showed that the most important factors for contracting *H. pylori* infection in the UK were worse socioeconomic conditions in childhood and number of siblings. The risk of infection rose with an increasing number of siblings (Moayyedi et al. 2002).

The prevalence of *H. pylori* infection in middle-aged adults was estimated as 74% in developing countries and 58% in developed countries (Parkin 2006). The same study suggested that 63.4% of gastric cancer cases worldwide were attributable to the bacterium, which translated to 5.5% of all cancers. *H. pylori* is most strongly associated with the non-cardia intestinal type adenocarcinoma and the rare gastric mucosa-associated lymphoid tissue-type (MALT) lymphoma (Wotherspoon et al. 1991).

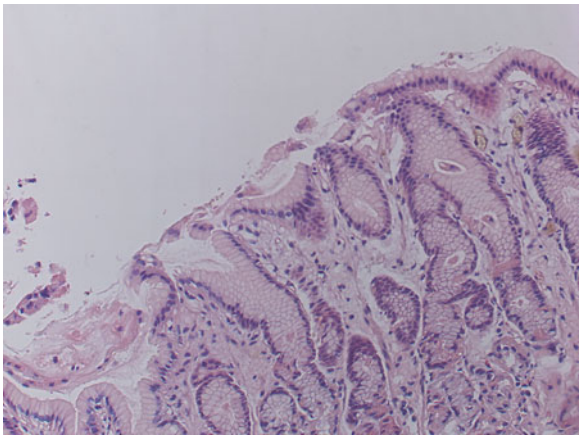

Fig. 1 Normal gastric mucosa (H & E 300× kindly supplied by Dr. Kevin West)

Other major risk factors for gastric cancer independent of *H. pylori* include pernicious anaemia, Epstein-Barr Virus (EBV) and previous gastric surgery. The following discussion relates to the complex interrelationship between *H. pylori*, inflammation and other associated risk factors in the pathogenesis of gastric adenocarcinoma.

2 Pathogenesis

Prior to the discovery of *H. pylori* it was well recognized that gastric inflammation and hypochlorhydria were associated with gastric cancer. Careful biopsy follow-up studies in high-risk populations had demonstrated the slow development of gastric atrophy over years and identified the importance of gastric body atrophy and intestinal metaplasia as risk factors for gastric cancer development (Siurala et al. 1966). Correa et al. (1975) developed the hypothesis that gastric cancer development was a slow and complex multistep process.

Fundamental to the model was the progression from normal gastric mucosa to non-atrophic gastritis, multifocal atrophic gastritis,and then the development of intestinal metaplasia and dysplasia before invasive cancer (Figs. 1, 2, 3, 4, 5, 6) (Correa et al. 1975).

Helicobacter pylori gastritis is fundamentally the host immune response to the bacteria with neutrophils, lymphocytes and macrophages. Despite the pronounced host humoral and cell-mediated response, colonisation persists for decades. The immune response is thus ineffective, raising issues regarding immune evasion (reviewed by Wilson and Crabtree 2007). Although *H. pylori* is the cause of the initial gastric inflammation only a tiny proportion of infected individuals will ultimately progress to cancer. An important determinant of cancer risk relates to the phenotype of the *H. pylori* gastritis. Subjects with an antral predominant gastric

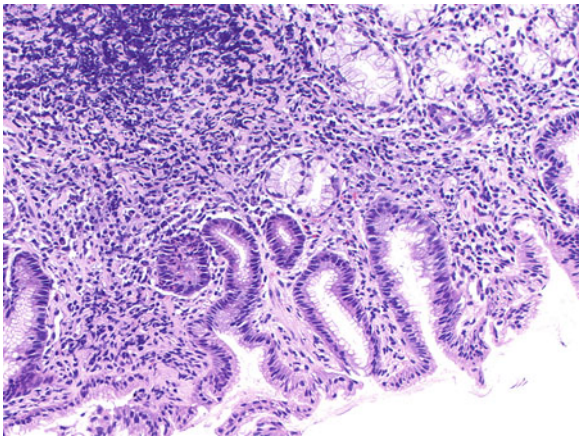

Fig. 2 *H. pylori* gastritis (H & E 300× kindly supplied by Dr. Kevin West)

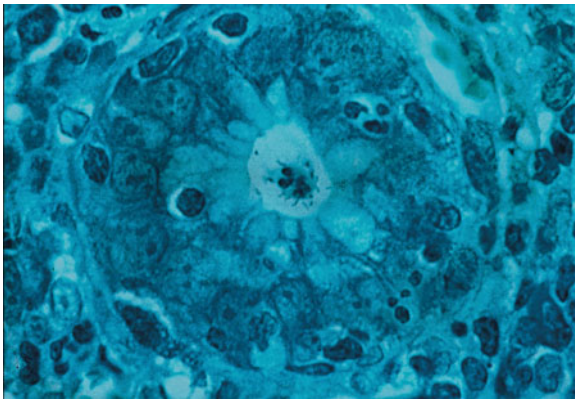

Fig. 3 *H. pylori* and polymorpholeukocyte in gastric pit (modified Giemsa stain 600× kindly supplied by Dr. Judy Wyatt)

inflammation maintain acid secretion and are not at risk of gastric cancer but are at risk of duodenal ulcer disease. Those individuals who develop an *H. pylori* pangastritis are at risk of developing multifocal atrophy, reduced acid secretion and an increased risk of gastric cancer. With decreasing acid secretion *H. pylori* colonisation may itself be lost.

Prior to the discovery of *H. pylori*, environmental factors were considered of utmost importance for gastric cancer pathogenesis. With the initial discovery of *H. pylori* the role of the bacterium took prominence. Recent studies have emphasised the importance of bacterial, host and environmental factors, and their complex interrelationship.

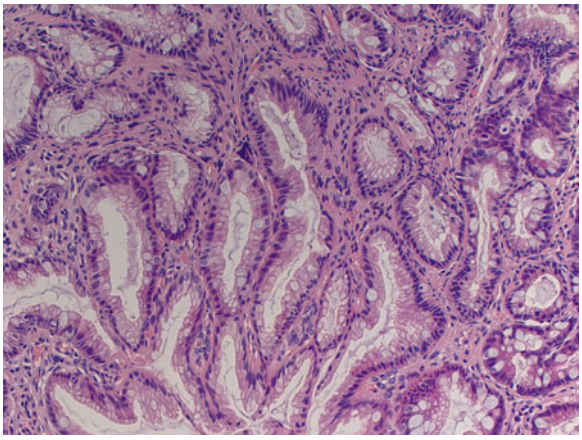

Fig. 4 Gastric intestinal metaplasia (H & E 300× kindly supplied by Dr. Kevin West)

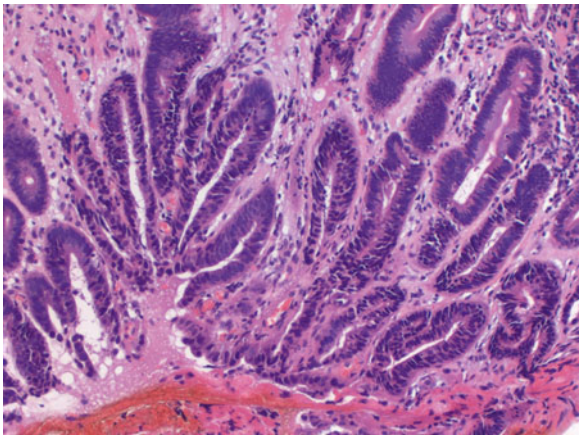

Fig. 5 Gastric low-grade dysplasia (H & E 300× kindly supplied by Dr. Kevin West)

2.1 Bacterial Factors

There is considerable heterogeneity in terms of *H. pylori* strains. A number of putative virulence factors have been identified and recently reviewed (Amieva and El-Omar 2008). Currently most interest centres on membrane proteins related to adherence, the cytotoxin associated gene A (CagA) and the cytotoxin VacA.

CagA is a 120–130 kDa protein initially identified by studies investigating the gastric humoral response to *H. pylori*. Only a proportion of colonised subjects had an immune response to this protein and the presence of such a response was associated with the degree of inflammatory activity and mucosal damage (Crabtree et al. 1991). Many studies have subsequently demonstrated that subjects with CagA positive

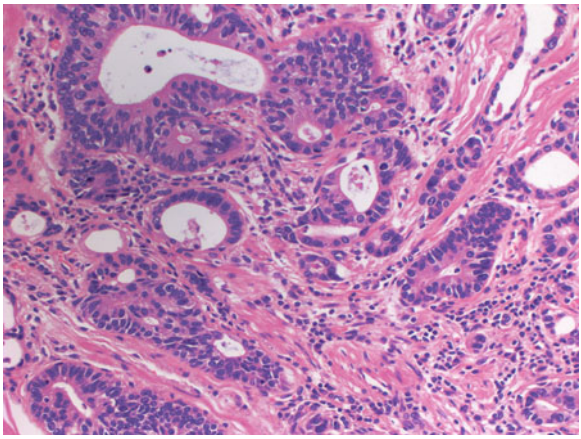

Fig. 6 Intestinal type gastric adenocarcinoma (H & E 300× kindly supplied by Dr. Kevin West)

strains are more associated with peptic ulcer disease and gastric cancer. The CagA gene is part of a large pathogenicity island (cag PAI). Genes from this island encode the CagA protein and a type IV secretion system. Following bacterial adhesion the secretion system is responsible for the translocation of CagA bacterial protein into the host cell (Segal et al. 1999; Odenbreit et al. 2000). Once translocated into host cytoplasm, CagA has complex effects on the host cell both directly and following phosphorylation.

CagA phosphorylation occurs on tyrosine residuals at its five amino acid EPIYA (Glu-Pro-Ile-Tyr-Ala) repeat region by host cell kinases. The tyrosine phosphorylated CagA interacts with a number of host proteins which can activate the ERK/MAPK pathway (Keates et al. 1999; Ding et al. 2008). The effect is to alter cellular signalling, cell proliferation and differentiation, programmed death, cytoskeletal organisation, stress and inflammatory responses (Backert et al. 2001; Chang et al. 2006).

The N-terminus of unphosphorylated CagA can complex with several junction proteins (*E*-cadherin, ZO1 and JAM) resulting in disruption of the epithelial cell apical junction complex, loss of polarity and proinflammatory and mitogenic responses (Bagnoli et al. 2005; Amieva et al. 2003). This may include the development of intestinal metaplasia (Murata-Kamiya et al. 2007).

CagA is a complex protein associated with dramatic effects on epithelial cells and associated with an increased risk of gastric cancer, yet the majority of subjects colonised with CagA positive strains will never develop gastric cancer.

The *H. pylori* bacterial cytotoxin VacA was initially described by Leunk et al. (1988) who demonstrated that *H. pylori* broth supernatents caused vacuolisation of cultured cells. The VacA gene occurs in all strains but has considerable variability in terms of cytotoxin activity. The major variations occur in the VacA signal region (types s1 and s2), the mid region (types m1 and m2) and the more recently described intermediate region (i1 and i2) (Atherton et al. 1995; Rhead et al. 2007).

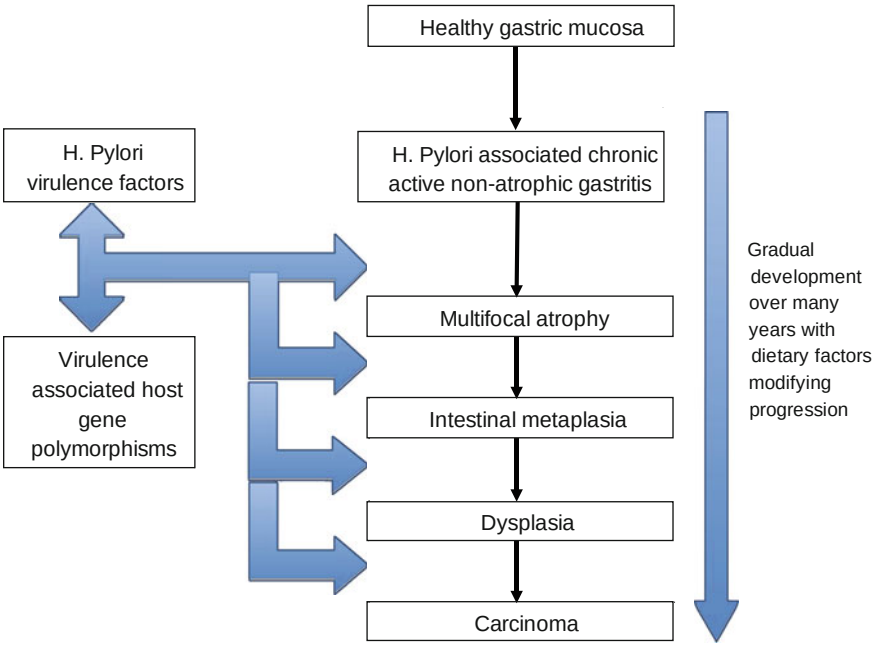

Fig. 7 Modified Correa gastric cancer pathway

When the toxin is secreted, approximately 50% remains associated with the bacterial cell surface and gains access to the epithelium with bacterial adherence (Ilver et al. 2004). The VacA toxin has multiple toxigenic properties. By affecting endosome maturation and function it causes cytoplasmic vacuolisation and impairment of antigen presentation (Molinari et al. 1998). Other effects include apoptosis, inhibition of T cell activation, disruption of tight junctions and cytochrome c release (Gebert et al. 2003; Galmiche et al. 2000; Papini et al. 1998; Willhite and Blanke 2004). The s1 m1 strains are the most toxigenic and the most associated with gastric cancer (Basso et al. 2008). Information on the intermediate type is limited although the i1 type has been associated with cancer risk in an Iranian population (Rhead et al. 2007).

Adherence seems a critical step with regard to *H. pylori* virulence with the highest density of adherence around intercellular junctions (Steer 1984). Several outer membrane proteins appear to act as adhesins. One of the most studied is BabA which is a 78 kDa outer membrane protein which binds through Lewis b blood antigens on the gastric epithelium (Ilver et al. 1998). Subjects expressing BabA have a higher density of colonisation which is associated with an enhanced mucosal inflammatory response (Rad et al. 2002). Two genes are associated with the encoding of BabA, although only BabA2 is functionally active. The expression of BabA2 is associated with the s1 VacA subtype and when associated with CagA it is associated with an enhanced risk of cancer formation (Gerhard et al. 1999).

The bacterial virulence factors described above are byzantine and intriguing in terms of benefit to the bacteria and cancer risk for the host. Adherence would appear to be a necessary prerequisite for significant mucosal damage. The BabA2, CagA and s1/m1 VacA virulence factors appear to act synergistically in promoting inflammation and the development of intestinal metaplasia (Zambon et al. 2003). An attempt to quantify the relative risk of the CagA and VacA bacterial factors confirmed the previously described CagA and VacA polymorphisms with disease but found that the single most important factor was the number of EPIYA-C segments and hence the magnitude of CagA phosphorylation (Basso et al. 2008).

The risk factors described above are all associated with an increased risk of gastric cancer and detailed studies are unravelling the mechanisms of increased risk.

Subjects colonised with the highest risk bacterial genotype are still unlikely to develop gastric cancer, again emphasising the multifactorial nature of this disease.

2.2 Host Factors

Helicobacter pylori colonisation is responsible for the increased mucosal expression of many cytokines, including several interleukins (IL-1β, IL-6, IL-8), tumour necrosis factor-α (TNFα) and interferon-γ (IFN-γ) (Calam 1999). These have all been described as affecting the parietal cells of the stomach, inhibiting acid secretion. There are multiple polymorphisms in inflammation-associated genes, for example in the IL-1 gene cluster that results in an increased expression of IL-1β, and in the TNF-A gene, resulting in increased expression of TNF-α (Kusters et al. 2006). Polymorphisms present in the interferon-γ receptor 1 (IFNGR1) gene have been found to increase host susceptibility to *H. pylori*-associated gastric cancer development (Canedo et al. 2008), and it was postulated that an increased receptor presence would result in increased number of proinflammatory cytokines. IL-8 is an important cytokine responsible for activating and attracting neutrophils and lymphocytes and for the induction of proinflammatory cytokines. The IL-8-251 gene polymorphism is associated with increased IL-8 production in *H. pylori*-associated gastric mucosa with an increased risk of premalignant change (Li et al. 2010). The presence of three or four proinflammatory gene polymorphisms in association with *H. pylori* infection has been shown to increase the risk of gastric cancer substantially (El-Omar et al. 2003).

In addition to the cytokine polymorphisms described above, other polymorphisms have been associated with an increased risk of gastric cancer in association with *H. pylori*. These include polymorphisms of the Toll-like receptor TLR4, a type I transmembrane protein expressed in the gastric epithelium which acts as a lipopolysaccharide (LPS) receptor and activates proinflammatory pathways. Certain polymorphisms in TLR4 have been shown to increase the risk of premalignant and malignant change in *H. pylori* colonised individuals (Hold et al. 2007; Hishida et al. 2009). The gene selenoprotein S (SEPS1) encodes a protein in

the endoplasmic reticulum which protects cells from oxidative damage and has a role in cytokine release (Curran et al. 2005).

A promoter polymorphism which results in reduced expression of SEPS1 has recently been associated with gastric cancer in a Japanese population (Shibata et al. 2009).

There is increasing interest in the potential role of bone marrow derived cells (BMDCs) as cancer stem cells in gastric neoplasia, reviewed by Correa and Houghton (2007). The bacterial and host factors resulting in ongoing inflammation and atrophy theoretically produce a situation where local stem cells are depleted. Mouse studies have demonstrated tissue stem cell depletion with ongoing inflammation and injury, and recruitment of bone marrow cells into the tissue stem cell niche. With continued inflammation and injury the BMDC-derived gastric mucosa developed metaplasia and dysplasia and subsequent invasive neoplasia (Houghton et al. 2004). The relevance and implications of these studies for human disease are as yet unclear.

2.3 Environmental Factors

Similar to other parts of the GI tract, the stomach has prolonged contact with ingested food material. The varying rates of gastric cancer geographically and chronologically have promoted an interest in the potential role of diet. Characteristically populations at high risk of gastric cancer have diets low in animal fats and proteins, low in fresh fruit and vegetables, high in starches and carbohydrates and high in salt and nitrates (Judd 1988). Particular interest has been paid to N-nitroso compounds as these have been found to induce tumours in the glandular stomachs of experimental animals and to the antioxidant vitamin C (ascorbic acid) as a potential protective factor.

Dietary N-nitroso compounds are typically found in cured meat, fish and beers, and have been associated with populations at an increased risk of gastric cancer (Judd 1988). There is also the potential for the formation of N-nitroso compounds in the stomach itself with gastric nitrate-reducing bacteria acting on dietary nitrates. *H. pylori* is not nitrate reducing, but if the *H. pylori* related inflammation involves the gastric body and results in decreased acid secretion, progressive atrophy can occur, raising the gastric pH. In these circumstances a range of other bacteria can colonise the stomach including nitrate-reducing bacteria and as a result higher levels of potentially carcinogenic N-nitroso compounds can be detected in gastric juice.

The recent EPIC-EUROGAST study demonstrated an association between endogenous formation of N-nitroso compounds and gastric cancer risk (Jakszyn et al. 2006). Paradoxically as the gastric pH rises, *H. pylori* colonisation is often lost, which could be due to a less favourable environment or competition from other bacteria. In practice *H. pylori* is absent from the stomach in most patients at the time when their cancer is diagnosed, emphasising the potential for varying factors to be important at different points in the natural history of gastric cancer.

Dietary ascorbic acid as an antioxidant has the potential to reduce carcinogenic *N*-nitrosamine formation in the stomach. Studies looking at gastric juice vitamin C levels demonstrated higher concentrations than would be expected from simple oral intake. These high levels are due to active gastric secretion of vitamin C. The mechanism for the secretion is unclear but like acid secretion it decreases with inflammation and atrophy (Rathbone et al. 1989; Sobala et al. 1989). Furthermore as the gastric luminal pH rises the proportion of the vitamin C available in its active form decreases thus increasing the risk of potentially carcinogenic N-nitroso compounds formation.

Another often overlooked environmental factor for gastric cancer is smoking with the EPIC study estimating that 17.6% of gastric cancer is related to smoking (González et al. 2003). A study looking at *H. pylori* status and smoking demonstrated a strongly increased risk of gastric cancer in those patients who were infected with CagA positive *H. pylori* and smoked, although the mechanism is unclear (Brenner et al. 2002).

3 Treatment

Both gastric cancer incidence and *H. pylori* infection rates are declining in many countries with the most probable reasons relating to improved living conditions. Important factors for this almost certainly include improved sanitation, water quality, food hygiene and a reduction in smoking. Despite the well-described association of gastric cancer risk with diets poor in vegetables and fruit, related intervention studies have failed to show a convincing benefit (Forman and Burley 2006).

A Cochrane analysis of randomised trials of anti-oxidant supplementation failed to demonstrate any evidence for a preventative effect against gastric cancer (Bjelakovic et al. 2008).

One of the sad facts regarding gastric cancer is the poor improvement in survival figures with treatment over the past 50 years. Clinical symptoms of gastric cancer generally occur late when the tumour is advanced and treatment options are limited. In the USA, Europe and China survival rates are only 20–25% whereas in Japan the equivalent survival rate is 52% (Parkin 2001). This difference is largely attributable to Japanese screening programmes to detect early disease. The current position regarding different treatment modalities has recently been well reviewed by Hartgrink et al. (2009).

With the important role *H. pylori* has in the early stages of the pathogenesis of gastric cancer it is compelling to believe that eradication programmes would be beneficial. The major problem here is that approximately 50% of the world's population is infected with *H. pylori*, and only a tiny minority of these are at risk of gastric cancer. Certainly in low-risk populations such as the UK and most of Europe, broad-based screening and treatment programmes are unlikely to be cost effective. In high-risk populations such as Japan and Columbia the cost benefit ratios are much better and the Asia–Pacific consensus guidelines on gastric cancer

prevention recommend screening and eradication in high-risk populations (Fock et al. 2008).

Current treatment regimens are not perfect with major problems regarding drug resistance which dramatically alters eradication rates. Where population treatment intervention studies have been carried out the results have been conflicting (Fuccio et al. 2007; Ito et al. 2009). For those with established atrophy and intestinal metaplasia, eradication is not so reliable in reducing cancer risk and endoscopic-histological screening is necessary to detect progression. This is demonstrated in a study on early gastric cancer patients treated by endoscopic mucosal resection and then treated with *H. pylori* eradication or placebo. At three years 3.3% of the eradicated patients had developed a metachronous gastric cancer compared to 8.8% of the placebo-treated patients (Fukase et al. 2008).

Clearly, advances in treatment regimens may alter the cost effectiveness of potential population treatment strategies but the development of an effective vaccine would be the best option for the future, but concerns remain regarding *H. pylori*'s immune evasion.

4 Conclusion

Gastric cancer remains a major health problem and is paradigmatic of a cancer which develops from inflamed mucosa. There is a clear pathway of development with many different factors operating. Despite a steady decline in the western world it remains a very significant cause of cancer deaths worldwide. Our understanding of the pathogenesis as described above has improved dramatically over the past thirty years however this has not translated into improved outcomes as yet.

The Correa pathway was an important concept in understanding the stepwise histological progression leading to invasive gastric cancer. Since this was described, the most fundamental breakthrough was the discovery of *H. pylori* and its role in causing histological chronic gastritis. Intensive research across the world has uncovered an astonishingly complex host–bacterial interaction and identified a number of interrelated factors pertinent to cancer risk (Fig. 7). The presence of *H. pylori* causes chronic mucosal inflammation early in the pathway. In the stepwise histological progression there appears to be a point of no return after which *H. pylori* plays no role and indeed colonisation is often lost. Eradication of or vaccination programmes against *H. pylori* in high-risk populations potentially offer a reduction in gastric adenocarcinoma and therefore the worldwide burden of cancer.

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Inflammatory Bowel Disease and Colon Cancer

Noor Jawad, Natalie Direkze and Simon J. Leedham

Abstract

The inflammatory bowel diseases (IBD); Crohn's and Ulcerative colitis, result from an altered host response to intestinal flora. Recurrent inflammation with ulceration and tissue restitution confers an increased risk of cancer in both UC and Crohns, and genome wide searches have identified a number of disease susceptibility alleles. The carcinogenesis pathway in colitis-associated colorectal cancer (CACRC) is less clearly understood than it's sporadic counterpart. Clonal ordering experiments have indicated the order and timing of chromosomal instability and common genetic mutations. Epigenetic changes such as DNA methylation and histone modification are thought to play an increasingly important role in inflammation induced carcinogenesis. Clonal expansion of procarcinogenic mutations can lead to large fields of mutant tissue from which colitis associated cancers can arise (field cancerisation). Endoscopic screening is the mainstay of surveillance in high-risk patients although the development of appropriate, clinically applicable biomarkers remains a research priority. Despite the expanding field of biological therapy in inflammatory bowel

N. Jawad
Blizard Institute of Cell and Molecular Science,
Bart's and The London School of Medicine and Dentistry,
Queen Mary, University of London,
London, UK

N. Direkze
Frimley Park Hospital NHS Foundation Trust, Portsmouth Road,
Frimley, Surrey, GU16 7UJ, UK

S. J. Leedham (✉)
Wellcome Trust Centre for Human Genetics, University of Oxford,
Roosevelt Drive, Oxford, OX3 7BN, UK
e-mail: simon.leedham@cancer.org.uk

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disease the ASA compounds remain the best-studied and most efficacious chemopreventive agents. Colitis associated CRC appears to have a different aetiology, carcinogenesis pathway and clinical course to its sporadic counterpart. Further research including long-term follow up of patient cohorts taking biological therapies will improve the detection and treatment of these important, inflammation-induced malignancies.

Contents

1 Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) both have a prevalence of two per 1,000 people in northern Europe, with an incidence of 10 and 6 per 100,000 people per year, respectively, in Western countries (Shivananda et al. 1996). Clinically the conditions are characterised by chronic, relapsing inflammation affecting the colon only in UC or any portion of the gut in CD. The aetiology of these conditions is unclear, but it is often presumed that they result from an altered host response to normal intestinal flora.

Inflammatory bowel disease (IBD) confers a high risk of development of a number of malignancies especially colorectal cancer, with a standardised incidence ratio of 2.4 (95% CI 0.6–6.0) in patients with extensive or pan UC. This risk is associated with longer disease duration, an earlier age of onset (Ekbom et al. 1990) the greater the severity of inflammation (Rutter et al. 2004), and the presence of concomitant inflammatory conditions such as primary sclerosing cholangitis (PSC). This suggests that the acquired cancer risk is a consequence of the inflammatory process which results in cycles of recurrent ulceration and tissue restitution. It is now accepted that the cancer risk in both Crohn's disease and UC is approximately

the same if similar disease patterns are compared (Gillen et al. 1994), and this is further evidence for similar inflammation-related tumour biology.

2 Genetic Epidemiology of IBD Aetiology

Genetic and environmental factors both appear to be important in the development of IBD. At least 13 genome-wide linkage studies have been completed since 1996, but the results have varied widely. The most frequently reported linkages were designated the IBD loci (Table 1).

A recent genome-wide association study (GWAS) of UC has recently identified susceptibility loci that provides the first genetic link between UC and colorectal cancer (Barrett et al. 2009). The strongest new association intervals include *CDH1* on chromosome 16q22, which encodes E-cadherin, a transmembrane glycoprotein and one of the main components of the adherens junction. It is a key mediator of intercellular adhesion in the intestinal epithelium and also plays a key role in epithelial restitution and repair following mucosal damage. The observation of correlated association signals at the *CDH1* locus in both colorectal cancer and UC is significant.

This is the first time that variants within genetic loci encoding epithelial barrier genes have shown association with IBD at rigorous genome-wide significant thresholds and provide further evidence for the re-emerging concept that altered epithelial barrier function and may be a strategic factor in UC pathogenesis. Additional fine mapping and functional studies are undoubtedly necessary to explore this association; however this study provides strong scientific rationalisation for the investigation of novel therapeutic targets pertinent to epithelial barrier function.

2.1 Genetic Variation in Inflammation-Related Genes

Associations between CRC and genetic variation in genes involved in inflammation-related pathways provide support to the mounting body of evidence that suggests inflammation-related pathways are important in the aetiology of CRC. Of potential importance are genes such as IL6, which is a critical regulator of inflammation signalling (Slattery et al. 2007). Polymorphisms in the IL6 gene promoter have been found to be associated with levels of circulating C-reactive protein, an important biomarker for pro-inflammatory status in several diseases (Ferrari et al. 2003). A study by Slattery et al. (2007) suggests that IL6 genotype may influence risk of CRC. Individuals with the C allele of the c.572G > C SNP and the GG genotype for the c.174G > C polymorphism were at a slightly reduced risk of colon cancer, but possibly at a slightly increased risk of rectal cancer. Associations were comparable for men and women and for all age groups and appeared to be modified by use of aspirin or non-steroidal anti-inflammatory drugs

Table 1 Genetic aetiology of IBD

IBD locus	Chromosome location	Condition	Candidate genes	References
IBD1	16q12	CD	*NOD2/CARD15*	Hugot et al. (1996)
IBD2	12q13	UC	*VDR*, *IFN-γ*	Satsangi et al. (1996)
IBD3	6q13	CD, UC	*MHC I*, *II*, *TNF-α*	Hampe et al. (1999)
IBD4	14q11	CD	*TCR a/d* complex	Ma et al. (1999)
				Duerr et al. (2000)
IBD5	5q31–33	CD	*IL-3*, *-4*, *-5*, *-13*, *CSF-2*	Rioux et al. (2000)
IBD 6	19p13	CD, UC	*ICAM-1*, *C3*, *TBXA2R*, *LTB4H*	Rioux et al. (2000)
Other loci (pre GWAS)	1p36	CD, UC	*TNF-R* family, CASP9	Cho et al. (1998)
	7q	CD, UC	*MUC-3*	Satsangi et al. (1996)
	3p	CD, UC	*HGFR*, *EGFR*, *GNA12*	Satsangi et al. (1996)
	8q	CD	*Beta 2 defensins*	Fellermann et al. (2006)
GWAS 2008	Multiple loci	UC, CD	*IL23R*, *IL12B*, *HLA-DQ/DR*, *NKX2-3*, *MST1*	Fisher et al. (2008)
GWAS 2008	Multiple loci	UC, CD	*HERC2*, *CCNY*	Franke et al. (2008)
		UC	*STAT3*, *PTPN2*	
GWAS 2009	Multiple loci	UC	Multiple genes including *HNF4A*, *CDH1*, *LAMB1*	Barrett et al. (2009)
GWAS 2009	Multiple loci	UC	Genes including *IL-27*, *SULT1A1*, *SULT1A2*, *EIF3C*	Imielinski et al. (2009)

(continued)

Table 1 (continued)

IBD locus	Chromosome location	Condition	Candidate genes	References
GWAS 2009	Multiple loci	UC	Multiple genes including: *FCGR2A*, *SLC26A3*, *INSL6*, *INSL4*, *JAK2*	Asano et al. (2009)

Summary table of the major IBD susceptibility loci detected by linkage and genome-wide association studies since 1996. Abbreviations—*IL23R*—interleukin 23 receptor; *IL-12B*—interleukin 12 B (natural killer cell stimulatory factor 2, cytotoxic lymphocyte maturation factor 2, p40; *HLA DQ/DR*—human leucocyte antigens DQ/DR; *NKX2-3*—NK2 transcription factor related, locus 3; *MST1*—macrophage stimulating 1 (hepatocyte growth factor-like); *HERC2*—hect domain and RLD2; *CCNY*—cyclin Y; *STAT3*—signal transducer and activator of transcription 3 (acute phase response factor); *PTPN2*—protein tyrosine phosphatase, non-receptor type 2; *HNF4A*—hepatocyte nuclear factor 4, alpha; *CDH1*—cadherin 1, type 1, E-cadherin (epithelial); *LAMB1*—laminin, beta 1; *IL-27*—interleukin 27; *SULT1A1*—sulfotransferase family, cytosolic 1A, phenol-preferring, member 1; *SULT1A2*—sulfotransferase family, cytosolic 1A, phenol-preferring, member 2; *EIF3C*—eukaryotic initiation factor 3, carrier subunit C; *FCGR2A*—Fc fragment of IgG, low affinity IIa, receptor (CD32); *SLC26A3*—solute carrier family 26, member 3; *INSL6*—insulin-like 6; *INSL4*—insulin-like 4 (placenta); *JAK2*—Janus-kinase 2

(NSAIDs): especially for colon cancer (Macarthur et al. 2005). In addition, if users had a C allele in either IL6 polymorphism, they had a greater reduction in risk of colon cancer (Slattery et al. 2007). Although these data are supportive of genetic involvement in an inflammation-related pathway, additional work is necessary that will encompass more genes in this pathway to obtain a better understanding of the associations between inflammation and genetic factors and CRC development.

3 Comparison of Carcinogenesis Pathways in Sporadic and Colitis-Associated Colorectal Cancer

There are several distinguishing clinical features when comparing colitis-associated colorectal cancer (CACRC) to sporadic colorectal carcinoma (SCRC). Firstly, CACRC arises in a younger population, often from flat, not polyploid dysplasia and has a more proximal distribution. Furthermore, there is a greater frequency of mucinous or signet cell histology and a higher incidence of multiple synchronous lesions (Itzkowitz and Yio 2004). From a histological perspective, sporadic tumours tend to follow the adenoma-carcinoma sequence (Vogelstein et al. 1988), whereas CACRC progresses from no dysplasia to indefinite dysplasia, usually through low (LGD) and high-grade dysplasia (HGD) to carcinoma. The stepwise accumulation of genetic mutations in onco- and tumour suppressor genes that underpins the SCRC carcinogenesis pathway is well established and has significantly altered worldwide clinical practice (Vogelstein et al. 1988). The CACRC carcinogenesis pathway is less explored and significantly differs in the requirement and timing of genetic and epigenetic alterations (Fig. 1).

3.1 Genetic Instability

3.1.1 Chromosomal Instability

In sporadic cancer carcinogenesis, chromosomal instability leading to aneuploidy, detectable by both image and flow cytometry, is rare in established precursor lesions before the development of high-grade dysplasia or cancer (Sieber et al. 2002). Yet, in ulcerative colitis, chromosomal instability (CIN) can be detected in histologically non-dysplastic tissue from high-risk patients (extensive disease distribution and long duration of disease), by comparative genomic hybridisation (Willenbucher et al. 1997), image (Keller et al. 2001) or flow cytometry and is thought to precede the development of dysplasia in these patients (Rubin et al. 1992; Lofberg et al. 1992; Befrits 1994). It has been suggested that CIN occurs as a consequence of the effect of inflammation and reactive oxygen species encouraging telomere shortening, permitting chromosomal end fusion. This results in cycles of chromatin bridge breakage and fusion, promoting the accumulation of chromosomal aberrations (O'Sullivan et al. 2002).

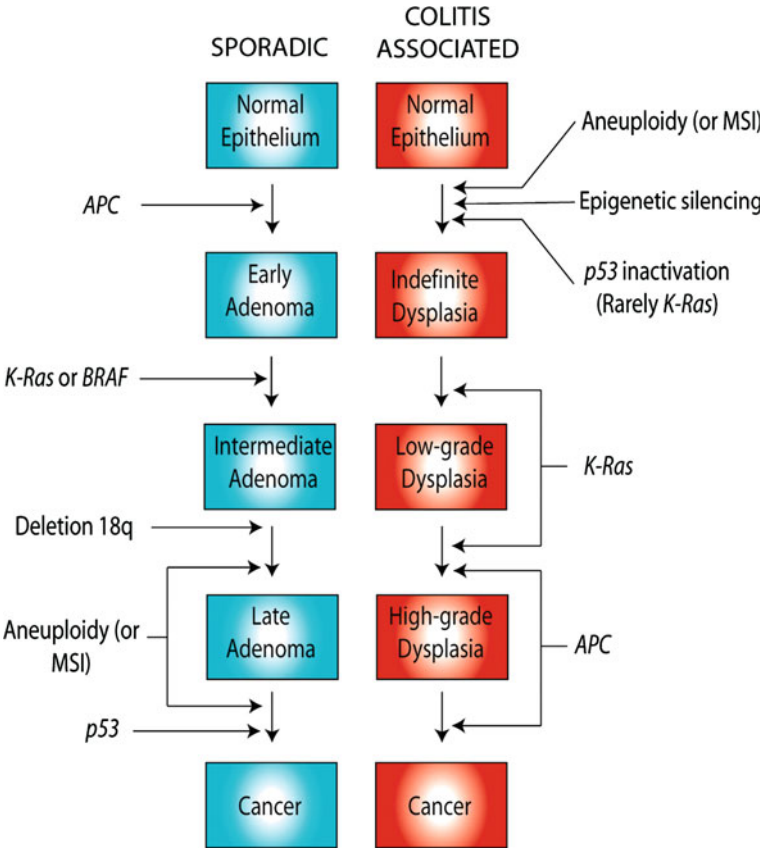

Fig. 1 *Comparison of Colitis Associated and Sporadic Colorectal Cancer Pathways.* Both types of cancer show multistep development with sequential mutation in tumour suppressor and oncogenes. The main differences between the pathways are in the timing of these mutations. Abbreviations: *APC*, *Adenomatous Polyposis Coli*; *DCC*, *Deleted in Colon Cancer*; LOH, loss of heterozygosity; MSI, Microsatellite instability

3.1.2 Initiating Genetic Mutations

In sporadic CRC carcinogenesis, mutations in *APC* are found in about 60% of sporadic adenomas and 80% of tumours (Powell et al. 1992) and are considered to be the gate-keeping, initiating mutations (Kinzler and Vogelstein 1996). It is now becoming clear that the inflammation and restitution processes that underly IBD, select for alternative initiating genetic mutations in CACRC. A recent clonal ordering study determining the spatial distribution of shared mutations in UC-associated neoplasia allowed insight into the timing of genetic mutations (Leedham et al. 2009). *p53* was the most common single founding mutation with *K-RAS* mutations as the only other detected unique gate-keeping mutation. *APC*

mutations were uncommon suggesting that *APC* is unlikely to have a gatekeeper function in colitis. This is consistent with other work. Point mutations in the *p53* gene can be detected in non-dysplastic tissue from patients with UC preceding the development of aneuploidy and LOH, and appear to be linked to the presence of inflammation (Brentnall et al. 1994; Hussain et al. 2000). Additionally, LOH for *p53* correlates with malignant progression, occurring in 6% of non-dysplastic biopsies, 33% of LGD, 63% of HGD and 85% of cancers (Burmer et al. 1992). The mutation spectrum in *p53* is dominated by transition mutations (Yin et al. 1993; Hussain et al. 2000; Yoshida et al. 2003), and this is likely to reflect the effect of the inflammatory process causing oxidative DNA damage and deamination of 5-methylcytosine, promoting G:C to A:T transitions (Hussain et al. 2000; Seril et al. 2003). It is simple to comprehend why *p53* may act as an initiating mutation in colitis. If underlying chromosomal instability throughout the colon is the main tumourigenic driving force in colitis (Chen et al. 2003, 2005), early *p53* would be selected for, on the basis that disruption of a mitotic checkpoint would permit the survival and selection of clones with gross chromosomal changes.

3.2 Role of Inflammation in Cancer Epigenetics

One of the many potential processes by which inflammation can contribute to carcinogenesis includes alterations in epigenetic events and subsequent inappropriate gene expression.

3.2.1 DNA Methylation and Transcriptional Silencing

CpG island hypermethylation often starts in normal mucosa as a function of age and is markedly increased in cancer (Issa et al. 2001). Such silencing is clonal and is thought to be physiologically irreversible in somatic cells. Neoplastic cells often display aberrant promoter region methylation with epigenetic silencing of multiple genes including genes that regulate critical processes such as cell cycle control, DNA repair and angiogenesis. In the colon, CpG islands methylated in cancer have been divided into two groups: those that display cancer-restricted methylation (type C), and those that are methylated initially in aging normal epithelial cells (type A). It has been proposed that age-related methylation contributes to an acquired predisposition to colorectal neoplasia because methylation alters the physiology of aging cells and tissues (Issa et al. 2001). This hypothesis predicts that higher levels of age-related methylation are associated with a heightened susceptibility to developing colorectal cancer, and it may be present in conditions of rapid cell turnover that mimic premature aging such as IBD.

Issa et al. (2001), investigated the methylation status of 4 genes in patients with UC versus controls *(ER*, *MYOD1*, *CSPG2* and *p16*). All four genes were highly methylated in dysplastic epithelium from patients with colitis-associated HGD or cancer. In addition, three of the four genes (*ER*, *MYOD* and *p16*) were also highly methylated in the normal appearing (non-dysplastic) epithelium from these same HGD/cancer patients, indicating that methylation precedes dysplasia and is

widespread in these patients. These results are consistent with the hypothesis that age-related methylation marks (and may lead to) the field defect that reflects acquired predisposition to colorectal neoplasia. More recently, Kukitsu et al. (2008) identified hypermethylation and subsequently reduced *p16* gene expression in aberrant crypt foci (ACF) in UC. These are the earliest detectable lesions in the CACRC pathway and suggest that aberrant methylation of tumour suppressor genes may be an early event in CACRC carcinogenesis.

3.2.2 Histone Modification

A well-proven epigenetic mechanism of gene expression control involves chromatin remodelling via histone modification. Transcriptional regulation of a variety of cancer-related genes are controlled by two contrasting classes of enzymes—histone deacetylase (HDAC) and histone acetyl transferases (HATs). The acetylation of lysine residues on the N-terminus of histones by HATs activates gene transcription, while removal of an acetyl group from lysine residues in histone tails by HDACs results in transcriptional repression. Therefore, HDACs and HATs, in general, act as transcriptional co-repressors and co-activators, respectively. In chronic inflammatory responses and carcinogenesis the inappropriate activation/inactivation of HDACs and HATs has been implicated. In a study by Cao et al. (2007), the exposure of human bronchial epithelial cells (BEAS-2B) to the diesel exhaust particulate matter induced the transcriptional activation of a representative pro-inflammatory gene cyclooxygenase-2 (COX-2) by promoting acetylation of histone-4 by degradation of HDAC-1. Similarly, the activation of NF-κB and expression and release of IL-8 and IL-6 in human alveolar (A549) cells by H_2O_2 were associated with augmented acetylation of histone-4 and diminished expression and activity of HDAC-2 (Cao et al. 2007). Thus histone modification and the ensuing upregulation of COX-2 and NF-κB demonstrates that inflammation induced modification in cellular epigenetic apparatus may also contribute to the genetic instability of cancer cells.

4 Mutator Phenotype versus Clonal Expansion

Multiple aneuploidy detection techniques have shown gross chromosomal changes occurring in non-dysplastic tissue in UC (see Sect. 3.1). Chen et al. (2003, 2005) used arbitrarily-primed (AP-PCR) and inter-simple-sequence repeat PCR (ISSR-PCR) genetic fingerprinting techniques to further analyse genomic instability in colitis. The identification of DNA fingerprint abnormalities throughout normal and dysplastic areas of the colon allowed the subdivision of patients with IBD into UC progressors: patients with identifiable genomic instability who are likely to progress to dysplasia or cancer, and UC non-progressors, patients with normal DNA fingerprints who are not (Chen et al. 2003). The authors proposed that this colon-wide genomic instability in UC progressors provides a field from which dysplasia develops, and is evidence of a mutator phenotype where mutations in genes maintaining genetic stability result in an increased mutation rate driving

colitis-associated tumourigenesis (Chen et al. 2003, 2005; Loeb and Loeb 1999). This is a controversial subject and proponents of an evolutionary theory of carcinogenesis argue that the mutator phenotype theory underestimates the power of natural selection (Tomlinson and Bodmer 1999; Bodmer 2008). The recent identification of colitis-associated neoplasia clonality with *p53* as the commonest initiating mutation (Leedham et al. 2009) lends weight to a Darwinian model where natural selection and clonal expansion are the dominant forces driving CACRC evolution—the somatic mutation theory of carcinogenesis. The close association between the cell cycle and DNA repair suggests that a number of genes involved in the cellular response to DNA damage, such as *p53* may have a two-fold responsibility in controlling DNA repair and growth. Consequently mutations in these genes may provide both a selective growth advantage and an increased mutation rate driving selection and the mutator phenotype simultaneously, although evolutionary geneticists argue that the mutator phenotype component is a coincidental by-product of direct selection of mutation of these genes for their anti-apoptotic effects (Bodmer 2008). The debate continues!

5 Field Cancerisation

The term field cancerisation was proposed by Slaughter et al. (1953) to explain the presence of multifocal head and neck cancers developing out of a field of pre-cancerous change that had developed following carcinogen exposure. Braakhuis et al. (2003) expanded this theory and proposed that the field was actually a clonally expanded area of mutated cells. Clonally expanded mutated patches have been documented previously in dysplastic and phenotypically normal mucosa of colitis patients (Lyda et al. 1998, 2000). Leedham et al. (2009) identified field cancerisation in one interesting patient when they demonstrated that three left-sided tumours and some of the intervening chronically inflamed but phenotypically non-dysplastic mucosa shared the same founder mutation, suggesting widespread clonal expansion of a progenitor clone from which the three spatially independent tumours arose. Niche succession and crypt fission are likely to be the mechanisms behind clonal expansion in CACRC. Occasional symmetrical division of individual crypt stem cells results in the extinction or amplification of one cell lineage (Kim and Shibata 2002). This process will occur faster if the mutation provides a growth advantage. Crypt fission has been shown to be responsible for the spread of individual clones into daughter crypts in the colon (Greaves et al. 2006) and this process is a histological feature of colitis and dysplasia (Park et al. 1995). Chen et al. (2005) used a fluorescent in situ hybridisation technique to demonstrate the spread of p53 mutations into the daughter crypts of a crypt in the process of fission in UC. The suggestion of field cancerisation in this condition has possible clinical implications, raising questions about the use of molecular genetic analysis of non-dysplastic tissue in high-risk cancer patients to detect fields from which future tumours may arise.

6 Screening and Detection

6.1 Endoscopic Screening

Early detection and screening is the mainstay of reducing cancer morbidity and mortality in the IBD population. As the risk of CRC is influenced by the extent and duration of the disease current European guidelines suggest an initial assessment colonoscopy 8–10 years after the onset of symptoms in UC (Moum et al. 1999). The development of PSC is an independent risk factor and patients should be offered yearly surveillance as soon as PSC is diagnosed. Many dysplastic lesions in colitis are flat rather than polypoid (Allen et al. 1985). These are more difficult to detect endoscopically, which leads us to the question of how many random biopsies to take to maximise the chance of detecting dysplasia? Current recommendations suggest that four biopsies should be taken every 10 cm with additional biopsies in strictured, raised or other abnormal areas of the colon; however this is time consuming for patients, nurses, colonoscopists and histopathologists. There are gradual moves towards a more focused approach to obtain targeted biopsies aided by the use of chromendoscopy with indigo carmine or methylene blue. This has shown to give a superior yield in the detection of dysplasia (Biancone et al. 2008; Eaden and Mayberry 2002; Winawer et al. 2003). The role of other methods of targeting biopsies—such as trimodal, autofluoresence and narrow band imaging are also being studied and may feature in future recommendations (East et al. 2006; van den Broek et al. 2008; Dekker et al. 2007).

6.2 Biomarkers

A biomarker is an indicator of a pathological process that may be measured or used to assess the response to therapeutic intervention. At present the histological detection of dysplasia in a biopsy sample is the only marker that has entered widespread clinical practice, and the detection of high-grade dysplasia is an indication for endoscopic resection or colectomy. The discomfort, difficulty and expense of obtaining histological samples mean that the development of a biomarker detectable in stool is a research priority. As yet studies on calprotectin (von Roon et al. 2007) and SFRP2 hypermethylation (Huang et al. 2007) from stool samples have failed to show the sensitivity and specificity required for clinical applicability.

7 Chemoprevention

Prevention is the best strategy to minimise the impact of cancer and may be theoretically achieved by good disease control and reduction of modifiable risk factors. A number of pharmacological agents have been proposed to have a chemopreventive role and these include 5-amino salicylic acid (5-ASA) compounds.

The efficacy of these agents may only be partially explained by anti-inflammatory effects of these drugs as other more potent anti-inflammatory agents such as glucocorticoids and immunomodulators such as azathioprine have a less significant cancer protective effect. Additional chemopreventative effects of 5-ASA compounds include; modulation of inflammatory cytokine production (Zimmerman and Jewell 1996), inhibition of cyclooxygenase (Allgayer 2003), inducible NO synthase (Hasko et al. 2001; Kennedy et al. 1999) and nuclear factor KB (Greten et al. 2004; Wahl et al. 1998) as well as activation of peroxisome proliferator activated receptor (PPAR) gamma (Dubuquoy et al. 2006; Rousseaux et al. 2005). In addition to this 5-ASA's scavenge oxygen free radicals and have an antimicrobial action (Swidsinski et al. 2005). 5-ASA compounds can also act as an inhibitor of protein phosphatase 2A—which can reduce the activity of the Wnt pathway (Bos et al. 2006). Although, theoretically these mechanisms could help to prevent cancer, there are no prospective randomised controlled trials to confirm the protective effect of 5-ASA in cancer chemoprevention in colitis. The best evidence to support their use comes from the meta-analysis by Velayos et al. (2008) that revealed a reduced risk of the development of cancer or dysplasia in UC patients on regular 5-ASA (pooled odds ratio of 0.51 (95% CI 0.38–0.69)).

8 Future Perspectives

8.1 Biological Therapies and Cancer

With the advent of the use of biological therapies, we have seen the medical management of IBD patients who are refractory to steroids and revolutionised immunomodulators (Rutgeerts et al. 2009). As yet there is no evidence to suggest that biologics offer any cancer chemoprevention. In fact, data from the British Society of Rheumatology Biologics Registry show that patients with pre-existing cancer have an increased risk of recurrence with the use of biologics, and those without pre-existing cancer have no increased incidence except in two cohorts—teenagers and young adolescents (in particular with the risk of hepatosplenic T-cell lymphoma) (Rosh et al. 2007). There remains many unanswered questions about the mechanism of action, appropriate time to use biologics and their long-term safety profile and as more long-term data emerges, our understanding of these novel therapies will expand.

8.2 Stem Cell Therapy

It is now appreciated that bone marrow-derived stem cells have a dynamic role in inflammation and cancer throughout the body. Bone marrow-derived cells contribute to myofibroblast populations in the colon and small intestine of mice and humans (Brittan et al. 2002) as well as in mouse models of colitis where they also contribute to vascular lineages (Brittan et al. 2005). Not only this, but also in the

IL-10 knock-out model, the colitis that develops can be ameliorated by transplantation of wild-type bone marrow (Bamba et al. 2006). Bone marrow has been shown to contribute to stromal cell populations in cancer (Direkze et al. 2004) and this may offer an alternative route to target therapies to control not only IBD itself but also CACRC, a finding that has been seen in mouse cancer models (Studeny et al. 2002; Nakamizo et al. 2005). In the human, case reports of amelioration of IBD in haematopoietic stem cell (HSC) transplant recipients for co-incident haematologic malignancy prompted interest in stem cell therapy for IBD (reviewed in (Lanzoni et al. 2008)). More recently adipose-derived mesenchymal stem cells have been successfully used in the treatment of refractory perianal fistulae (Garcia-Olmo et al. 2005) and a European-wide phase III trial on the effect of autologous stem cell transplantation in CD is underway (ASTIC trial). Whether the beneficial effect of stem cell therapy arises from concomitant immunosuppressive therapy or from a 'resetting' of the colonic stem cell niche remains to be seen.

There is increasing evidence that CACRC has a different aetiology, carcinogenesis pathway and clinical course to its sporadic counterpart. Genome-wide association studies have revealed new susceptibility loci and opened up new lines of investigation. The recognition that intestinal immune system dysregulation provokes chronic inflammation with resultant carcinogenesis has already shifted the focus of management of the IBD. The development of further biological treatments including stem cell therapy promises further tantalising insight into the pathogenesis of these, and other chronic inflammatory conditions.

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Primary Sclerosing Cholangitis

Piotr Milkiewicz and Ewa Wunsch

Abstract

Primary sclerosing cholangitis (PSC) is a chronic liver condition which may affect both intra and extrahepatic biliary tree. Etiology of PSC remains to be fully elucidated but genetic, autoimmune, inflammatory and possibly infective factors could all contribute to its development. More than two-thirds of patients are males and the most commonly associated condition is an inflammatory bowel disease which occurs in up to 70% of affected subjects. Endoscopic cholangiopancreatography (ERCP) and magnetic resonanse cholangiopancreatography (MRCP) remain a gold standard in the diagnosis of this condition. No curative treatment of PSC exists and a proportion of patients who develop liver failure or suffer from recurrent episodes of cholangitis requires liver transplantation. PSC is associated with increased risk of malignancies, in particular cholangiocarcinoma which may arise in 12% of patients. The main aim of this chapter is to review the current knowledge on pathogenesis and clinical aspects of PSC as well as its associated malignancies.

Keywords

Primary sclerosing cholangitis · Cholangiocarcinoma · Colorectal cancer

P. Milkiewicz (✉) · E. Wunsch
Department of Hepatology and Liver Transplantation,
M.Curie Hospital, Arkonska 4, 71-455 Szczecin, Poland
e-mail: p.milkiewicz@wp.pl

P. Milkiewicz
Liver Research Laboratories, Pomeranian Medical School,
Powstancow Wlkp. 72, 70-111 Szczecin, Poland

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Contents

1 Introduction

Sclerosing cholangitis comprises a group of chronic, progressive cholestatic liver diseases which may affect both intra and extrahepatic biliary tree. Secondary and rare causes of IgG4 related sclerosing cholangitis include portal bilopathy, sclerosing cholangitis, inflammatory hepatic pseudotumours, autoimmune pancreatitis, mast cell cholangiopathy or AIDS related cholangiopathy. The main focus of this chapter is primary sclerosing cholangitis (PSC), the commonest cause of sclerosing cholangitis. PSC is one of the most fascinating and challenging conditions in the contemporary hepatology. Its aetiology remains a mystery and although underlying immunological mechanisms play an important role, for many reasons PSC can not be called a typical autoimmune condition. In terms of diagnosis and treatment neither reliable serum markers to diagnose PSC exist nor is curative medical therapy available. Liver transplantation potentially cures the disease in many patients however the disease may recur after surgery even leading to graft loss. Significantly increased risk of both biliary and extrahepatic malignancies pose a real challenge in the management of this condition.

2 Epidemiology

Incidence of PSC seems to be higher in northern Europe and United States than in southern Europe or Asia. Unfortunately, the data on the epidemiology of PSC are scanty with only a few population-based studies existing in the literature. Boberg et al. (1998) in their study on Norwegian population found the mean annual incidence to be of 1.3/100000 and the point prevalence of 8.5/100.000. Bambha et al. (2003) who studied American population living in Minnesota showed an age-adjusted incidence of PSC of 1.25/100000 in males and 0.54/100.000 in females with the prevalence of 20.9/100.000 in males and 6.3/100000 in females. In another, population-based, Canadian study, Kaplan et al. (2007) found an annual incidence to be of 0.92/100.000 and more specifically for the small-duct variant of

PSC of 0.15/100000. A more recent study from the UK where the incidence rates during the period between 1991 and 2001 were analysed showed the rate of the disease to be 0.41/100000 person-years and the prevalence in 2001 of 3.85/100000. Authors found that the incidence of PSC over the analyzed period increased by 50% (Card et al. 2008).

3 Aetiopathogenesis

Without a doubt the aetiology of PSC is multifactorial with genetic, autoimmune, inflammatory and possibly infective factors all playing their role.

An increased prevalence of PSC has been found in first degree relatives (Bergquist et al. 2005) and recent, Swedish study on 678 patients with PSC showed the risk (with the hazard ratio and 95% confidence interval) of cholangitis in off spring, siblings and parents of these patients to be 11.5; 11.1 and 2.3, respectively when compared to the relatives of the control group (Bergquist et al. 2008).

HLA haplotypes B8 and DR3 which are both associated with autoimmune conditions occur with an increased frequency in patients with PSC. MICA genes are localised between HLA-B and TNFA in the MHC class I region and MCA*008 homozygocity showed the most significant association with PSC, reaching an odds ratio of 5.01 (Norris et al. 2001). It has also been postulated that haplotypes DRB1*0701, DRB1*0401 and MICA*002 may be related to decreased risk of PSC (Spurkland et al. 1999). With regard to non-MHC genes data are conflicting and almost as a rule are not reproducible (Kitiyakara and Chapman 2008).

An autoimmune background for PSC is suggested by its common coexistence with typical autoimmune conditions and inflammatory bowel diseases, predominantly ulcerative colitis. PSC is associated with various autoantibodies, including antinuclear antibodies (ANA), smooth muscle antibodies (SMA) or perinuclear-staining antineutrophil cytoplasmic antibodies (p-ANCA). They occur with various frequencies but are unlikely to be directly involved in the pathogenesis of this condition (Angulo et al. 2000).

A potential role of infection as a possible trigger of an immune-mediated inflammation has been considered since the late 1980's. Several studies have shown a high prevalence of various microorganisms obtained from either bile or bile ducts tissues (Kahana et al. 2003; Fox et al. 1998; Olsson et al. 1998; Kulaksiz et al. 2006). However, these organisms have been found mainly in patients with immunodeficiency syndromes or PSC-like secondary cholangitis. On the other hand Olsson et al. reported a significant prevalence of hepatobiliary infection in patients with PSC caused by colonic bacteria, predominantly α-haemolytic Streptococci (1998). A potential role of infectious agents can also be suggested by the fact that combined therapy with metronidazole and ursodeoxycholic acid (UDCA) improves both liver biochemistry and histology in patients with PSC (Farkkila et al. 2004).

Well known close linkage between PSC and chronic inflammatory bowel disease (IBD) led to the hypothesis that portal bacteremia associated with increased permeability of the inflamed colon could be a potential source of biliary inflammation. This hypothesis is strengthened by the works on animal models where hepatic injury similar to PSC were observed after intraportal injection of intestinal non-pathogenic bacteria (Kono et al. 1988) or in experimental small bowel bacterial overgrowth (Lichtman et al. 1990). These findings, however, do not explain why PSC occurs more frequently in patients with ulcerative colitis than with Crohn's disease and also human studies have not confirmed portal bacteraemia or portal vein phlebitis in patients with ulcerative colitis. However, recently published studies support the idea that intestinal permeability, secondary to IBD could play a role in the pathogenesis of PSC. According to this hypothesis, colonic bacteria or bacterial antigens can trigger ANCA formation and autoimmune reaction in the genetically susceptible host by molecular mimicry in a cross-reaction with human autoantigens (O'Mahony and Vierling 2006). In this context induction of bile duct inflammation neither require a direct microbial presence in the biliary ducts nor the portal bacteraemia. However, unlike in primary biliary cirrhosis (PBC) where ubiquitous, xenobiotic-metabolising bacteria, *Novosphingobium aromaticivorans* has been suggested as a source of molecular mimicry (Selmi et al. 2003), there is no conclusive evidence which bacterial antigens might cross-react with human autoantigens and thereby play the trigger's role of autoimmunity phenomena in PSC. Recently, the bacterial cell division protein FtsZ has been proposed as a potential antigen for p-ANCA in patients with PSC and AIH (Terjung and Spengler 2009).

In patients who undergo proctocolectomy for ulcerative colitis symptoms of PSC may occur many years later. This fact led to the hypothesis that PSC can be triggered by/through long-lived memory cells, primary recruited during active inflammation of the colon (Grant et al. 2002). These T lymphocytes undergo enterohepatic circulation and can cause an organ specific immune response. The described lymphocyte tropism is a result of the unique expression of many adhesion molecules restricted to the particular locations. It has been shown that there is an overlapping expression of mucosal addressin cell adhesion molecule-1 (MAdCAM-1) and vascular adhesion protein-1 (VAP-1) between the mucosal and hepatic endothelium, especially during episode of inflammation (Grant et al. 2001). It may suggest that mucosal memory cells may be able to enter the liver using both VAP-1 and MAdCAM-1 and, when activated by the particular stimulus or stimuli, can trigger the chronic liver inflammation even after resolution of colitis.

4 Clinical Features and Diagnosis

Up to 71% of patients are male. A significant proportion (between 21 and 44%) of affected subjects are asymptomatic at the diagnosis and their disease is diagnosed as a consequence of further investigations of accidentally found disturbance of

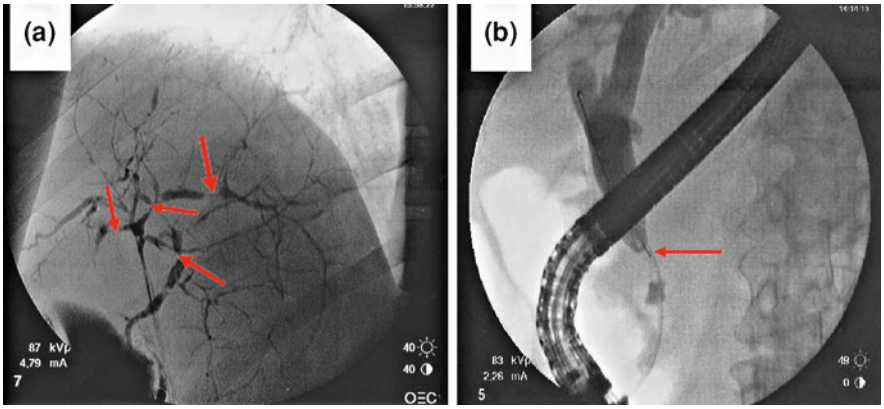

Fig. 1 **a** Primary sclerosing cholangitis with typical multiple stenoses seen in the biliary tree leading to a beaded pattern (*arrows*). **b** cholangiocarcinoma (CCA) of the common bile duct (*arrow*)

liver biochemistry. The most common complaints in those who are symptomatic are abdominal pain, icterus, skin itching and recurrent episodes of fever (Weismuller et al. 2008). The prevalence of chronic fatigue, an extremely troublesome symptom in chronic cholestasis, (Milkiewicz and Heathcote 2004) is controversial. Bjornson et al. found it to be lower than in general population (Bjornsson et al. 2004) whereas in most recent study Al-Harty et al. were able to detect it in more than 90% of patients (Al-Harthy et al. 2009).

Inflammatory bowel disease (IBD) is the most commonly associated condition which can occur in up to 73% of patients. Ulcerative colitis is diagnosed in majority of affected subjects and colonic Crohn's disease comprises between 1 and 14%. Among other associated diseases of an autoimmune background, most common are insulin dependent diabetes mellitus (IDDM), thyroid conditions and psoriasis.

Liver biochemistry demonstrates cholestasis with elevated alkaline phosphatase (ALP). Mild/moderate elevation of serum transaminases can also be commonly seen. Bilirubin levels tend to fluctuate and increase during episodes of cholangitis. In patients who developed liver cirrhosis features of impaired synthetic function with decreased levels of albumin and prolonged prothrombin time can be seen. Autoantibodies, with an exception of p-ANCA (36) play neither diagnostic nor prognostic role (Terjung and Spengler 2005).

ERCP remains a gold standard in the diagnosis of PSC. As ERCP can be associated with complications such as pancreatitis or cholangitis, MRCP is a modality of choice in an early assessment, particularly in patients without clinical and biochemical symptoms suggesting the necessity of endoscopic intervention. Except non-invasiveness of MRCP, its clear advantage is a visualisation of bile ducts localised after significant stenoses where contrast medium may not penetrate. However, interpretation of MRCP scans requires advanced radiological expertise, for sure not available in all hospitals thus ERCP with its ability of direct

diagnostic (brush cytology) and therapeutic (stenting) interventions holds its role as a gold standard. Typical ERPC image in patient with PSC is shown in Fig. 1a.

It has been recently shown that overall complications rate after ERCP in patients with PSC was comparable to these without PSC. However the risk of cholangitis was significantly higher and the duration of the procedure was significantly longer in subjects with PSC (Bangarulingam et al. 2009).

Unlike in many other liver disorders, histology plays a minor role in the diagnosis of PSC as it shows significant variability and it affects clinical management in just 1.3% of patients (Olsson et al. 1995; Burak et al. 2003). It is however useful in the assessment of the stage of fibrosis and for confirming cirrhotic. A small proportion of patients have normal ERCP/MRCP, suffer from IBD and have histology compatible with PSC. This variant is called small-duct PSC and carries significantly better prognosis in terms of progression of the disease and the risk of CCA (Bjornsson et al. 2002). It has been recently shown that unlike in PSC with typical ERCP/MRCP picture the presence of IBD exerts no significant effect on the long-term prognosis in patients with small-duct variant (Bjornsson et al. 2008).

PSC can be overlapped with AIH, more commonly in children (Gregorio et al. 2001). Also in adults it has been postulated that all patients with AIH who have elevated ALP or GGT should be screened for PSC with MRCP (Abdalian et al. 2008). This approach permits detection of PSC in 10% of adult patients with AIH (Abdalian et al. 2008).

5 Malignancies in PSC

5.1 Cholangiocarcinoma

Cholangiocarcinoma (CCA) is a tumour with extremely bad prognosis and has similar incidence and mortality rates (Khan et al. 2008). Incidence shows geographical relationship with the highest rates observed in Thailand and being 100 higher than in Europe. In East Asia CCA is associated with infestation with hepatobiliary flukes, in particular *Opisthorchis viverrini* and *Clonorchis sinensis* (Shaib and El-Serag 2004; Watanapa 1996) and in Japan and Taiwan with hepatholithiasis (Okuda et al. 2002). In Europe, PSC is the most common risk factor. Over last decades mortality rates of CCA show a significant increase of intrahepatic CCA and no increase or even fall in extrahepatic form. These data have to be interpreted with caution due to a potential error related to misclassification of hilar (Klatskin) tumour which in terms of its localisation is extrahepatic but in ICD-0-2 classification it received a code of an intrahepatic one.

In terms of pathogenesis, CCA is clearly related to processes induced by inflammation and cholestasis. Undoubtedly, like in many other tumours, interleukin-6 (IL-6) is a key signalling cytokine involved in the pathogenesis of CCA (Wise et al. 2008). Its receptor subunit gp-130 is overexpressed in CCA (Yokomuro et al. 2000) and production facilitated by other inflammatory agents

(Park et al. 1999). Increased IL-6 not only causes CCA resistance to cytotoxic treatments (Isomoto et al. 2007; Kobayashi et al. 2005) but also leads to an activation of the family of mitogen activated protein kinases (MAPK) such as p44, p42 and p38 playing a crucial role in proliferation of CCA cells (Park et al. 1999). IL-6 produced by CCA cells in an autocrine fashion upregulates anti-apoptotic molecule Mcl-1 via STAT3 and AKT cascades (Kobayashi et al. 2005).

Various other cytokines enhanced by both inflammation and cholestasis in PSC trigger the cascade of inducible nitric oxide synthase (iNOS) activation followed by reactive nitrogen oxide species (RNOS) production which leads to DNA damage and mutagenesis (Blechacz and Gores 2008). Persistent activation of epidermal growth factor receptor (EGFR) by bile salts has been observed in CCA (Werneburg et al. 2003). This phenomenon facilitates proliferation of CCA cells. EGFR phosphorylation (along with the effect of bile acids, oxysterol and iNOS) triggers MAPK leading to increased expression of cyclooxygenase-2 (COX-2) in CCA cells and inhibition of apoptosis (Endo et al. 2002; Han et al. 2004). Growth of CCA cells is also affected by various other factors such as estrogens, neuropeptides or neuroendocrine hormones. 17 beta estradiol, one of the most toxic metabolites of estradiol (Milkiewicz et al. 2001) facilitates proliferation of CCA cells, an effect clearly inhibited by tamoxifen (Sampson et al. 1997). On the other hand stimulation of alpha2 receptors, gamma-aminobutyric acid and gastrin seem all decrease proliferation of CCA cells via various mechanisms (Wise et al. 2008; Kanno et al. 2002; Fava et al. 2005). Figure 2 shows in a simplified scheme of the development of CCA.

A large study on the relationship between PSC and CCA comprising about 400 patients from 5 European centres showed that 12.2% of patients with PSC were found to have CCA (Boberg et al. 2002). In 50% of them CCA was diagnosed within first year of the diagnosis of PSC and in a further 27% CCA was found during liver transplant assessment. Typical ERCP image of CCA is shown in Fig. 1b. Interestingly, although symptoms of jaundice, pruritus or abdominal pain were more pronounced in these who developed CCA, this relation was not seen when these patients were excluded from the analysis. Also, patients who developed CCA suffered from ulcerative colitis significantly longer than these who did not (17.4 years vs. 9 years). More recently a Dutch study found the 10 and 20 year rate of CCA in patients with PSC to be of 9% and 9%, respectively (Claessen et al. 2009a). Detailed description of diagnosis, staging and treatment of CCA is not an aim of this chapter. Authors would recommend recent review on it by Blechacz and Gores (Blechacz and Gores 2008).

5.2 Other Malignancies in PSC

5.2.1 Colorectal Cancer

An increased risk of colorectal dysplasia and cancer in patients with PSC and IBD has been demonstrated in several studies with their cumulative risk being 9, 31 and 50% after 10, 20 and 25 years as compared to 2, 5 and 10%, respectively for

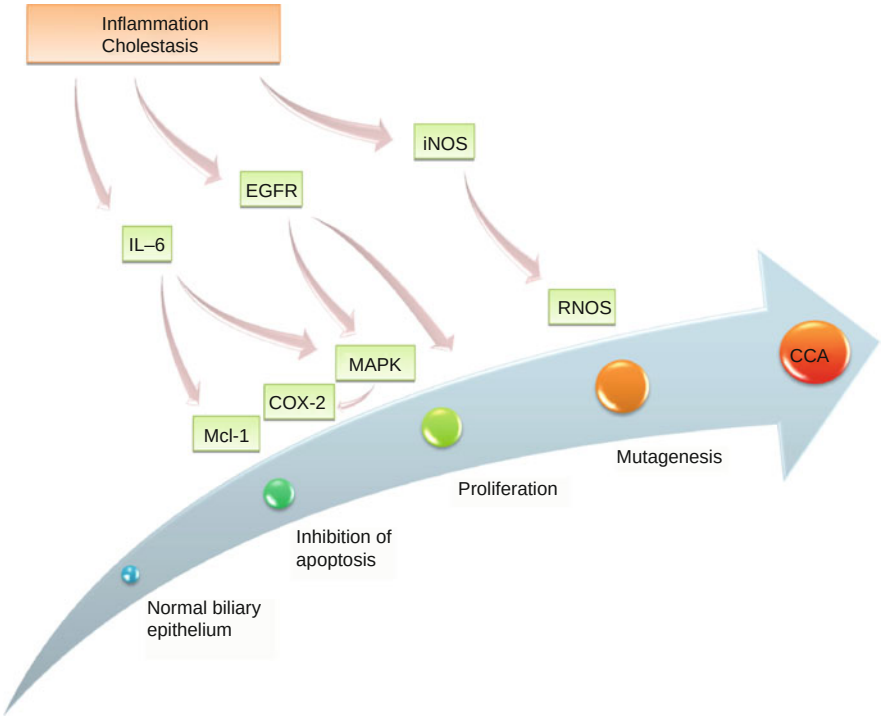

Fig. 2 Processes involved in the pathogenesis of cholangiocarcinoma in PSC (detailed description in the text). Inflammation and cholestasis with accumulation of several toxic agents lead to activation of various signaling cascades promoting inhibition of apoptosis, proliferation and mutagenesis of biliary epithelial cells. Abbreviations: interleukin-6 (IL-6), mitogen activated protein kinases (MAPK); inducible nitric oxide synthase (iNOS); reactive nitrogen oxide species (RNOS); cyclooxygenase-2 (COX-2); epidermal growth factor receptor (EGFR)

patients with UC only (Broome et al. 1995). In patients who underwent liver transplantation for PSC cumulative risk of developing colonic cancer was 14 and 17% after 5 an 10 years, respectively as compared to 0% at 10 years in these who have PSC without IBD (Vera et al. 2003). The natural history of colitis seems to be different in patients with PSC where it presents itself more frequently as pancolitis with less active course (Joo 2009). Thus the duration of colitis may be longer in these subjects, increasing the risk of colorectal carcinogenesis (Sokol et al. 2008). Also colonic cancer in patients with PSC and IBD is more frequently localised to the right colon (Claessen et al. 2009b). It is now widely recommended that patients with PSC and IBD should undergo colonoscopies on an annual basis (Kitiyakara and Chapman 2008). Those who do not have symptoms of colitis should undergo initial colonoscopy at their diagnosis of PSC but no clear guidelines exist as to the further follow-up in this subgroup of patients.

5.2.2 Gallbladder Cancer

When compared to general population, gallbladder polyps represent an increased risk of neoplastic transformation in patients with PSC, with as many as 57% of polyps found malignant in one series (Buckles et al. 2002). In another study, gallbladder adenocarcinomas were found in 14% of patients with PSC and an increased risk of gallbladder neoplasia in subjects with coexisting IBD or intrahepatic biliary cancer was also observed (Lewis et al. 2007). Thus regular examination of the gallbladder in PSC patients has been recently suggested and cholecystectomy recommended when gallbladder lesion is found, regardless of its diameter (Said et al. 2008).

5.2.3 Other Malignancies in PSC

Increased risk of two other gastrointestinal tract tumours, namely pancreatic (standard incidence ratio 9.7) and stomach cancer (standard incidence ratio 2.5) were observed in a large study from Sweden comprising more than 600 patients with PSC (Bergquist et al. 2002).

5.3 Chemoprevention

In patients with PSC and UC colonic cancer is more frequently localised to the right side of the colon suggesting a potential pathogenic role of agents to which proximal colon is exposed first. Among good candidates are secondary bile salts such as deoxycholate, a metabolite of primary bile salt (cholate), sythesised in the gut after the exposure of cholate to intestinal bacteria. Deoxycholate has a well proven toxic and cancerogenic properties (Bernstein et al. 2005; Rosignoli et al. 2008). In this context, a potential, chemopreventive role of UDCA, a hydrophyllic, secondary bile acid, commonly prescribed in PSC may be of importance. UDCA expresses several hepatoprotective properties not only by replacing toxic bile salts from a total bile acid pool but also inducing choleresis by triggering various intracellular signalling pathways (Paumgartner and Beuers 2004; Beuers et al. 2001; Milkiewicz et al. 1999, 2002; Wimmer et al. 2008). Our recent study applying gene array technique showed that UDCA is a potent modulator of genes involved in cell cycle and apoptosis (Chen et al. 2008). In terms of a potential chemopreventive effect of UDCA on the development of CCA the literature is equivocal (Olsson et al. 2005; Brandsaeter et al. 2004) but certainly it is affected by the fact that majority of CCA manifests itself shortly after the diagnosis of PSC thus the preventive effect of UDCA is difficult to prove. However, clear trends towards positive effect of UDCA have been seen by some authors with one study showing that no single case of CCA has been diagnosed in patients treated with UDCA longer than 8 years (Rudolph et al. 2007). More convincing evidence exists for chemoprevention of UDCA on colonic cancer (Wolf et al. 2005; Tung et al. 2001; Pardi et al. 2003). Additionally, these data is strengthened by the study on patients with another chronic, cholestatic liver condition, PBC where significant reduction of colonic polyps was observed in

Table 1 Studies on chemoprevention with ursodeoxycholic acid (UDCA) in primary sclerosing cholangitis (PSC)

Author/year of publication	Tumour/ lesion	Type of study	Results/conclusions
Tung et al. 2001	Colonic dysplasia	Cross-sectional	Significant decrease of colonic dysplasia in patients using UDCA
Pardi et al. 2003	Colonic dysplasia/ cancer	Prospective	Significant reduction of the risk of colonic dysplasia/cancer in patients taking UDCA
Wolf et al. 2005	Colonic dysplasia/ cancer	Retrospective	1. Not significant reduction of the incidence of colonic dysplasia/cancer in patients taking UDCA
			2. Significant reduction of overall mortality in patients taking UDCA
Brandsaeter et al. 2004	Hepatobiliary tumours	Prospective	Increased risk of hepatobiliary cancers in patients who did not take UDCA
Olsson et al. 2005	CCA	Randomised, placebo-controlled	Not significant effect of high dose of UDCA on the incidence of CCA but study did not reach an enrolment target
Rudolph et al. 2007	CCA	Prospective	Annual incidence of CCA lower than expected in patients taking UDCA

these who took UDCA (Serfaty et al. 2003). Literature data on the chemopreventive studies with UDCA in patients with PSC is summarised in Table 1.

5.4 Treatment

5.4.1 Medical

Historically, several agents have been assessed for the treatment in PSC. These include colchicine, cyclosporine A, methotrexate, *d*-penicillamine, budesonide, mycophenolate mofetil, pentoxifylline, pirfenidone, tacrolimus and prednisone (Cullen and Chapman 2006). Almost all these therapies are now considered ineffective or associated with significant side effects. The only exception is perhaps steroids. Boberg et al. (2003) have shown that a subgroup of patients with PSC may benefit from steroids in terms of long-term survival. They comprised a group of young subjects with significantly higher levels of ALT and some histological features of AIH. Nevertheless, all subjects from these study had their PSC confirmed with cholangiography.

Ursodeoxycholic acid (UDCA) has been widely used in patients with PSC however recent study has shown that long-term, high-dose therapy with this compound did not improve survival and was in fact associated with higher rates of

serious adverse events (Lindor et al. 2009). Following these findings American Association for the Study of Liver Disease (AASLD) has recommended against using UDCA in patients with PSC (Chapman et al. 2010) and most recent European Association for the Study of Liver Disease (EASL) Guidelines suggested that UDCA may be used in patients with PSC but in these who also suffer from advanced colitis (EASL Clinical Practice Guidelines 2009). Despite these controversies many experts may still consider using UDCA in a lower dose (13–15mg/ kg b.w.) recommended in patients with PBC (Chapman 2009).

A recent, pilot study showed a significant improvement of alkaline phosphatase and Mayo risk score in patients treated with minocycline (Silveira et al. 2009). This effect was attributed to anti-inflammatory and immunomodulatory rather than antimicrobial properties of minocycline. Certainly, these results have to be validated in larger cohorts of patients.

Major steps in our understanding of the pathogenesis of cholestasis and elucidation of the role of nuclear orphan receptors such as PXR, FXR, VDR, CAR or PPAR may have an important therapeutic consequences in future, however at this point the data is limited to experimental works on laboratory animals (Beuers et al. 2009).

5.4.2 Endoscopic

Endoscopic treatment plays an important role in the management of patients with PSC. Unfortunately, due to a lack of randomised studies comparing different endoscopic approaches no guidelines on applying therapeutic endoscopy in PSC exist. In principle, endoscopy is of particular use in restoring a bile flow in patients who developed a dominant stricture, especially in a common bile duct. Most commonly this can be managed with either balloon dilatation or stenting (Bjornsson et al. 2004; Bjornsson and Olsson 2004; Baluyut et al. 2001). As CCA may manifest itself as a dominant stricture material for cytology and if possible histology should be taken during the procedure (Weismuller et al. 2008). A recent, retrospective study on a large cohort of patients treated endoscopically showed that their survival at 3 and 4 years was significantly better than the one predicted by Mayo model (Gluck et al. 2008).

5.4.3 Liver Transplantation

PSC is a good indication for liver transplantation however the timing of the operation poses a significant challenge. This is mostly due to the high risk of CCA which once developed is a contraindication for the transplantation in a majority of centres. Patients with PSC usually comprise a population of relatively young and frequently clinically stable subjects thus liver transplantation on the grounds of the fact that 10–15% of them will develop CCA is not justified. As effective surveillance for CCA does not exist the decision of transplantation is difficult. There is no doubt that patients with recurrent episodes of cholangitis in whom endoscopic management is not effective are good candidates. Patients with Candida positive bile cultures may be at particular risk and should be referred for liver

transplantation early (Rudolph et al. 2009). Also those who developed liver cirrhosis with features of end stage liver disease should be considered for transplantation according to the same principles applied in other patients with cirrhosis. On occasion, intractable pruritus can be in itself an indication for surgery, but this is more common in PBC than in PSC.

A recent study, based on UNOS database obtained between 1995 and 2006 showed that despite the constant increase of the overall number of liver transplants in the US, the number of transplants for PSC showed no change over the analysed period (Lee et al. 2007). Interestingly, a clear trend towards a decrease of placing patients with PSC on the waiting list was seen. Interpretation of these phenomena is difficult as the static number of transplants performed for PSC may be related to the low MELD (Model of End Stage Liver Disease) scores of these patients and, in terms of numbers on the waiting list, it may reflect better pre-transplant treatment (UDCA, endoscopic methods). Prognosis after transplantation is favourable with 5 and 10 years survivals of 79 and 78% (European liver transplant registry 2009). PSC may recur after surgery in up to 37% of patients. Data are accumulating that the presence of intact colon poses a significant risk of the recurrence. Vera et al. (2002) have shown that cumulative, 10 years risk of PSC recurrence after grafting in patients in whom their colons were removed before or during transplantation was 0.1 as compared to 0.7 in those who had their colons intact. They also showed that in small number of patients, recurrence may lead to graft loss and re-grafting. These findings have been recently confirmed by the same group on a significantly larger cohort of patients (Alabraba et al. 2009).

As already mentioned, the presence of CCA is a clear contraindication for transplantation in PSC. However, authors from Mayo Clinic designed protocols which include meticulous staging and neoadjuvant chemoradiation which permit transplantation in carefully selected patients with PSC and CCA reaching 5 years survivals in 82% of patients as compared to 21% in these who underwent resection (Rea et al. 2005).

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Chronic Inflammation and Hepatocellular Carcinoma

Malcolm R. Alison, Linda J. Nicholson and Wey-Ran Lin

Abstract

Hepatocellular carcinoma (HCC) invariably develops within a setting of chronic inflammation caused by either hepatotropic viruses, toxins, metabolic liver disease or autoimmunity. Mechanisms that link these two processes are not completely understood, but transcription factors of the NF-κB family and signal transducer and activator of transcription 3 (STAT3), cytokines such as IL-6 and IL-1α and ligands of the epidermal growth factor receptor (EGFR) family are clearly pivotal players. HCC may have its origins in either hepatocytes or hepatic progenitor cells (HPCs), and HCCs, like other solid tumours appear to be sustained by a minority population of cancer stem cells.

Keywords

Cell senescence · Chronic inflammation · STAT3 · NF-κB · Fibrosis · Cirrhosis · Hepatic progenitor cells · Hepatocytes · Oval cells · Stem cells

Contents

M. R. Alison (✉) · W.-R. Lin
Centre for Diabetes, Barts and The London School of Medicine and Dentistry, BICMS, 4 Newark Street, London, E1 2AT, UK
e-mail: m.alison@qmul.ac.uk

L. J. Nicholson
Division of Cancer Studies, King's College London, London, UK

W.-R. Lin
Chang Gung Memorial Hospital, Chang Gung University College, Taipei, Taiwan

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1 Introduction

Hepatocellular carcinoma (HCC) is the 5th most common cancer worldwide and the 3rd most common cause of cancer death with approximately 600,000 deaths annually. Most HCCs (70–90%) arise in a cirrhotic liver, i.e., a situation where there has been repetitive hepatocyte damage leading to regenerative hyperplasia and chronic inflammation (hepatitis) leading to fibrosis and cirrhosis (Fig. 1) (Schutte et al. 2009; Neuveut et al. 2010). Hepatic fibrosis leading to cirrhosis is the liver's connective tissue response to iterative hepatocyte injury, mediated by activated hepatic stellate cells (Bataller and Brenner 2005). Both HBV and HCV infection cause increased hepatic production of Hh ligands that can stimulate stellate cells to produce collagen and hepatic progenitor cells (HPCs) to divide (Pereira et al. 2010). Cell proliferation is of course a necessary pre-requisite for successful tumour initiation, which together with inflammation-induced oxidative stress makes a fertile background for genomic mutation. There are huge geographical variations in the incidence of HCC, with the highest incidence in areas such as Eastern Asia and sub-Saharan Africa where chronic hepatitis B virus (HBV) infection is a major risk factor (Brechot 2004). In Europe and the USA, the incidence of HCC is low but slowly increasing, probably due to the rise in people infected with HCV. Apart from hepatotropic viruses, the other major risk factors for HCC are other factors leading to cirrhosis such as alcohol abuse and metabolic liver disease, and mutagens such as aflatoxins that are toxic metabolites of the food mould *Aspergillus species.*

Cholangiocarcinomas (CC) are believed to arise from biliary epithelium that is either within the liver (intrahepatic) or extrahepatic. The tumour is much less common than HCC, but its incidence and associated mortality has been increasing steadily over the past 2–3 decades, with most tumours arising in persons over 50 years of age, suggesting carcinogenesis is a protracted and (possibly) multistep process (Sirica 2005). Injury to the biliary epithelium with chronic inflammation, together with impedance of bile flow, are common factors in high-risk conditions for CC such as primary sclerosing cholangitis, hepatolithiasis (gall stones) and liver fluke infestation by *Opistorchis viverrini* and *Clonorchis sinensis*.

2 Hepatitis B virus

Worldwide, Hepatitis B virus (HBV) infection is a major problem with between 350 and 400 million people chronically infected. After acute hepatitis B infection, about 90% of adults achieve complete immune clearance of the virus, but 10%

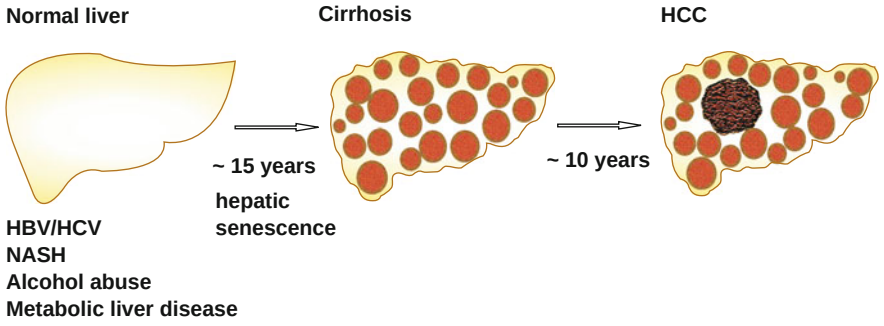

Fig. 1 Simplified overview of events leading to HCC development

develop chronic infection. Viral hepatitis plays a key role in up to 80% of all HCC, with HBV being responsible for two-thirds of all cases. Areas of high risk for HCC are mostly endemic for HBV, though the relevance of HBV infection is waning with the introduction of universal prophylactic vaccination programmes for infants, pioneered in Taiwan. Chronic active hepatitis occurs because viral hepatitis B surface antigen (HBsAg) is expressed on the hepatocyte's cell surface, stimulating the host's immune system and triggering inflammation thus generating reactive oxygen species (ROS) leading to oxidative DNA damage (Hussain et al. 2003), probably the main factor driving liver carcinogenesis (Kremsdorf et al. 2006; Brechot et al. 2010). Nuclear factor erythroid2-related factor (Nrf2), the transcription factor that positively up-regulates the expression of a large number of cytoprotective genes (detoxifying enzymes, biotransformation enzymes and transporters) by binding to antioxidant response elements, protects liver cells from such oxidative/electrophilic stress (Klaassen and Reisman 2010).

The carcinogenic process will of course be aided by a host of chemokines and cytokines produced by the inflammatory cell infiltrate that foster cell survival and proliferation (Coussens and Werb 2002). Other drivers of the carcinogenic process may well include direct viral DNA integration into the host genome leading to the likes of genomic instability, chromosomal loss and inappropriate gene activation (insertional mutagenesis) (reviewed in Brechot et al. 2010), while the virally encoded pleiotropic Hepatitis B virus X protein (HBx) has been implicated in anti-apoptotic pathways by blocking p53 function and transactivating promoters and enhancers with NF-κB binding sites. HBx also causes DNA re-replication by de-regulating expression of the DNA replication licensing factors Cdt1 and Cdc6, and geminin, the inhibitor of re-replication, so leading to an aberrant DNA content that propagates genomic instability and up-regulation of cell cycle genes such as the mitotic kinase called polo-like kinase (Plk1) (Studach et al. 2009), whose activity is required for entry into mitosis. The importance of these mechanisms other than the immune response to HbsAg in the carcinogenic process is illustrated by the fact that patients who spontaneously clear HBsAg can still develop HCC many years later, albeit at a lower rate than persistent carriers (Simonetti et al. 2010).

Treatments that delay or block disease progression include interferons and antiviral nucleoside analogues that block viral DNA replication.

3 Hepatitis C virus

Globally, 25% of HCC deaths can be attributed to Hepatitis C virus (HCV) (McGivern and Lemon 2009). In contrast to HBV-associated HCC, HCV-associated HCC has been increasing steadily since the 1960 s mainly because of contaminated blood supplies and widespread intravenous drug abuse (shared syringe needles). HCV is the leading cause of end-stage liver disease worldwide and the most common indication for liver transplantation in the United States and Europe. Unlike HBV-associated acute hepatitis, acute hepatitis C resolves in only about 10–40% of cases and effective vaccination against HCV is currently not available. The sequelae of histological changes are broadly similar to those associated with HBV infection, namely hepatocyte death, inflammation, steatosis and progressive fibrosis leading to cirrhosis and HCC, thus regenerative hyperplasia in the setting of oxidative stress leading to DNA damage is likely to be a major cause of malignant transformation (reviewed in Bartosch et al. 2009; Castello et al. 2010). The HCV genome is a single stranded RNA molecule of approximately 9600 nucleotides in length, but lacks a reverse-transcriptase, so with no DNA intermediate, does not integrate into the host genome, so mechanisms of hepatocarcinogenesis by HCV proteins are likely to be indirect, perhaps activating cell signalling pathways such as STAT3, PI3 K and NF-κB or by direct interaction with key cell cycle regulators such as pRb and p53. In HCV core transgenic mice, both spontaneous and diethylnitrosamine (DEN)-induced HCC hepatocarcinogenesis can be effectively abolished by knockdown of c-Jun (AP-1) and STAT3, transcription factors that are collectively required for stimulating cell proliferation, suppressing apoptosis and impairing oxidative DNA damage repair (Machida et al. 2010). Additionally there are many pro-carcinogenic co-factors such as co-infection with HBV, obesity linked to non-alcoholic steatohepatitis (NASH) and insulin resistance that lead to more inflammation favouring hepatocarcinogenesis. In a murine model of HCV hepatocarcinogenesis, enteric bacteria promote tumourigenesis related to NF-κB signalling in both the intestine and liver, seemingly associated with activation of the innate and Th1-type adaptive immunity (Fox et al. 2010). Treatments for HCV infection centre on pegylated interferon-α and ribavirin (a nucleoside analogue) that interfere with viral replication among other things.

4 Signalling Pathways Linking Inflammation and HCC Development

Apart from infectious agents with their own transforming oncoproteins, bacteria and viruses can activate cells of the innate immune system (particularly macrophages) through pattern recognition receptors (PRRs), specifically those belonging

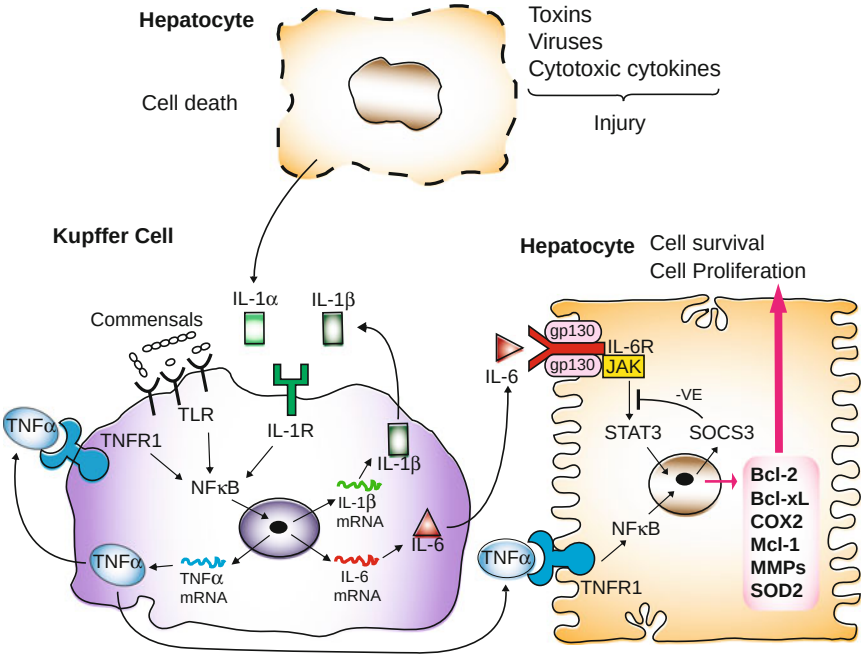

Fig. 2 Some of the intracellular signaling pathways linking hepatocyte cell death with inflammatory cells and hepatocyte survival and proliferation. COX-2, cyclooxygenase 2; MMPs, matrix metalloproteinases; SOD, superoxide dismutase (see text for further details)

to the Toll-like receptor (TLR) family. Receptor occupancy triggers the production of chemokines and cytokines to amplify the response, while others, notably IL-6 and TNF-α can stimulate normal and malignant liver cell proliferation (Fig. 2).

Among the signalling pathways, the one controlled by NF-κB appears pre-eminent. NF-κB can be activated downstream of TLRs through the adaptor molecule, myeloid differentiation primary response gene 88 (MyD88) or by TNF-α or IL-1β. NF-κB provides a mechanistic link between inflammation and cancer, being a major factor in determining the ability of normal, preneoplastic and malignant cells to avoid apoptosis (Karin and Greten 2005; Karin 2006; Elsharkawy and Mann 2007). Before stimulation most NF-κB dimers are retained in the cytoplasm bound to specific inhibitors—the inhibitors of NF-κBs (IκBs) (Moynagh 2005) (Fig. 3). Cell stimulation activates the IκB kinase (the IKK complex) composed of two catalytic subunits (IKKα and IKKβ) and a regulatory subunit (IKKγ/NEMO). Activated IKK phosphorylates the NF-κB-bound IκB protein, targeting it for polyubiquitination and ultimately proteasomal destruction; freed NF-κB dimers translocate to the nucleus where they coordinate transcriptional activation of several hundred target genes.

A prototype of inflammation-associated HCC is the *Mdr2*$^{-/-}$ mouse with homozygous deletion of the multidrug resistance 2 gene that encodes a P-glycoprotein. This animal model develops cholestatic hepatitis leading to dysplasia and eventually

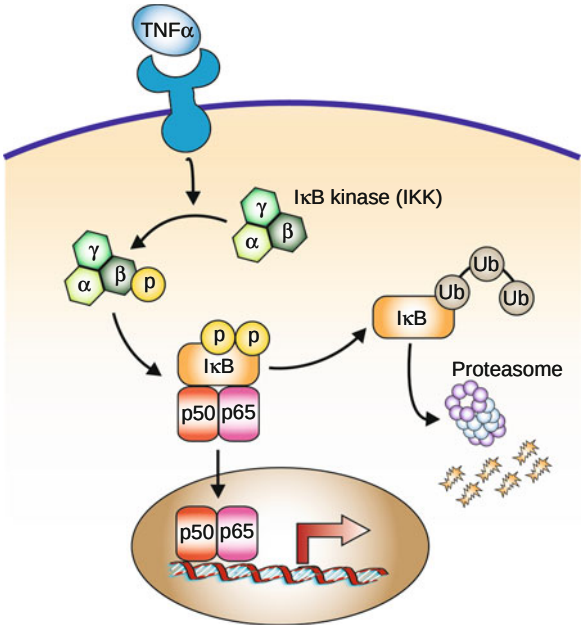

Fig. 3 Simplified diagram illustrating just one of the pathways involved in promoting NF-κB signaling

HCC after 8–10 months, and here TNF-α produced by adjacent inflammatory cells and endothelial cells leads to up-regulation of NF-κB signalling in hepatocytes, thereby promoting HCC formation, while an anti-TNFα treatment suppressed HCC formation (Pikarsky et al. 2004). The pro-tumourigenic effect of NF-κB appeared to be related to a suppression of apoptosis in premalignant and malignant hepatocytes through up-regulation of inhibitors of apoptosis such as Bcl-X_L and IAPs. Tumour development could also be blocked by over-expression of the degradation-resistant form of the so-called IκB super repressor.

However, while NF-κB inactivation late on in the process of tumour progression appears to **impede** growth because the tumour cells need the pathway for cell survival, inactivation of the pathway at an early stage in some models actually **promotes** hepatocarcinogenesis, seemingly because normal hepatocytes are now more sensitive to apoptosis which triggers a compensatory hyperplasia under inflammatory and oxidative stress, thus increasing the risk of malignant transformation. For example, mice (*Nemo/Ikkγ*$^{\Delta hep}$) lacking IKKγ/NEMO, the regulatory subunit of the upstream kinase, have spontaneous liver damage, with enhanced steatohepatitis and HCC development (Luedde et al. 2007). Likewise, mice lacking IKK*β* specifically in hepatocytes (*Ikkβ*$^{\Delta hep}$ mice) also show enhanced HCC formation following DEN exposure, thought to be linked to increased ROS production, more hepatocyte death and thus more regenerative hyperplasia (Maeda et al. 2005). In both these latter two murine models, liver injury is a key event, and

with hepatocyte-specific deletion of IKKγ/NEMO it appears that this is mediated by NK cell damage via TRAIL/DR5 signalling leading to the familiar cascade of cellular events, inflammation, steatohepatitis, fibrosis, cirrhosis and HCC development (Beraza et al. 2009). On the other hand, in the *Mdr*$^{-/-}$ mouse, HCC development depends on low-grade chronic inflammation with no liver injury, and in this situation NF-κB signalling is pro-tumourigenic, however if hepatocyte injury is superimposed upon this model by a two-thirds partial hepatectomy then long-term tumourigenesis is enhanced, probably because hepatocytes harbouring DNA damage replicate increasing genomic instability (Barash et al. 2010).

An equally important inflammation-associated signalling pathway is that controlled by STAT3 triggered by cytokines such as IL-6 that bind to cytokine receptor-associated Janus family kinases (JAKs). The pathway is negatively regulated through transcriptional up-regulation of SOCS3 (suppressor of cytokine signaling 3), a molecule that prevents further interaction of JAKs with STAT3, thus blocking further JAK-mediated phosphorylation (activation) of STAT3. The importance of STAT3 to inflammation-associated hepatocarcinogenesis is illustrated by the fact that hepatocyte-specific deletion of SOCS3 in a mouse model of DEN-induced HCC results in larger and more numerous tumours (Ogata et al. 2006). Interestingly, up to 60% of hepatic adenomas associated with inflammation can have gain-of-function mutations in the *IL6ST* gene that encodes the signaling co-receptor gp130 (Rebouissou et al. 2009); these mutants can activate STAT3 in the absence of ligand. IL-6/STAT3 also promotes survival in established HCC cells, and blocking STAT3 can sensitise cells to doxorubicin (Liu et al. 2010). Blocking c-Met specifically in hepatocytes also results in increased sensitivity to potentially damaging bile duct obstruction (Giebeler et al. 2009).

As highlighted, signalling via NF-κB is complex, being anti-tumourigenic in *Nemo/Ikk*$\gamma^{\Delta hep}$ and *Ikk*$\beta^{\Delta hep}$ mice where liver injury occurs (and is needed for initiation via compensatory regeneration), but being pro-tumourigenic in *Mdr*$^{-/-}$ mice that depend upon chronic low-grade inflammation for tumour progression. But, NF-κB signalling can also be anti-tumourigenic in the context of tumour progression. In a further twist, NF-κB signalling has been found to negatively regulate STAT3 by preventing accumulation of ROS (He et al. 2010). Transplanting DEN-initiated hepatocytes into a mouse liver with ongoing indigenous hepatocyte loss, it was found that deletion of IKKβ in the transplanted cells caused increased accumulation of ROS leading to JNK and STAT3 activation and accelerated tumour development. As noted by the authors, human hepatocarcinogenesis is likely to involve both inflammatory and injury/regeneration responses, thus all of the above model systems probably have some relevance to human HCC development.

Other promoters of HCC development include the cytokines lymphotoxin (LT) α and β produced by $CD3^+$ and $CD20^+$ lymphocytes that act primarily on hepatocytes expressing the LTβR, resulting in the release of chemokines that cause an influx of inflammatory cells that then contribute to hepatocyte death. These molecules are notably up-regulated in HBV- and HCV-induced hepatitis and HCC, and LTβR inhibition in LT$\alpha\beta$-transgenic mice with hepatitis suppresses HCC formation (Haybaeck et al. 2009). Signalling through EGFR ligands is a further

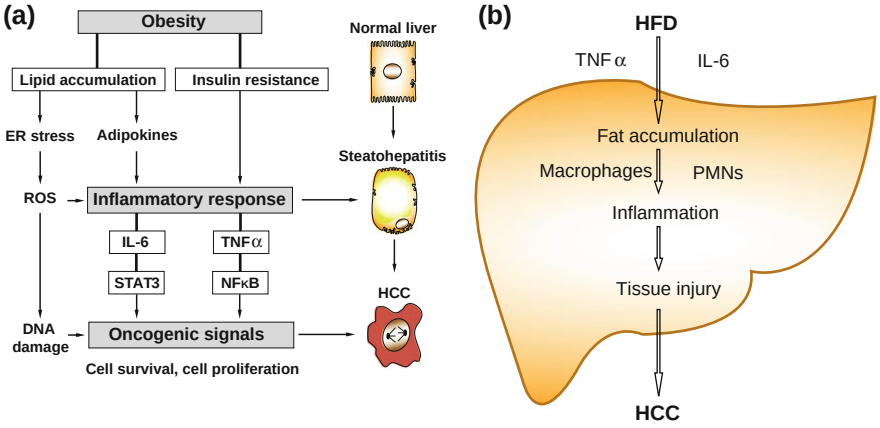

Fig. 4 **a** The principal cellular events linking obesity with the promotion of HCC development. **b** Summary of how a high fat diet (HFD) leads to liver inflammation, hepatocyte damage and compensatory hyperplasia rendering the liver at greater risk of neoplastic transformation

stimulus to the growth of premalignant and malignant hepatocytes (Berasain et al. 2009).

5 Obesity

The relative risk of death from HCC increases dramatically with obesity (men with a BMI of 35–40 have a 4.5-fold increase in relative HCC risk), particularly in those patients who develop non-alcoholic steatohepatitis (NASH) and cirrhosis. Increases in intracellular lipid puts a heavy metabolic demand on the endoplasmic reticulum (ER) leading to ER dysfunction and production of excessive ROS leading to oxidative stress and activation of inflammatory pathways (e.g., NF-κB). Studies in mice suggest that IL-6 and TNF-α are the main molecular triggers linking obesity with hepatocarcinogenesis (Fig. 4a). Mice treated with the carcinogen diethylnitrosamine (DEN) and fed with a high fat diet (HFD) have a higher tumour burden than those fed with a normal diet after DEN injection, seemingly linked to the abnormal proliferation of hepatocytes with damaged DNA (Park et al. 2010). It was thought unlikely that this effect resulted from carcinogens or tumour promoters in the HFD since leptin-deficient mice (Lep^{Ob}) that spontaneously develop obesity, also had a higher tumour burden after DEN than wild-type mice when both are fed on a low fat diet. Obesity was associated with elevated levels of the inflammatory cytokines IL-6 (and associated STAT3 activation), TNF-α and IL-1β in the liver, produced by the likes of Kupffer cells, hepatocytes and inflammatory cells, and an ingress of inflammatory cells (macrophages and neutrophils); moreover studies with IL-6 knock-out mice confirmed the requirement for IL-6 for tumour promotion in

this scenario. In turn, TNF signalling was required for IL-6 expression since TNFR1 ablation almost completely abolished obesity-induced tumour promotion, and both IL-6 and TNF were required to trigger NASH. Thus, either dietary or genetic obesity seems to be a *bona fide* liver tumour promoter, at least in mice (summarised in Fig. 4b).

6 Liver Regeneration and Carcinogenesis

In postnatal animals and humans, hepatocytes are highly differentiated cells with multiple synthetic and metabolic functions. Notwithstanding, hepatocytes are the cells that normally shoulder the burden of regenerative growth after liver damage, so they can be considered as the functional stem cells under most circumstances. Normally the liver exhibits a very low level of cell turnover, but when hepatocyte loss occurs, a rapid regenerative response is elicited from all cell types in the liver to restore the organ to its pristine state (Alison et al. 2009). More severe liver injury, particularly long-standing iterative injury in the context of chronic inflammation (e.g., chronic viral hepatitis leading to fibrosis) is often associated with replicative senescence (Marshall et al. 2005; Ikeda et al. 2009), activating a facultative stem cell compartment located within the intrahepatic biliary tree, giving rise to cords of bipotential transit amplifying cells (named oval cells in rodents and hepatic progenitor cells [HPCs] in man), that can ultimately differentiate into hepatocytes and biliary epithelial cells.

As already noted, chronic liver injury is invariably accompanied by progressive fibrosis, involving the activation of hepatic stellate cells (HpSCs) that proliferate and synthesise and secrete interstitial collagens. HpSCs express α smooth muscle actin (α-SMA), the histological hallmark of myofibroblasts, and they are thought to be the pivotal in the pathogenesis of liver fibrosis. As in other responses, Kupffer cells seem to be key cells, activating HpSCs by secretion of TGF-β, and bacterial products such as lipopolysaccharide (LPS) engage Toll-like receptors on stellate cells, down-regulating the inhibitory TGF-β pseudoreceptor Bambi, allowing unrestricted activation of HpSCs by the Kupffer cells (Seki et al. 2007). Regenerative nodules, a key feature of cirrhosis, have been traditionally considered to be formed through the simple fibrotic dissection of the liver parenchyma by the deposition of collagens, but we have proposed an alternative explanation (Lin et al. 2010). It is well recognised that the fibrotic septae in cirrhosis also harbour HPCs, derived from biliary ducts, that comprise the ductular reaction (schematically illustrated in Fig. 5a), and by using mitochondrial DNA (mtDNA) mutations in the cytochrome *c* oxidase gene as clonal markers we have found that the regenerative nodules are invariably monoclonal and derived from HPCs (Fig. 5b).

So are HPCs, the cells that are so common in circumstances of chronic inflammation in the liver, the founder cells for HCC? Certainly, hepatocytes are implicated in some instances of HCC, for example, in some mouse models of HCC, oncogenic transgenes are driven by albumin promoters. The direct involvement of hepatocytes

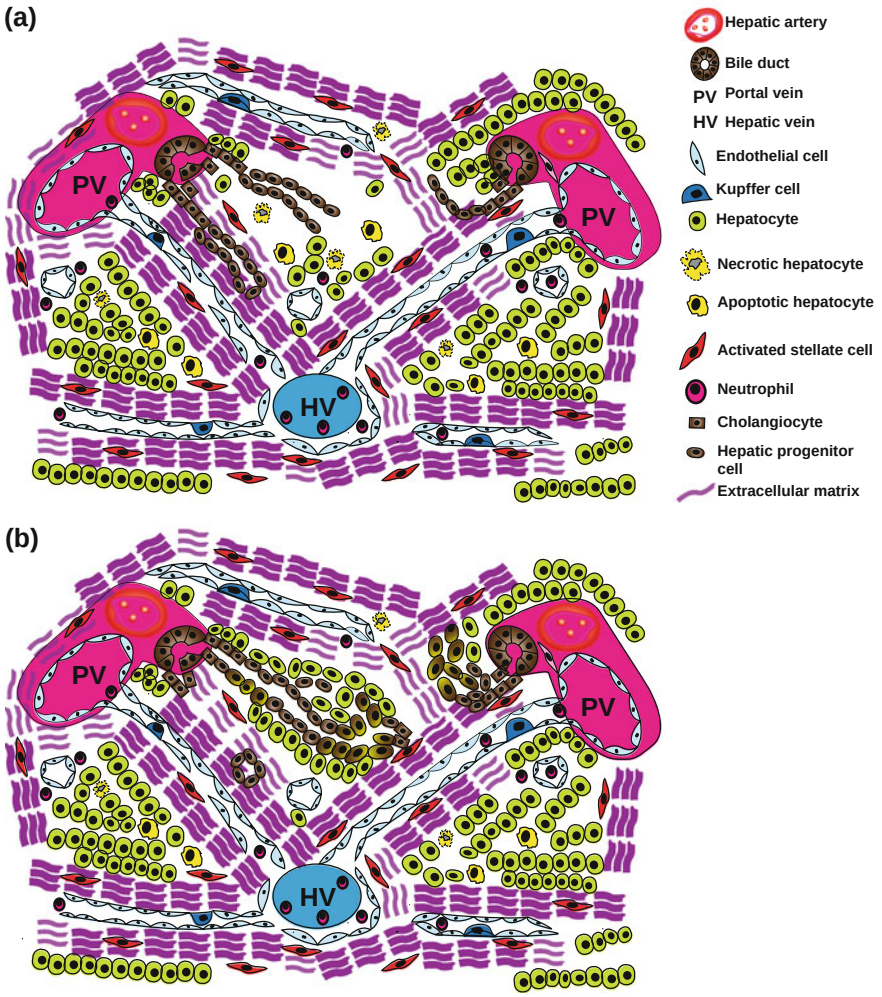

Fig. 5 Scheme for the development of regenerative nodules in the chronically inflamed human liver. **a** Chronic hepatocyte damage leads to the replacement of the liver parenchyma by fibrous tissue; commonly a ductular reaction composed of hepatic progenitor cells is located within these fibrous septae. **b** If these hepatic progenitor cells differentiated into hepatocytes they could form the nidus of a regenerative nodule. Hepatic progenitor cells could also be the founder cells for HCC (see text for experimental details)

in hepatocarcinogenesis has been established in rats. Gournay et al. (2002) found that some preneoplastic foci (expressing gamma glutamyl transpeptidase and the placental form of glutathione-S-transferase) were directly descended from hepatocytes. This was established by stably labelling hepatocytes at day one after a two-third partial hepatectomy (PH) with β-galactosidase using a recombinant retroviral vector containing the β-galactosidase gene; subsequent feeding with 2-acetylaminofluorene

lead to foci, some of which were composed of β-galactosidase-expressing cells. Using the same labelling protocol, Bralet et al. (2002) observed that 18% of hepatocytes expressed β-galactosidase at the completion of regeneration after a two-third PH; subsequent chronic treatment with diethylnitrosamine (DEN) resulted in many HCCs of which 17.7% of the tumours expressed β-galactosidase, leading to the conclusion that a random clonal origin of HCC from mature hepatocytes was operative in the model.

As discussed above, there is now compelling evidence that HPCs are capable of giving rise to both hepatocytes and cholangiocytes. The fact that the ductular cell reaction precedes the development of HCC in almost all models of hepatocarcinogenesis and invariably accompanies chronic liver damage in humans makes it almost certain that the mature hepatocyte is not the cell of origin of all HCCs, indeed perhaps only a small minority of HCCs are derived from mature hepatocytes. An origin of HCC from HPCs is often inferred from the fact that many tumours contain an admixture of mature cells and cells phenotypically similar to HPCs (Alison et al. 2009). Cells with an HPC phenotype have also been noted in a relatively rare subset of hepatic malignancies where there are clearly two major components, an HCC component and a cholangiocarcinoma component, again suggestive of an origin from a bipotential progenitor (Theise et al. 2003).

Direct evidence of a role for HPCs in the histogenesis of HCC can be found; Dumble et al. (2002) isolated HPCs from p53 null mice and when the cells were transplanted into athymic nude mice they produced HCCs. If tumours do arise from HPCs, then this would suggest a block in HPC differentiation, a process termed 'stem cell maturation arrest'. Along these lines of thought, in humans four prognostic subtypes of HCC have been identified, corresponding to a hierarchy of liver cell lineages (Yamashita et al. 2008). Those with the poorest prognosis possessed a sizeable proportion of either $EpCAM^{+}AFP^{+}$ cells (hepatoblast-like) or $EpCAM^{-}AFP^{+}$ cells (HPC-like), whereas those with $EpCAM^{-}AFP^{-}$ cells (mature hepatocyte-like) or $EpCAM^{+}AFP^{-}$ cells (cholangiocyte-like) had a more favourable outcome. Moreover, gene expression profiling has identified a subset of HCCs with a poor prognosis that have a profile consistent with an origin from HPCs (Lee et al. 2006), and simple enumeration of CK19-positive cells in HCC can identify a patient group who have a shorter time to disease recurrence (Durnez et al. 2006).

There is a growing realisation that many, if not all cancers contain a minority population of self-renewing stem cells, the *cancer stem cells* (CSCs) which are entirely responsible for sustaining the tumour as well as giving rise to proliferating but progressively differentiating cells that are responsible for much of the cellular heterogeneity that is so familiar to histopathologists (Alison et al. 2011). We have suggested that many liver tumours probably have their origins in normal liver stem cells, particularly HPCs, but do liver tumours have CSCs? The answer is probably yes, with CD133, CD90, ALDH and the side population being variously advocated as liver CSC markers (Alison et al. 2011).

7 Conclusions

This chapter has summarised our current knowledge of how the liver responds to chronic liver damage, regenerating after both acute and more chronic iterative damage. Under normal circumstances the differentiated parenchymal cells (hepatocytes) are the functional stem cells, but in more extreme circumstances a 'potential' stem cell compartment can be recruited into action, providing HPCs from the intrahepatic biliary system that can differentiate into hepatocytes. Most cases of HCC arise within a cirrhotic setting, a scenario associated with hepatocyte replicative senescence and HPC activation. The signaling pathways centred on NF-κB and STAT3 appear to be central to the hepatocarcinogenic process. Observation and experimental evidence points to a possible origin of HCC from either HPCs or hepatocytes, and HCC itself appears to have a minority population of CSCs.

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Gastrointestinal Cancer: Current Screening Strategies

Hugh Barr

Abstract

The prevention and chemoprevention of cancer is based on identifying a pre-neoplastic lesion and altering the outcome by early intervention. Many of the gastrointestinal epithelial cancers are related to chronic inflammatory conditions for many years prior to cancer development. It is clear that treatment of the inflammatory condition can prevent and indeed reverse changes that predispose to cancer. This is most notable for helicobacter pylori infection of the stomach. Screening for Barrett's oesophagus and other conditions are more controversial and the results of large scale clinical trials are awaited. Nevertheless preventive strategies are highly attract health care interventions and are being actively considered.

Contents

H. Barr (✉)
Cranfield Health, Gloucestershire Royal NHS Foundation Trust,
Great Western Road, Gloucester, GL13NN, UK
e-mail: hugh.barr@glos.nhs.uk

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1 Introduction

The association between chronic inflammation and cancer in the gastrointestinal tract now has a very strong scientific basis (Marx 2004; Quante and Wang 2008; Clemons et al. 2007). Inflammatory cells, in particular macrophages, infiltrate tumours and promote tumour growth liberating TNF-α, NF-κB and COX-2. Cytokines can also influence and indeed instruct cell lineage choice (Rieger et al. 2009). The evidence base remains incomplete but data is accumulating that the progression to cancer may be preventable, if patients are detected early and measures are put in place to detect and control inflammation, and screening for pre-invasive neoplasia in those with chronic inflammatory conditions (Velayos et al. 2006).

There is now accumulating evidence, from epidemiological studies, that regular usage of aspirin and non-steroidal anti-inflammatory drugs reduces the risk of developing and also progressing to cancer. These trends are so important that they are being explored in large, long-term clinical trials of secondary chemoprevention at the present time (Raj and Jankowski 2004; Wong and Fitzgerald 2005; Jankowski and Barr 2006; Jankowski and Hawk 2006). However, these issues are complex, and it is important to note that there is some data to indicate that if the sequence of inflammation to cancer is very advanced, then chemopreventative strategies may not help. For example, a recent multicentre, randomised, placebo-controlled trial explored the effect of long-term administration of a COX-2 inhibitor in patients with Barrett's oesophagus who had with established diagnosis of dysplasia. This anti-inflammatory intervention did not appear to prevent progression to cancer after 48 months of therapy (Heath et al. 2007).

Nevertheless, the purpose of screening of patients with chronic inflammatory conditions is to detect the degeneration to cancer at a pre-invasive histopathological stage and at an appropriately early clinical stage. The premise is that early intervention will alter the clinical course and prevent premature mortality from invasive cancer. Therefore the strategies of screening and subsequent intervention must be well tolerated and without substantial risk. Repeated assessment may be necessary and required over prolonged time scales and the health care resource required must be measured.

2 Oesophageal Cancer

The outcomes from this disease are very poor with incidence paralleling to mortality and very little evidence that intervention for symptomatic disease has had much impact on overall disease mortality. Despite these dreadful outcomes, any screening programme must be completely informed by the very marked geographic and ethnic variation in the incidence of oesophageal cancer. In the white population of the USA the incidence per 100,000 is 5.8, in the United Kingdom it is 4.8, and in the Netherlands it is 2.5. In the West, there are some areas of moderately high occurrence including parts of France (25.5), and among the black population of Southern USA (20.5). However, in many of the

low-incidence countries, adenocarcinoma of the gastro-oesophageal is increasing dramatically. Adenocarcinoma in white Americans has increased 463% and 335% in men and women, respectively, over the past three decades (Brown et al. 2008).

The biggest problem appears to be in the United Kingdom with marked regional variation with a particular problem in Scotland around Glasgow (Wong and Fitzgerald 2005).

Overall, in the worldwide problem is that of squamous cell cancer, which predominates, particularly in the Caspian area and in Kazakhstan, where the recorded incidence reaches 547.2 per 100,000; also in the Transkei region of South Africa (357.2 per 100,000) and in Linxian Province in China (379 per 100,000). The other main high-risk groups include those with the rare autosomal dominant condition tylosis, which is associated with a 70% chance of developing a squamous cell carcinoma. Some 3–7% patients with achalasia, develop squamous cell carcinoma. Similarly, patients with an aerodigestive tract squamous cell carcinoma are more prone to develop further cancers in the region with a cumulative risk of 25% at 5 years (Barr 2008).

3 Screening for Oesophageal Squamous Cancer

Inflammation in the oesophagus caused by fumonisin, (a maize fungal toxin) nitrosamines and aflatoxins are the probable causative inflammation producing agents in Chinese patients. There is a programme for screening occurrence in the high-risk regions of China. It is based on the use of the lawang (a balloon covered with a mesh, when swallowed, it is inflated and pulls up the oesophagus) to obtain cells for cytological analysis. Those found to have neoplastic cells proceed to confirmatory endoscopy and biopsy. This regimen has a sensitivity of 80% and cure is possible in 90% of patients with early asymptomatic cancer (Barr 2008).

Food stasis and bacterial infection in achalasia cause chronic inflammation in the squamous mucosa, leading to a sequence through dysplasia to invasive cancer. Screening of these patients has demonstrated that early detection and cure is possible. The overall incidence of squamous carcinoma is 3.4/100 patients per year, being a 33-fold increase over controls (0.104/1,000 patients a year) (Katz 2008). A population-based study of 1,062 patients followed for 9,864 patient years showed a 16-fold increase in cancer. However, it was calculated that 406 endoscopies would be required to detect one cancer in men and 2,220 endoscopies for the detection of cancer in women (Sandler 1992). Therefore, endoscopic screening is left to the clinician's discretion, but it is recommended by the American Society for Gastrointestinal Endoscopy in patients with untreated achalasia for 15 years at 1–2 years intervals. If patients have had successful treatment then screening and surveillance is not recommended.

It is now becoming established that screening for early neoplastic lesion after treatment of squamous cancer can detect lesions at an early stage, and screening occurs in those interested in early detection and therapy but is not fully established

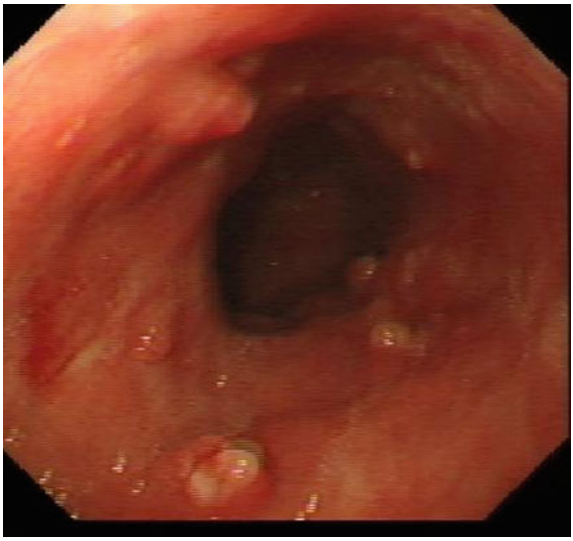

Fig. 1 Endoscopic picture of an segment of Barrett's oesophagus with the development of multiple areas of intramucosal cancer. Patient had a history of over 30 years of gastro-oesophageal reflux

(Grosjean and Monnier 1998). Certainly the few patients found to have tylosis should be screened.

4 Screening for Oesophageal Adenocarcinoma (Barrett's Cancers)

There is a clear link between gastro-oesophageal reflux disease producing chronic mucosal inflammation at the gastro-oesophageal junction progressing to adenocarcinoma. A very influential case–control Swedish study showed that recurrent reflux symptoms had an odds ratio of 7.7 for oesophageal adenocarcinoma and two for adenocarcinoma of the gastric cardia when compared with asymptomatic patients. Severe prolonged symptoms produced an odds ratio of 43.5 and 4.4 for oesophageal and cardia adenocarcinoma, respectively (Lagergren et al. 1999). The proposed sequence is of inflammation leading to metaplasia (Barrett's oesophagus), dysplasia and cancer. The aetiology of the intense inflammation at the gastro-oesophageal junction in these patients is a subject of intense study. It is now apparent that there is a highly toxic inflammatory mixture based on nitrosative chemistry that is produced may be the driver of the inflammatory mutagenesis (Suzuki et al. 2005) (Figs. 1 and 2).

Adenocarcinoma in Barrett's oesophagus has an incidence of 800 per 100,000, and should be compared with an incidence of lung cancer in men over 65, of 500 per 100,000. There is continued intense controversy over the need for screening and surveillance to detect oesophagitis, Barrett's oesophagus and early cancer, and the subsequent management of any detected pre-neoplastic lesions and early cancers (Fernando et al. 2009). More importantly national guidelines indicate that there is no evidence that screening or surveillance will save lives (Wang and Sampliner 2008).

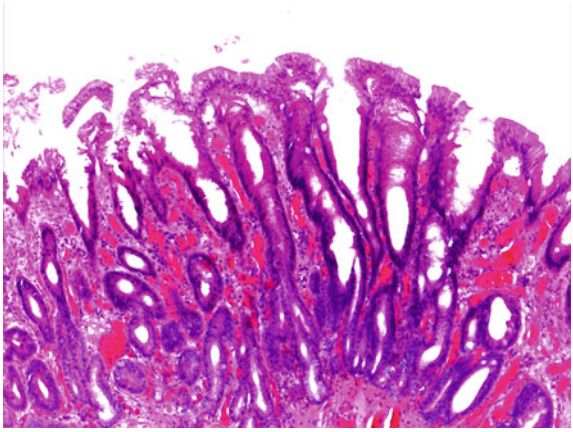

Fig. 2 Histopathological section of an area of Barrett's oesophagus showing inflammation and dysplasia

It is now clear that many patients will have no reflux symptoms prior to the diagnosis of an oesophageal adenocarcinoma (Tomizawa and Wang 2009). The two issues of whether protocol surveillance with 2 yearly endoscopy and biopsy, and if chemoprevention with aspirin and proton pump inhibitors are being addressed by large United Kingdom randomised trials. The Aspirin and Esomeprazole chemoprevention trial (ASPECT) has successfully recruited over 2,500 patients and the Barrett's Oesophagus surveillance Study (BOSS) is open and recruiting well and both will report in a few years (Jankowski and Barr 2006). Currently patients should be entered into studies and screening and surveillance is left to clinicians and patient's choice but it is suggested to occur every 2 years with protocol biopsy. Certainly in the United Kingdom there is little conviction and adherence to protocol surveillance until randomised evidence is forthcoming (Das et al. 2008).

5 Screening for Adenocarcinoma and Lymphoma of the Stomach

In worldwide terms gastric adenocarcinoma is still a common cancer. There is also marked geographical variation. The epidemic is based in Japan (70 per 100,000), with a cumulative risk of developing this cancer by the age of 75 as high as 11%. The incidence is falling in the United Kingdom and USA (age-adjusted mortality for men is 10 per 100,000). The anatomical location is also changing with carcinoma occurring at the cardia rather than more distally in the stomach. Infection and chronic gastric inflammation with *Helicobacter pylori* (*H. pylori*) is a clear causative agent (Graham and Shiotani 2005). The mechanism is through chronic inflammation, metaplastic change and then gastric atrophy. Screening by using antibody status to detect *Helicobacter* and serum pepsinogen as a marker for gastric atrophy has been examined. A group of 9,293 participants were assessed in a mass health appraisal programme and 6,983 were followed up and gastric cancer

was detected by annual endoscopy. Those with normal pepsinogen and negative for *H. pylori* had an annual incidence rate of 0.04% (0.02–0.09) compared with 0.35 (0.23–0.57) for those with 'atrophic' pepsinogen and *H. pylori* positive, and those with 'atrophic' pepsinogen and negative for *H. pylori* 0.60 (0.34–1.05). Thus screening may be possible using this combination to identify high-risk groups (Watabe et al. 2005).

In Japan, gastric cancer caused 50,562 deaths (15.85% of all cancer deaths) in 2004. The Health Service Law for the Aged was introduced in 1983 with the support of the national government. This is a mass programme of radiographic and endoscopic screening, available for all residents aged 40 or over. The method of initial screening is photofluorography. However, only 25% of cancers detected are as a result of this programme. Most cancers and early cancers are detected by an aware population requesting endoscopy in response to symptoms or health screening. The number of cancers detected at as early gastric cancer has risen from 2 to 63%, and after 25 years of the programme there has been a corresponding fall in the death rate (Hamashima et al. 2008).

In a study population of 1,625 patients over 40 in the National Cancer Screening Survey in Korea there is a preference (67%) for endoscopy when compared with radiology (33%) (Choi et al. 2009). Overall in Asia gastric cancer is the second most common cause of death, and few countries have national screening programmes. Most will adopt opportunistic screening of high-risk groups and there is few quality data to recommend changing practice (Leung et al. 2008). Prophylactic eradication of *Helicobacter* is to be recommended in patients after an endoscopic resection of an early gastric cancer as an important preventative for developing metachronous lesions (Fukase et al. 2008). Overall 2–8% of gastric malignancies are lymphomas. *Helicobacter* infection causes inflammation followed by the excessive development of mucosal associated lymphoid tissue (MALT), and lymphoma. Screening and eradication of *H. pylori* is essential. In the United Kingdom routine screening and surveillance does not form part of guidelines. However, in high-risk groups annual screening endoscopy should be considered for patients who have atrophic gastritis, dysplasia and adenomatous polyps (Allum et al. 2002).

6 Small Intestine Inflammation: Crohns Disease

The two chronic inflammatory conditions that progress to cancer are coeliac and Crohns disease. Long standing ileal inflammation is the major risk factor.

Patients with small bowel Crohns disease have an increased risk of small bowel adenocarcinoma but all series are very small and have wide variability, often including other areas of the gastrointestinal tract (3–91 fold) (Friedman 2006). The Olmsted data indicates an increased risk of 40.6 (95% CI, 8.4–118) in patients with small bowel disease (Jess et al. 2006). A meta-analysis of population-based studies reported the risk as 33.2 (95% CI, 15.9–60.9) (Canavan et al. 2006). There is perhaps a borderline increase in lymphoma and leukaemia but the data is conflicting and may be related to treatment. There is an increased risk of anal squamous cell cancers in

patients with chronic inflammation related to Crohns disease of the perineum and anal fistulae (Friedman 2006). No screening or surveillance programme is placed.

For over 40 years it has been recognised that the chronic inflammation associated with coeliac disease is associated with enteropathy-associated T-cell lymphoma (EATL). These neoplastic lesions are usually diagnosed symptomatically and after a clinical relapse and the overall survival is poor with most patients dying within 3 years. The risk of malignancy is 40–100 times higher than in the general population (Logan 2009). Recent data from a large population-based survey in Finland of 8,000 subjects demonstrated no difference in the overall risk of malignancy in the prognosis in undiagnosed coeliac subjects compared with known patients (Lohi et al. 2009). There is no basis at present for a mass screening programme to detect celiac disease at an earlier stage.

7 Inflammatory Bowel Disease: Colorectal Adenocarcinoma

The extent of the colitis is clearly an independent risk factor for the development of colorectal cancer although most cancers occur in the sigmoid colon and rectum. Pancolitis or extensive disease (variably defined as beyond hepatic flexure) has a standardised incidence ratio for developing colorectal cancer of 14.8% (95% CI, 11.4–18.9) compared with 2.8 (95% CI, 1.6–4.4) for left-sided colitis (Ekbom et al. 1990; Rubin and Kavitt 2006).

The risk of colorectal cancer in patients with inflammatory bowel disease is also related to the duration of the disease. The overall prevalence is estimated to be 3.7% (95% CI, 3.2–4.2%). For the first 10 years the incidence rate is 2/1,000 patient years, for the second decade 7/1,000 patient years, for the third decade 12/1,000 patient years. The geographical variation is important being 2/1,000 patient years in Scandinavia, 4/1,000 patient years in the United Kingdom and 5/1,000 patient years in the United States (Eaden et al. 2001).

Primary sclerosing cholangitis (PSC) confers a five-fold increase risk. This estimate follows a meta-analysis of 16,844 patients with ulcerative colitis and 560 patients with PSC (Soetikno et al. 2002; Konda and Duffy 2008). Ureterosigmoidoscopy is associated with increased incidence of colorectal cancer by 2–15%, and annual sigmoidoscopies should be performed starting 10 years after the surgery (Konda and Duffy 2008). Patients with asymptomatic HIV infection have a higher incidence of neoplastic lesions odds ration 3 (95% CI, 1.83–4.93) (Bini et al. 2009).

In the United Kingdom recommendations are for patients with ulcerative colitis and Crohns colitis. Screening should be by colonoscopy with the initial screening at 8 years for patients with pancolitis and 15 years for left sided colitis; timings are from the onset of symptoms. The frequency is 3 yearly in the second decade and 2 yearly in the third decade. If the patient has PSC and colitis, colonoscopy should be annually at the diagnosis of PSC with biopsy for every 10 cm. Following uretero-sigmoidoscopy a flexible sigmoidoscopy should be performed annually,

10 years post surgery (Cairns and Scholefield 2002). Patients who have an ileoanal pouch may be at increased risk of neoplasia in the pouch following pouchitis, and in any retained mucosal rectal cuff. In the latter circumstance should have repeated biopsies (Rubin and Kavitt 2006).

8 Conclusions

The prospect of secondary cancer prevention by intervention to control inflammation is very attractive. Trials are underway that will inform the decisions as to whether screening for and of these inflammatory conditions will prevent mortality from cancer. Ultimately the answer may well be prevention with control of inflammation at an early stage.

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Targeted Drug Therapies and Cancer

K. L. Yim and D. Cunningham

Abstract

With the progress of research in molecular biology and greater understanding of cell signalling systems emerge an increasing array of potential targets for the therapy of cancer. While traditional chemotherapy aims to elicit tumour cell death, it also produces undesirable side effects on physiologically proliferating cells. By isolating cell surface receptors which link specific intracellular secondary messenger pathways, researchers are increasingly able to define the biological network which drives cellular function. Of importance are routes involved in malignant transformation, proliferation, survival and angiogenesis. Thus targeted therapy is directed to specific differential growth processes particular to malignant tumours. The principle mode of action generally involves the "lock-and-key" mechanism and identifying the "Achilles' heel" for drug action. Various targeted agents have been studied and many have translated into significant clinical benefit. This chapter will describe some examples which illustrate the role of this approach in gastrointestinal cancers.

Contents

K. L. Yim (✉) · D. Cunningham
The Royal Marsden NHS Foundation Trust, Downs Road, Sutton, Surrey SM2 5PT, UK
e-mail: yimkl2000@hotmail.com, kein.yim@rmh.nhs.uk

D. Cunningham
e-mail: david.cunningham@rmh.nhs.uk

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1 Cell Signalling Networks and Potential Targets

Examples of potential targets include: (1) cell surface receptors, (2) receptor tyrosine kinase (RTK), (3) intracellular downstream signalling proteins and (4) anti-angiogenic agents.

Targeted therapies may thus be directed towards inhibition of the receptor-intracellular signalling axis via:

1. Inactivation of ligands by preventing binding to receptors triggering signalling pathways.
2. Binding of extracellular receptor domain and interfering with activation by the effector ligand.
3. Inhibition of intracellular tyrosine kinase domain by interrupting phosphorylation and downstream messenger initiation.
4. Disruption of intracellular signalling pathways by disrupting the downstream signal transfer circuit.

Principle of therapy extends to treatments which may also trigger apoptotic pathways and modify inflammation pathways. The range of potential targets is wide but in this chapter clinical data is highlighted to illustrate the translational approach using selected targeted therapies in the management of gastrointestinal cancer (Fig. 1).

2 Targeted Therapies in Gastrointestinal Cancers

2.1 Tyrosine Kinase Cell Receptor Families

2.1.1 EGF Receptor Inhibitor

EGFR is a member of the ErbB family of trans-membrane tyrosine kinase receptors which consists of ErbB1 (HER1, EGFR), ErbB2 (HER2, neu), Erb3 (HER3) and ErbB4 (HER4). Investigation into signal transduction processes involving EGF receptors has linked these receptors to the Ras-Raf-MEK-MAPK, PI3 K-Akt and STAT pathways leading to DNA transcription, cell cycle progression and cellular proliferation. Overexpression of EGFR occurs in a variety of tumours including head and neck, colorectal, pancreatic, lung, breast, kidney, prostate and bladder carcinomas; and HER2 in breast and gastric cancers.

Drugs which target EGFR are monoclonal antibodies (mAb) which competitively bind to the extracelluar domain, inhibiting receptor activation and downstream signalling. Cetuximab and panitumumab are two mAb EGFR inhibitors which are commonly investigated in clinical trials.

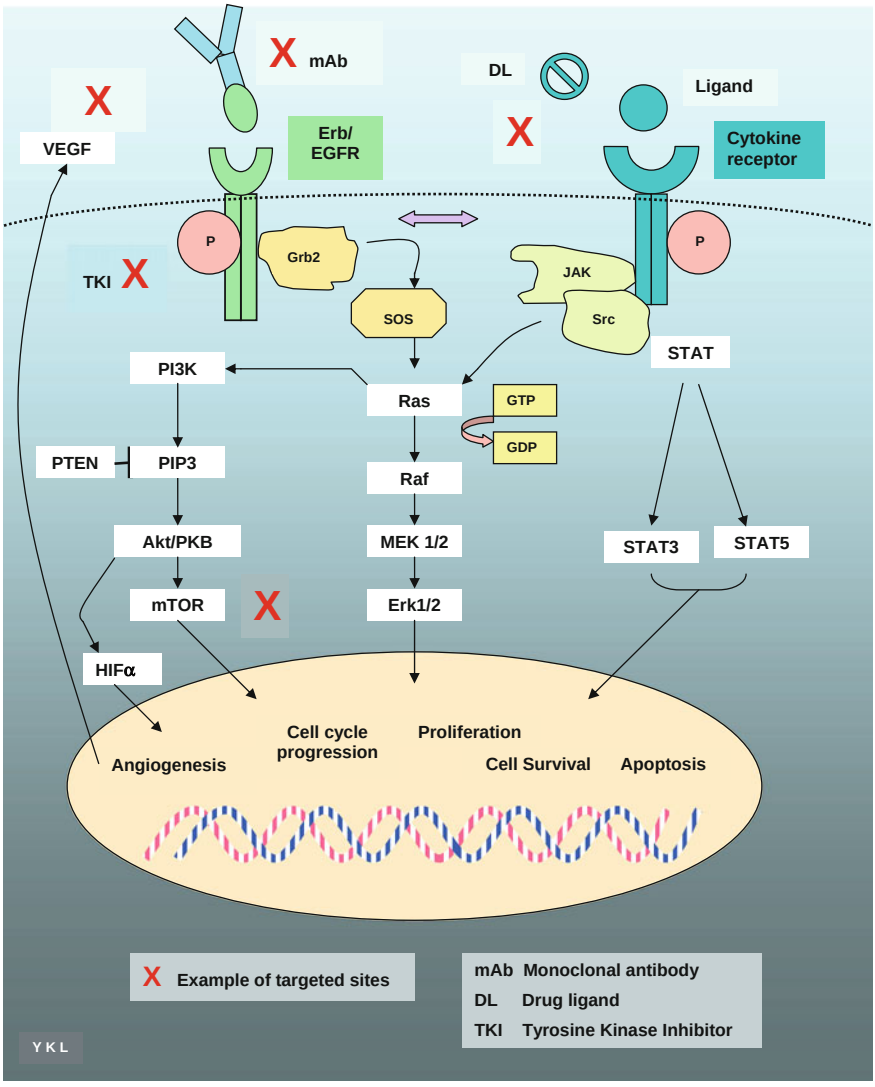

Fig. 1 Simplified example of cell surface receptors and their interaction with intracellular signalling pathways

EGFR inhibitors have been investigated in several large phase III metastatic colorectal cancer (mCRC) trials, initially in the chemo-refractory setting and more recently as part of first-line therapy. However, activating somatic *KRAS* mutation occurs in around 40% of cases and commonest mutations occur in codons 12, 13 and 61 (approximately 82, 17 and 8%, respectively) (Edkins et al. 2006). Subgroup analyses of the major randomised EGFR-therapy trials demonstrate lack of clinical benefit in tumours with *KRAS* mutation due to the evasion of upstream

EGFR blockade (Karapetis et al. 2008). This highlighted the importance of an intact receptor-signalling axis and illustrates the proof of concept in targeted therapies.

The synergistic effect of cetuximab when used in combination with chemotherapy was first reported in the BOND trial which compared single agent cetuximab with cetuximab in combination with irinotecan for patients who had progressed on irinotecan-based regimens. Significant improvement in response rate (22.9 vs. 10.8%), median time to progression (4.1 vs. 1.5 months) and median survival (8.6 vs. 6.9 months) (Cunningham et al. 2004) was achieved, demonstrating the ability of cetuximab to reverse irinotecan-resistance and also for monotherapy activity in patients resistant to conventional lines of treatment. In the EPIC (Sobrero et al. 2008) trial, cetuximab was used in combination with irinotecan in the second-line treatment of patients with mCRC. Compared to irinotecan alone, statistically significant improved response rate and progression-free survival (PFS) were achieved (16.4 vs. 4.2% and 4.0 vs. 2.6 months, respectively), reinforcing the concept of reversal of chemo-resistance. However, there was no difference in overall survival (OS). This may have been due to treatment cross over between the arms.

The importance of *KRAS* mutation emerged from subsequent trials. When treatment with cetuximab monotherapy plus best supportive care (BSC) was compared to BSC alone, patients with *KRAS* wild-type (wt) tumours had significantly better median OS (9.5 vs. 4.8 months, $p < 0.0001$) and median PFS (3.7 vs. 1.9 months, $p < 0.0001$) (Karapetis et al. 2008) with EGFR therapy. Similar improvement in PFS but not OS was also found in *KRAS* wt patients treated with panitumumab monotherapy versus BSC (3.0 vs. 1.8 months, $P < 0.001$) (Amado et al. 2008).

Subsequent trials incorporated EGFR-targeted therapy with combination chemotherapy in the first-line treatment of patients with mCRC. The CRYSTAL trial (Van Cutsem et al. 2009a) randomised 599 chemotherapy-naïve patients with mCRC to FOLFIRI with cetuximab versus 599 patients to FOLFIRI alone. Treatment with EGFR inhibition led to an improved hazard ratio for PFS in patients with *KRAS* wt (0.68; 95% CI, 0.50–0.94) but not *KRAS* mutant (mt) tumours. The OPUS trial randomised 337 patients to cetuximab in combination with FOLFOX-4 versus FOLFOX-4 alone. Benefit was again limited to the *KRAS* wt, but not *KRAS* mt group confirming an improved chance of response (ORR = 61 vs. 37%; odds ratio = 2.54; $p = 0.011$) and a lower risk of disease progression (HR 0.57; $p = 0.0163$) in favour of anti-EGFR therapy.

However, no significant survival benefit from the addition of cetuximab to FOLFOX was seen in the UK MRC COIN trial (Adams 2009). Early data suggested a trend to inferior survival in patients with *KRAS* mt tumours treated in the cetuximab combination arm. Together with a significantly shortened PFS in the subgroup of patients with *KRAS* mt tumours from the OPUS trial, this suggests a possible negative interaction between oxaliplatin and anti-EGFR-targeted therapy not seen with irinotecan-based combination chemotherapy (see Sect. 3).

Resection of liver-only metastases in CRC improves survival (Giacchetti et al. 1999; Adam et al. 2004). In patients with unresectable hepatic metastases, cetuximab was shown to increase response rate when added to FOLFIRI (59.3%) (Van Cutsem et al. 2009a). However, when added to FOLFOX, overall response rate of up to 85% was achieved in the randomised phase II CELIM trial, leading to a resection rate of 40%. Activity in *KRAS* wt tumours was 79% with cetuximab. In the UK, the use of cetuximab combined with chemotherapy in patients with liver-only metastases has been approved by NICE.

2.1.2 HER2 Receptor Inhibitor

It is estimated that between 6 and 35% of oesophageal and gastric malignancies overexpress HER2. In the first phase III prospective randomised ToGA trial (Van Cutsem et al. 2009b) investigating HER2 inhibition in locally advanced or metastatic gastroesophageal and gastric adenocarcinomas, histological samples were found to be HER2 positive in 22.1% of 3,807 patients (Bang et al. 2009). A modified HER2 score was used and HER2 positivity was defined as IHC 3+ and/or FISH positive.

Five hundred and ninety two patients with HER2 positive tumours were randomised to receive 3-weekly fluoropyrimidine (5-fluorouracil or capecitabine) with cisplatin alone or in combination with trastuzumab. Results were in favour of the trastuzumab arm showing a significant improvement in OS of 2.4 months (13.5 vs. 11.1 months, respectively) with no unexpected adverse events or difference in symptomatic congestive cardiac failure. Results of this trial suggest the benefit of anti-HER2 targeted therapy as a treatment option in the management of these patients.

2.1.3 IGFR-1 Receptor Inhibitor

Another class of tyrosine kinase transmembrane receptor is the IGF-1R which is activated by IGF-1 and 2 ligands. It is structurally similar to the insulin receptor and share the IRS-1 and 2 substrate transmission proteins. However, IGF-1R has been shown to trigger the Ras-Raf-MEK-MAPK and PI3 K-Akt pathways leading to a wide range of cellular activity including growth, differentiation and survival. IGF-1R is known to be overexpressed in gastric, colorectal and pancreatic cancers leading to increased risk of metastases and worse clinical outcome.

In gastric cancer, a positive correlation between overexpression of IGF-1R and rate of nodal metastases in gastric cancer was found (Guo et al. 1995). IGF-1R is also implicated in the pathogenesis of colorectal cancers (Guo et al. 1995; Zecevic et al. 2006). The risk of developing bowel malignancy is known to be increased in patients with acromegaly. This is associated with high growth hormone and IGF-1 levels. IGF-1 may be overexpressed in up to two-thirds of colorectal cancer and can independently affect tumour progression (Scartozzi et al. 2009).

Various IGF-1R receptor antibodies and tyrosine kinase inhibitors (TKI) have been developed and are undergoing clinical trials. In vitro study using IR3 antibody directed to IGF-1R in gastric carcinoma resulted in a reduction of cell colony count (Pavelic et al. 2002). In colorectal cancer, a current phase II/III trial investigates anti-IGFR antibody MK-0646 in conjunction with cetuximab and irinotecan.

This combination was found to be tolerated and further results on PFS and correlation with *KRAS* status is pending (Watkins et al. 2009). In pancreatic cancer, a dose-response relationship was found in cell line studies when treated with single agent IGF-1R antibody and synergistic effect was observed when IGF-1R blockade was combined with gemcitabine and panitumumab (Beltran et al. 2009).

IGF-1R also interacts with other receptors involved in malignant transformation including EGFR and VEGF. A study on surgical specimens demonstrated correlation between membrane-dominant dominant EGFR/cytoplasmic-dominant IGF receptor with lower grade and prognosis, and cytoplasmic-dominant EGFR/membrane-dominant IGF receptor with higher grade and worse prognosis. In addition, IGF-1R and EGFR overexpression was also more frequent in liver metastases compared to pancreatic primaries (Ueda et al. 2006).

2.2 VEGF Inhibitor

With tumour cell proliferation, angiogenesis is essential for continued growth. This process is mediated by the VEGF family of circulating angiogenic ligands (VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and placenta growth factor PlGF). The ligands exert its effect on corresponding VEGF receptors VEGF-R1 (migration, invasion), VEGF-R2 (proliferation, survival) and VEGF-R3 (lymphangiogenesis). These processes are not only involved in inflammation and wound healing, but are also activated in malignant angiogenesis. In addition, VEGF plays an important role in the orchestration of tumour survival including inhibition of apoptosis, migration and invasion.

Bevacizumab is an antibody to VEGF-A. Curbing the development of tumour blood vessels, it also normalises vessel blood flow through vasoconstriction, reducing hypoxia and expression of HIF-1. It was one of the earliest to be studied in colorectal cancer improving median survival from 15.6 to 20.3 months when used in combination with first-line 5-fluorouracil and irinotecan (Hurwitz et al. 2004). A recent Cochrane analysis showed overall benefit of bevacizumab (Wagner and Moehler 2009). Although it has little efficacy as monotherapy, improvement in PFS and OS was evident when used in combination with chemotherapy. The benefit of anti-VEGF therapy was also found in patients $\geq$65 years old (Kabbinavar et al. 2009). Allowing for heterogeneity in treatment duration and chemotherapy platform using oxaliplatin-based regimens, improvement in HR for PFS (0.61; 95% CI 0.45–0.83) and OS (0.81; 95% CI 0.73–0.90) was demonstrated in the first-line setting. The value of continuing bevacizumab beyond progression was suggested by the observational BRiTE study. Although not randomised, median OS rates were 12.6 months in patients who stopped treatment, 19.9 months in those who continued chemotherapy without bevacizumab and 31.8 months in those who continued chemotherapy with bevacizumab (Grothey et al. 2008). In the second-line setting, a large trial using FOLFOX with bevacizumab demonstrated a in HR for PFS (0.61; 95% CI 0.51–0.73) and OS (0.75; 95% CI 0.63–0.89) (Giantonio et al. 2007).

However, early data on bevacizumab in the adjuvant scenario showed it to be of little advantage. The NSABP-C08 phase III trial comprising of 2,672 patients with stage II or III colorectal cancer did not demonstrate benefit in terms of PFS after a year of anti-VEGF treatment in addition to FOLFOX (Wolmark et al. 2009).

2.3 Small Molecule TK Inhibitor

Signal transduction occurs at the intracellular domain when the receptor is triggered by their complementary ligand. This leads to downstream phosphorylation of substrate proteins and subsequently activates the network of signal transduction pathways which regulate important cell functions. TKI block this key activation process and were the forerunners of targeted therapy in cancer.

2.3.1 Imatinib

Kit and PDGFR display extensive structural homology and are members of the type III tyrosine kinase receptor family. TKIs which act on these sites were among the earliest described and provided the initial proof of concept. STI-571 (Imatinib mesylate) has binding activity to sites including Kit, Bcr-Abl and PDGF domains. It was tested in advanced GIST tumours which express the kit (Hirota et al. 1998) mutation. Early studies showed a response rate exceeding 80% and median PFS of over 24 months could be achieved. The commonest mutation occurs at the exons 11 (70%) and 9 (15%) but other sites including exons 13, 14, 17 as well as PDGFRA (exons 12, 18) (Heinrich et al. 2003) have been reported. Exon 11 encodes for the intracellular autoinhibitory juxta-membrane domain, while exon 9 encodes for the distal part of the extracellular domain. In addition, exons 13/14 and 17 encode for the drug/ATP binding pocket and activation loop, respectively. Differential mutations at these sites predict for objective response—71.7% for exon 11 versus 44.4% for exon 9, as well as clinical outcome where presence of exon 11 mutation led to a 5-year recurrence-free survival of 89 $\pm$ 10%, but only 40 $\pm$ 8% if other mutations were found ($p = 0.03$) (Singer et al. 2002). Doubling the dose of imatinib to 800 mg improved PFS for patients with exon 9 mutation (Verweij et al. 2004; Van Glabbeke et al. 2007). Secondary mutations in exons 13/14 and 17/18 led to resistance. Clinical observation suggested that abrupt withdrawal of the drug could result in accelerated progression. This may reflect heterogeneity of the tumour where previously imatinib sensitive malignant clones encounter resurgence.

2.3.2 Sunitinib

Sunitinib is a TKI with activity against Kit (CD117), PDGF-Rβ and VEGFR. In patients with GIST who progress on imatinib, sunitinib has shown to be effective as second line therapy (Demetri et al. 2006, 2009; Heinrich et al. 2008). Although it demonstrated in vitro activity in wild-type, exons 11 and 9 mutated tumours, benefit in clinical trials translated into the exon 9 group. This is

postulated to be due to dimerisation of the receptor as a distinct mechanism of activation compared to exon 11 mutations (Dibb et al. 2004; Yuzawa et al. 2007). However, clinical trials will be required to test sunitinib TKI naïve GIST to exclude selection bias.

2.3.3 Sorafenib

Sorafinib is active against Raf kinase, PDGFR, VEGF receptor 2 and 3 kinases and c-Kit. Targeting signalling through the Ras-Raf-MAPK-Erk pathway, it has been studied in a phase III trial (Llovet et al. 2008) in advanced hepatocellular carcinoma (HCC). Patients were randomised to receive sorafenib or placebo. In the treated group, a significant improvement in median OS and median time to radiological progression was found (10.7 vs. 7.9 months, 5.5 vs. 2.8 months, respectively).

A separate analysis of signalling pathway proteins in HCC tumour samples show activation of Erk pathway correlated with low levels of nuclear β-cat and high levels of *p*-mTOR, distinguishing them from normal liver parenchyma. Better survival of 6 months or more in patients treated with sorafenib was linked to tumours with a high level of nuclear *p*-Erk with low level of *p*-Akt and *p*-GSK3β in tumour cells. In addition, high levels of VEGFR, *p*-Erk and *p*-Src in endothelial cells were associated with high microvessel density and micrometastases (Ji et al. 2009).

2.3.4 Erlotinib

Erolitnib was the first targeted therapy to show survival benefit in a phase III trial involving advanced pancreatic cancer. Compared to gemcitabine alone, it led to a modest but statistically significant improvement in survival at 12 months (23 vs. 17%) and HR for OS (0.82; 95% CI, 0.69–0.99, $p = 0.038$) (Moore et al. 2007).

However, interesting questions remain relating to the mechanism of action. *KRAS* mutation was found in 79% of patients in the trial and analysis of subgroups shows advantage irrespective of EGFR expression, given variation in laboratory testing techniques. One hypothesis is anti-EGFR therapy targets EGFR expressing endothelial cells in the tumour microenvironment stimulated by EGF-like peptides expressed at high levels in pancreatic cancer (Bruns et al. 2000; Normanno and De Luca 2007; Salomon et al. 1995). Although erlotinib may play a part in angiogenesis, it may be an early indirect effect on cellular proliferation that limits the condition conducive to VEGF production. This may explain the negative results in large phase III clinical trials where the addition of bevacizumab to gemcitabine and erlotinib did not result in OS benefit (Vervenne et al. 2008; Van Cutsem et al. 2009c). An on-going phase I/II TARGET trial addresses the efficacy and safety of gemcitabine and capecitabine together with erlotinib and bevacizumab.

2.3.5 Gefitinib

Overexpression of EGFR has been found to be a negative prognostic factor in oesophageal carcinoma (Yacoub et al. 1997). Several non-randomised phase II trials investigating gefitinib in patients with advanced oesophageal carcinoma

demonstrated an overall response rate between 30 and 58% (Janmaat et al. 2006; Ferry et al. 2004, 2007). Currently the UK COG trial is underway randomising patients between gefitinib and placebo in patients with relapsed advanced oesophageal cancer.

3 Network Interactions and Resistance to Targeted Therapies

The interaction of various signalling networks suggests that tumours may escape interruption of downstream signalling by TKI through by-passing blocked channels. This re-routing of messaging system enables continued progression despite disabling a known target. The interplay of signalling pathways is more elaborate than initially thought and appears to contribute to tumour progression and resistance to therapy.

However, current trials show that efficacy is not necessarily improved by combining targeted drugs. For example, two CRC trials using VEGF together with EGFR-directed mAbs and chemotherapy demonstrate a detrimental effect on survival (Hecht et al. 2009; Tol et al. 2009). Apart from increased toxicity, combining targeted agents worsened survival especially in patients with *KRAS* mt tumours as confirmed on meta-analysis (Zhou et al. 2009). The choice of chemotherapy platform may also have a negative impact. In the OPUS trial where FOLFOX-4 was used in combination with cetuximab, patients with *KRAS* mt tumours had a trend to reduced median PFS compared to FOLFOX-4 alone (5.5 vs. 8.6 months, HR 1.83; 95% CI, 1.095–3.056, $p = 0.192$) (Bokemeyer et al. 2009). A negative effect of reduced median PFS was again seen when FOLFOX was added to panitumumab in the PRIME study (7.3 months in chemotherapy-only arm vs. 8.8 months for combination treatment; HR 1.29; 95% CI, 1.04–1.62, $p = 0.0227$) (Douillard 2009). Early results from the COIN study (Adams 2009) investigating FOLFOX or CAPOX in combination with cetuximab suggested a worse outcome with the capecitabine combination. These trials indicate a negative interaction between targeted agents and oxaliplatin or capecitabine. The mechanism of interaction is of considerable interest and remains to be elicited.

Forty to seventy percentage of *KRAS* wt tumours do not benefit from EGFR inhibition. The varied permutation and interaction between signalling pathways is only beginning to be understood. In a report investigating chemorefractory mCRC found mutations in *KRAS*, *BRAF* and *NRAS* were mutually exclusive and occur independently in over 47% of cases (Lambrechts et al. 2009). *BRAF* wt also conferred a significantly better PFS and OS. Mutation in *BRAF* and *NRAS* occurred in 9.8 and 5% of *KRAS* wt CRC tumours, respectively. Best outcome was associated with *KRAS/BRAF/NRAS* wt. Twelve percentage carried the independent PI3 K mutation but this was not correlated with tumour response or outcome. In advanced gastric cancer, however, *KRAS* and *BRAF* status was not predictive of response to cetuximab (Stella et al. 2009). In addition, mutation rates of *KRAS*

were lower compared to colorectal tumours at 11.4 and 2.3%, respectively. Similarly *KRAS* mutation was found in only 12% of cholangiocarcinoma and did not impact on treatment response to EGFR inhibition (Gruenberger et al. 2009).

Interaction between receptors may also impact on prognosis. In a study of 66 patients with mCRC receiving irinotecan and cetuximab, partial response rate was 50% in *KRAS* wt and IGF-R1 tumours but 5% with a co-existent IGF-R1 mutation. A statistically significant difference in time to tumour progression was also found (11 vs. 3.2 months). Thus IGF-R1 status is implicated as a predictive factor for resistance in tumours treated with anti-EGFR therapy (Scartozzi et al. 2009).

Mutations in exons 13/14 may be coexpressed with exon 17 in GIST tumours. Although both are involved in signal transduction, they occur in distinct parts of the pathway. The former involves changes in the ATP binding site and the latter in the ATP activation loop. However, they are linked by a common primary mutation (V560D). Thus stopping treatment directed at one arm of the mutational defect due to development of a second defect at the first sign of resistance may also disinhibit activity in the second mutational site leading to rapid tumour growth. For example, although tumours resistant to imatinib due to secondary mutation in exons 13/14 were sensitive to second-line sunitinib, this mutation was coexpressed as a mutation in exon 17 (V560D) which was not susceptible to either drugs (Heinrich et al. 2008). Therefore removing collateral inhibition may result in tumour flare.

4 Conclusion

The role of targeted treatment in cancer has developed rapidly in recent years. With greater understanding of the biology of various cancers, new drugs have been developed and tested in the clinical setting demonstrating efficacy and extending the treatment paradigm beyond that of traditional chemotherapy. Current data bridging in vitro studies and clinical trials demonstrate the mechanism of action of targeted therapy is more than just a proof of concept in translational cancer research. However, understanding the interplay between numerous receptor-signalling pathways has presented to us a bigger challenge than simply addressing individual targets. More research is warranted both to greater understand the basic molecular biology of cancer as well as to develop further randomised clinical trials in addressing the potential of targeted therapy in this era of biological treatment in cancer.

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Genetics of Inflammation in the Gastrointestinal Tract and How it Can Cause Cancer

Mairi H. McLean and Emad M. El-Omar

Abstract

Genetic epidemiology is an important discipline that is helping to unravel the aetiology and pathogenesis of complex human diseases. In the context of gastrointestinal malignancy, the paradigm model of host genetic influence on disease outcome is *H. pylori*-associated gastric adenocarcinoma. This cancer represents a classic example of an inflammation-induced malignancy and highlights the importance of host genetics in disease development. This chapter gives an insight into how genetic epidemiology can play an important role in the development of gastric cancer. Increasing our understanding of host genetics in cancer development may allow particularly susceptible individuals to be targeted for screening or treatment to reduce risk of future malignant transformation.

Contents

M. H. McLean · E. M. El-Omar (✉)
Gastrointestinal Research Group, Division of Applied Medicine,
School of Medicine and Dentistry, Institute of Medical Sciences,
Aberdeen University, Foresterhill, Aberdeen, Scotland, AB25 2ZD, UK
e-mail: e.el-omar@abdn.ac.uk

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Almost 150 years ago, Rudolf Virchow identified leucocytes within neoplastic lesions and hypothesised that malignant change arose within areas of chronic inflammation. This was assumed to be a consequence of the cancer process per se. The last few decades, however, have seen a surge in research interest in the relationship between inflammatory pathways and malignancy (Balkwill and Mantovani 2001; Coussens and Werb 2002; Macarthur et al. 2004). Certainly, the understanding of the mechanisms of inflammatory activity and its role in development of disease has escalated and there can be no doubt that carcinogenesis in a variety of body sites are related to tissue inflammation. Concentrating on the gastrointestinal tract, this is particularly evident in *Helicobacter pylori* (*H. pylori*)-associated gastric cancer, chronic hepatitis C-associated hepatocellular carcinoma and inflammatory bowel disease associated colorectal cancer. This chapter is focussed on discussing the influence of genetic makeup on the process of inflammatory linked cell transformation to cancer.

It must be appreciated that genetic epidemiology is only one part of the jigsaw, a contributing part of the complex web of multifactorial influences determining outcome in human disease processes. A strategy employing population-based genetic epidemiology calculates risk of developing a particular disease in relation to host genotype, and has a particular role in unravelling the complexities of common chronic diseases with strong environmental influences. Specifically, single nucleotide polymorphisms of pro-inflammatory genes in the context of host susceptibility have been extensively studied. SNP's are 'common' mutations, occurring at >1% within the general population, and describes the variation in DNA sequence between the two alleles of a particular gene. Differing genotypes can manipulate phenotypic character and therefore be associated with risk of developing overt pathology, in specific racial groups. Overall, it is estimated that there are over 10 million SNPs within the human genome and this is probably just the tip of the iceberg, with a large number not identified as yet. Luckily, the majority of these will not have functional consequence, occurring either in non-coding sections of DNA or having no influence on the final protein product. However, it is appreciated that carriage of a particular genotype can lead to downstream functional consequences, either silencing or promoting the expression of downstream gene targets that can positively influence pathogenesis of disease.

1 *Helicobacter pylori* and Gastric Cancer

In the context of gastrointestinal malignancy, the paradigm model of host genetic influence on disease outcome is *H. pylori*-associated gastric adenocarcinoma. This cancer represents a classic example of an inflammation-induced malignancy, and highlights the importance of host genetics in disease development. For this reason, this chapter will focus on *H. pylori*-induced gastric cancer to illustrate the role of host genetics in the pathogenesis of disease. Gastric adenocarcinoma remains a major health problem worldwide, with 900,000 new cases diagnosed in 2002, mainly in Eastern Asia and with increasing incidence in the developing world

(Parkin et al. 2005). Eight thousand new cases are diagnosed each year in the UK, and despite advances in treatment, the prognosis remains poor. Clearly, this disease carries significant burden in terms of patient morbidity, mortality and healthcare economics. A major advance in the fight against this malignancy came with the recognition of the role of *H. pylori* infection in its pathogenesis. *H. pylori* is a Gram-negative, urease-positive bacillus, acquired during childhood, probably via the faecal–oral or gastric–oral routes. This infection persists throughout life unless treated with an antibiotic-based eradication regime. The bacteria mainly reside on the surface mucus gel layer, with little invasion of the gastric glands (Amieva and El-Omar 2008). Despite this, the host responds with an impressive humoral and cell-mediated immune response. In most cases, spontaneous clearance is rare leading to chronic carriage of the bacteria. Persistent carriage of the bacteria in itself does not necessarily lead to development of gastric malignancy, at least in the majority of cases. It is now well established that three separate phenotypic outcomes exist. The majority of individuals colonised with *H. pylori* will be asymptomatic and will not develop clinically significant disease. In this group, carriage of the bacteria could be described as commensal. In contrast, chronic carriage can be pathogenic, resulting in one of two significant disease phenotypes. The first of these is antral-predominant gastritis, associated with high gastric acid secretion and duodenal ulceration, the so called 'duodenal ulcer phenotype'. Alternatively, an infected individual may develop corpus predominant chronic inflammation, associated with gastric atrophy and hypochlorhydria, a pre-malignant phenotype, associated ultimately with the development of gastric adenocarcinoma (Amieva and El-Omar 2008).

These diverse clinical outcomes of chronic *H. pylori* infection are mutually exclusive with the development of the 'duodenal ulcer' phenotype protective against subsequent gastric malignancy (Hansson et al. 1996). Therefore, it is clear that infection with *H. pylori* can lead to several diverse disease phenotypes and it is now known that host genetic factors play a key role in determining outcome. Specifically, the course and magnitude of cytokine driven inflammatory response, induced and perpetuated by chronic *H. pylori* colonisation is largely dependent on genetic susceptibility. Overall, genetic variation in important cytokine genes, both pro- and anti-inflammatory, impart an important influence on the final outcome.

2 Role of IL-1β in Gastric Cancer Risk

The pro-inflammatory cytokine IL-1β is recognised as an important candidate in the development of gastric malignancy, reflecting profound pro-inflammatory action, up-regulation in response to *H. pylori* infection, and potent gastric acid suppression (El-Omar et al. 2001). The IL-1 gene cluster lying within a 430 kb region on chromosome 2q contains three related genes, namely, *IL-1A, IL-1B* and *IL-1RN*, which encode for pro-inflammatory cytokines IL-1α and IL-1β as well as their endogenous receptor antagonist IL-1ra, respectively. Three diallelic polymorphisms at the *IL-1B* loci have been reported, all representing C–T or T–C transitions, at positions −511, −31, and +3,954 bp from the transcriptional start

site (Bidwell et al. 2001). The *IL-1RN* gene has a penta-allelic 86 bp tandem repeat (VNTR) in intron 2.

In a Caucasian population of gastric cancer relatives, polymorphisms in the *IL-1* gene cluster were found to be associated with increased risk of developing pre-malignant gastric changes of gastric atrophy and hypochlorhydria, in response to *H. pylori* infection (El-Omar et al. 2000). Specifically, carriage of the *IL-1B*-31*C or -511*T and *IL-1RN**2/*2 genotypes were associated with these pre-malignant gastric changes. This association also extends into the development of overt non-cardia gastric adenocarcinoma, with an estimated odds ratio of 1.6 (95% CI, 1.2–2.2) and 2.9 (95% CI, 1.9–4.4) for carriage of IL-1β-31*C/-511*T and IL-1RN*2/*2, respectively (El-Omar et al. 2000). In terms of other upper gastrointestinal tract malignancies, this pro-inflammatory genotype was not associated with gastric cancer of the cardia, or oesophageal malignancy of either glandular or squamous origin (El-Omar et al. 2003). This is in keeping with the underlying physiological consequence of enhanced IL-1β signalling and particularly, reflects its potent acid inhibitory action. These findings have been confirmed independently in both Caucasian (Figueiredo et al. 2002; Machado et al. 2001) and other ethnic groups, specifically Asian (Furuta et al. 2002; Zeng et al. 2003) and Hispanic (Garza-Gonzalez et al. 2005) populations. Machado et al. were the first to confirm the association between *IL-1* gene markers and gastric cancer in Caucasians and reported similar results to those reported by the initial study. They then later investigated the combined influence of pro-inflammatory *IL-1* genotypes and *H. pylori* bacterial virulence factors (*cagA* positive, *VacA s1* and *VacA m1*), highlighting a potentially important interaction between host and bacterium in the pathogenesis of gastric cancer. Specifically, combination of high-risk bacterial/host genotype conferred the greatest risk of developing gastric malignancy (Figueiredo et al. 2002).

Not all studies, however, have confirmed the association between genetic variability of the *IL-1* loci and gastric cancer. There are a number of reasons why this may be the case. Firstly, the reported discrepancies may reflect issues with study design, such as use of inappropriate controls or an underpowered study population. Overall, within the last few years, meta-analyses of *IL-1B* genotype as a risk factor for gastric cancer have been published (Wang et al. 2007; Camargo et al. 2006; Kamangar et al. 2006) and these suggest that genetic variation in the pro-inflammatory gene, *IL-1*β, is a risk factor for developing gastric malignancy. However, it is likely that there are other influences. Positive associations between genotype and disease appear to be demonstrated more readily in low incidence compared with high incidence areas, suggesting that background prevalence of gastric cancer in a population is an important factor when carrying out these studies. The haplotype context is also an important consideration for genetic risk associations. This describes the relationship between haplotype structure and gene regulation. Specifically, the question of whether individual SNPs within a gene promoter region (*IL-1*β in this case) might affect promoter function was raised. It has also recently been recognised that potential synergy between two or more genetic markers may influence phenotypic outcome. For example, association between carriage of *IL-1B*-31C allele and gastric cancer was identified but only in individuals with *GTF2A1* GG genotype (Lee et al. 2007).

3 Confirmation of the Role of IL-1β in Gastric Cancer Pathogenesis

A crucial piece of evidence that confirmed the apparent role of IL-1β in *H. pylori*-induced gastric carcinogenesis came from a transgenic mouse model in which IL-1β overproduction was targeted to the stomach by the H +/K + ATPase beta promoter (Tu et al. 2008). With overexpression of IL-1β confined to the stomach, these transgenic mice had a thickened gastric mucosa, produced lower amounts of gastric acid and developed severe gastritis followed by atrophy, intestinal metaplasia, dysplasia and adenocarcinoma. Crucially, these IL-1β transgenic mice proceeded through a multistage process that mimicked human gastric neoplasia. These changes occurred even in the absence of *H. pylori* infection, which when introduced led to an acceleration of these abnormalities. Most interestingly, the pathological changes including the progression to gastric cancer were prevented by infusion of interleukin-1 receptor antagonist, proving beyond doubt that IL-1β is responsible for the pathological effects (Tu et al. 2008).

From these initial studies, interest in the role of host genetics in the pathogenesis of gastric cancer escalated to include other key inflammatory genes. The target candidate genes under investigation mirror the cytokine response evoked by *H. pylori* infection and included potent pro-inflammatory cytokines, such as TNFα, IL-8 and IL-6 and also genes encoding for key inflammatory mediators such as COX-2 and MMP-9. There have been many other studies. The list of genes investigated is extensive and we have chosen to expand on a few of these to illustrate the expanding knowledge base in this field.

4 Tumour Necrosis Alpha (TNF-α)

TNF was recognised as a potentially important cytokine in the development of gastric malignancy, due to its powerful pro-inflammatory action and acid-inhibitory effect, albeit weaker than that of IL-1β. The G > A polymorphism at position −308 within the TNF-α gene was identified as significant, with carriage of the pro-inflammatory A allele associated with a 2-fold increase in malignant phenotype (El-Omar et al. 2003). This finding was subsequently reproduced in a population of similar ethnicity (Machado et al. 2003), and a recent meta-analysis confirmed the role of *TNF-A* polymorphisms in gastric cancer risk (Gorouhi et al. 2008).

5 Interleukin-8

Interleukin-8 (IL-8) is a potent pro-inflammatory cytokine, involved not only in recruitment of neutrophils and macrophages at the site of inflammatory activity, but also exerting a multitude of additional functional capabilities, many central to

tumour biology. IL-8 is mitogenic and angiogenic, through linked expression of MMP's, namely MMP-2 and 9, influences tumour cell motility so that tumour invasion is enhanced. IL-8 has been found to be increased in a number of cancers, including those of the GI tract (Xie 2001). There is a well defined promoter T > A polymorphism on the IL-8 gene at position −251. Functionally, carriage of the A allele results in enhanced IL-8 expression within the gastric mucosa of *H. pylori* infected individuals. The association of this genotype to overt gastric malignancy however, remains under debate, with both positive and negative associations reported across different ethnic groups (Ohyauchi et al. 2005; Savage et al. 2006; Taguchi et al. 2005) .

6 COX-2 and MMP-9

Cyclo-oxygenase (COX) is the key rate limiting enzyme in arachidonic acid metabolism, resulting in the production of many active prostaglandins, prostacyclins and thromboxanes. There are two recognised isoforms. COX-1 is constitutively expressed in normal cellular homeostasis. In contrast, COX-2 is inducible and expressed in response to mainly inflammatory stimuli, growth factors and mitogens. It is now widely recognised that COX-2 activity is implicated in a wide range of cell processes that are central to cell function and life cycle, such as angiogenesis, cell proliferation, inflammatory response and apoptosis (Wang and DuBois 2006). Clearly, this could play an important role in the development of malignancy. COX-2 is known to be up-regulated in gastric carcinoma. Sitarz et al. (2008) found a positive association between gastric cancer and carriage of the G allele at position −765 of the COX-2 gene in a Dutch population. Overall, a systematic review published recently, assessing the role of 17 SNP's within the *COX-2* gene on gastrointestinal malignancy, concluded that the polymorphisms found at positions −1329A, −899C and *429TT were associated with up to a 3-fold increased risk of gastric cancer (Pereira et al. 2010).

MMP-9 is a key inflammatory mediator released from neutrophils and macrophages within areas of inflammatory activity and has been implicated in carcinogenesis at a number of body sites, mainly due to breakdown of collagen matrix, supporting tumour cell invasion and exerting a positive influence on angiogenesis. MMP-9 has been associated with pathobiological behaviour of gastric malignancy, including tumour size, invasion and lymphatic metastasis (Zheng et al. 2006). Recently, a study on a Chinese population identified a 3-fold increase risk of developing lymph node metastasis from primary gastric cancer with carriage of 2 SNP's within the *MMP-9* gene (Tang et al. 2008).

When concurrent carriage of pro-inflammatory alleles in up to four genes (*IL-1B*, *IL-1-RN*, *TNF-A and IL-10*) was assessed in relation to risk of gastric cancer, the risk progressively increased along with an increasing pro-inflammatory genotype, such that, when three or four of the pro-inflammatory alleles were present, risk increased to 27-fold (El-Omar et al. 2003).

7 Role of Innate Immune Response Genes

Overall, in considering the pathogenesis of this disease and the role of genetic variation within this process, researchers have investigated both bacterial variability within microbial virulence factors as well as the host cytokine response to persistent infection. In addition, the investigative focus has included the initial contact between bacterium and host, and it is now clear that genetic variation within the adaptive immune response involved in this initial interaction is an important consideration. Lipopolysaccharide (LPS) is found in the cell membrane of Gram-negative bacteria, including *H. pylori*, and binds to trans-membranous pattern-recognition receptor, *TLR*-4, expressed on immune cells of the adaptive immune system, namely macrophages. Binding to this receptor activates a signalling cascade involving MyD88, IL-1 receptor associated kinase and TRAF6, to activate NFkB and mitogen-activated protein kinase pathways, which results in a plethora of cytokines and pro-inflammatory mediators being released into the environment. There are functionally relevant polymorphisms in the *TLR*-4 gene that profoundly alters host response to bacterial challenge. In particular, an A–G transition at position +896 on exon 4 leads to change in protein sequence, specifically replacement of aspartic acid to glycine at position 299. This ultimately alters the extracellular domain of the receptor and its overall function, renders the cell hyporesponsive to an LPS challenge through inhibition of ligand binding, inhibition of protein binding and by altering transport of the receptor to the cell membrane, reducing capacity of the cell to deal with pathogenic challenges. Initially, the immune response is jaded. However, overall, due to a reduction in IL-10 secreting regulatory cells, the immune response that is able to be activated through alternative signalling mechanisms becomes overwhelming and exaggerated in magnitude. Not surprisingly, it was hypothesised that defective signalling through TLR-4/*H. pylori* interaction associated with carriage of genetic variation could lead to an exaggerated immune response to this organism and contribute to the development of gastric malignancy. Indeed, it has been found that carriage of *TLR-4* +896G confers an 8-fold increase for pre-malignant change in 3 Caucasian populations, with profound corporeal inflammation and gastric atrophy. In terms of malignancy per se, carriage of the variant allele conveys a doubling of risk (Hold et al. 2007).

Santini et al. (2008) showed that another *TLR4* polymorphism increases risk of intestinal-type gastric cancer. Thus, the *TLR4* Thr399Ile was associated with an increased hazard ratio of 5·38, 95% CI 1·652–8·145, $p = 0{\cdot}006$. More work is required on elucidating the full impact of polymorphisms within the toll-like receptors and their associated pathways.

There are other reports of innate immune response gene polymorphisms being associated with increased risk of gastric cancer. Mannose binding lectin is an antigen-recognition molecule involved in systemic and mucosal innate immunity. It is able to bind to a range of microbes and subsequently kill them by activating the complement system and promoting complement-independent opsonophagocytosis.

Baccarelli et al. (2006) showed that polymorphisms in the mannose binding lectin-2 gene (*MBL2*) were associated with increased risk of gastric cancer. In haplotype analysis, the HYD haplotype was associated with increased risk of stomach cancer when compared with HYA, the most common haplotype (OR = 1.9, 95% CI 1.1–3.2; $p = 0.02$). Further analyses to examine the joint effect of *MBL2* and *IL-1B* polymorphisms indicated that the combination of at-risk *IL-1B* genotypes (CT or TT at location −511) and HYD *MBL2* haplotype was associated with a 3.5-fold risk (OR = 3.5, 95% CI 1.6–7.6; $p = 0.001$). The findings suggest that the codon 52 D *MBL2* variant causing a cysteine > arginine replacement is specifically associated with gastric cancer risk.

8 Genome-Wide Association Studies and Gastric Cancer

Genotyping technology has advanced dramatically in the past 5 years and it is now possible to study hundreds and thousands of SNP's simultaneously. This approach, termed genome-wide association studies, was recently used by Sakamoto et al. (2008) to study gastric cancer in the Japanese population. Employing a two-stage genome-wide association study (stage 1: 85,576 SNPs on 188 cases and 752 references; stage 2: 2,753 SNPs on 749 cases and 750 controls) identified a significant association between an intronic SNP (rs2976392) in PSCA (prostate stem cell antigen) and diffuse-type gastric cancer (allele-specific OR = 1.62, 95% CI = 1.38–1.89, $P = 1.11 \times 10^{-9}$). Interestingly, the association was far less significant in intestinal-type gastric cancer. The PSCA gene is possibly involved in regulating gastric epithelial-cell proliferation and it will be very interesting to find out how it influences susceptibility to diffuse-type gastric cancer.

9 Overall Contribution of a Host Pro-Inflammatory Genetic Makeup to Pathogenesis of Gastric Cancer

It appears that subjects with a pro-inflammatory genetic makeup based on a combination of markers from cytokine/chemokine genes (e.g. *IL-1B*, *TNF-A*, *IL-10*, *IL-8*) and the innate immune response (e.g. *TLR4*, *MBL2*), respond to *H. pylori* infection by creating a gastric environment that is chronically inflamed and with reduced acidity. The damage is exacerbated if the infecting organisms are particularly virulent, and particularly if CagA positive. This bacterial protein has recently been shown to act as an oncoprotein and there is no doubt that its presence heightens the inflammatory process further (Ohnishi et al. 2008). This environment is conducive to the growth of other non-*H. pylori* bacteria within the gastric milieu, leading to sustained inflammation and oxidative/genotoxic/oncogenic stress. Subjects with the same pro-inflammatory polymorphisms may respond in the same exaggerated manner to these non-*H. pylori* bacteria, thus maintaining the pro-neoplastic drive. This may explain why *H. pylori* is not required in the later stages of gastric carcinogenesis and why it is often absent from gastric tumor tissue.

10 Conclusion

Overall, this chapter has given a brief insight into how host genetics can play an important role in the development of malignancy. Clearly, this is only part of the story, yet an important strand of the complex web of disease pathogenesis. Increasing our understanding of host genetics in cancer development may allow particularly susceptible individuals to be targeted for screening or treatment to reduce risk of future malignant transformation, for example, targeting those people who may benefit from *H. pylori* eradication therapy in an attempt to reduce gastric cancer prevalence. However, this benefit may be theoretical as the outcome in altering the natural progression of this disease is unknown. In addition, the currently recognised genetic risk markers are very common and do not carry the necessary degree of specificity required for a screening test. As the genetic revolution continues and technology advances, it will be possible to define a much more robust genetic profile that could be used for screening. This would certainly be a worthwhile achievement.

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Endoscopic Methods

Gaius Longcroft-Wheaton and Pradeep Bhandari

Abstract

Endoscopic methods to recognise and treat early gastrointestinal malignancies have increased in recent years. This has resulted in more lesions being diagnosed at an early stage and a shift away from invasive surgery towards endoscopic resection. However, it is necessary for the endoscopist to understand the key principles behind advanced endoscopic diagnosis and the new therapeutic options available. This chapter will review the advances in endoscopic techniques and methods which are changing the way we diagnose and treat these cancers. It will examine the general principles behind advanced endoscopy and then examine their application in Barrett's neoplasia, gastric cancer and the dysplasia associated lesions or masses associated with ulcerative colitis. It will focus on the best techniques for each of the above pathology.

Contents

G. Longcroft-Wheaton · P. Bhandari (✉)
Department of Gastroenterology, Queen Alexandra Hospital,
Cosham, Portsmouth, PO6 3LY, UK
e-mail: pradeep.bhandari@porthosp.nhs.uk

G. Longcroft-Wheaton
e-mail: gaius@gaius.wanadoo.co.uk

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1 Endoscopic Methods

Gastrointestinal malignancies represent a significant health burden, with the three main malignancies, colon, oesophageal and stomach cancer, representing the 3rd, 9th and 10th most common malignancies in the UK. With the exception of pancreatic cancer, survival has improved significantly over the last three decades, with survival rates doubling for oesophageal and colonic malignancies. This chapter will look at the advancing endoscopic techniques which are contributing to this, both in terms of diagnosis, staging and new novel treatments.

2 Inflammation and Cancer

As most gastrointestinal cancers develop on a background of inflammation it is important to differentiate inflammation from dysplasia. This is not always possible for the endoscopist or for the pathologist and is a common cause for confusion. The best approach is to always ensure that inflammation is completely treated prior to assessment of the mucosa for cancer. The diagnostic value of endoscopy for the detection of early cancer or dysplasia is very low in the presence of oesophagitis, gastritis or colitis. When these conditions are encountered a decision to fully treat inflammation and rescope at a later date should be made to assess for early neoplasia.

3 Fundamental Endoscopic Principles

3.1 Preparation

Good preparation is central to quality endoscopy, and the process starts before performing the endoscopy. In the upper gastrointestinal tract a mucolytic drink is recommended to enable subtle abnormalities to be evaluated. Pronase and Gascon ingested as a drink prior to gastroscopy has been shown to significantly improve mucosal visibility (Bhandari et al. 2010). Improving mucosal visibility should translate into improving early detection of cancers but that remains to be proven. Pronase is not licensed for ingestion, so a 50 ml solution containing 5 ml of 10% *N*-acetyl cysteine (NAC) with 5 ml of infacol can be used as an alternative in Western countries. This should be given as a drink 5 min prior to the procedure. Likewise, in the colon bowel preparation is central to visualising lesions. It is important that patients are informed of the importance of the bowel preparation,

including the timing of the sachets and the importance of consuming a significant volume of water with most of the commonly available preparations. When performing the procedure any residual debris should be washed away and the lens kept clean. Mucous and bubbles hinder the use of advanced techniques like spectral imaging and dye spray. A cap on the end of the endoscope can improve image quality when examining a lesion by stabilising the mucosa.

3.2 Basic Concepts

When visualising the oesophagus, stomach and colon, the mucosa should be inspected after insufflation and then again as the organ is deflated. It is during deflation of the lumen that many abnormalities become visible. Therefore, if inspection is only performed with complete inflation pathology will be missed. All areas should be visualised, with particular care taken to look beneath folds. There are key areas in each organ where pathology is most likely to be found, which will be discussed later. It is becoming recognised that a rapid procedure is not necessarily a good procedure. This has been demonstrated in studies looking at polyp detection in the colon, where a withdrawal time of >6 min is mandatory, and the same principle applies to the upper gastrointestinal tract. Likewise use of hyoscine during gastroscopy and colonoscopy can flatten the folds and help improve the pickup rate of early neoplasia.

3.3 Philosophy of Endoscopy

Most patients have an endoscopy because they are symptomatic and the endoscopist performs the procedure to look for a cause for the patient's symptoms. These causes are likely to be due to findings like peptic ulcer, oesophagitis or cancer in gastroscopy, and colitis or cancer during colonoscopy. However, these are very obvious findings during the procedure so endoscopists should pay special attention when no gross findings are seen. They should spend enough time to look at the fine details of the mucosa to identify subtle mucosal changes suggestive of the presence of early cancerous changes. Endoscopists in Japan have a very good understanding of these changes and also pay special attention looking for the changes. This might explain the higher incidence of early cancers detected in Japan. We believe that the endoscopist should spend more time looking at the mucosa and spend less time taking random untargeted biopsies.

3.4 Oesophageal Cancer

Oesophageal cancer is the ninth most common cancer in the UK, with 7,800 people diagnosed every year, accounting for 5% of all cancer deaths in the UK. Rates have increased by 50% over the last 30 years. It is more common in men

than women, with an incidence of 8.8–14.1 per 100,000 in men, and 4.8–5.7 per 100,000 in women (Barr 2007). The vague early symptoms have traditionally led to late diagnosis, leading to an overall 5 year survival of 9% (Hellier et al. 2006). However, recent endoscopic advances have made it easier to diagnose the condition at an earlier stage.

A significant risk factor for adenocarcinoma of the oesophagus is Barrett's epithelium, an acquired pre-malignant condition, caused by reflux of gastric contents into the oesophagus. The gastric acid damages the normal squamous epithelium, becoming replaced by a columnar epithelium. The newly updated definition by the British society of Gastroenterology defines Barrett's as 'an endoscopically apparent area above the oesophagogastric junction that is suggestive of Barrett's which is supported by the finding of columnar lined oesophagus on histology' (Playford 2006; Watson et al. 2005).

Barrett's oesophagus effects up to 1.6% of the general population (Ronkainen et al. 2005). It is found in 15–20% of gastrointestinal endoscopies performed for symptoms of reflux. The incidence is increasing in the West (Blot et al. 1991; Pera et al. 1993). It has the potential to progress into adenocarcinoma. Risk factors for this include male gender, age >45, extended segment (>8 cm) disease, duration of reflux history, early age of onset of GORD, duodeno-gastrooesophageal reflux, mucosal damage and family history (Watson et al. 2005).

There is not a national screening policy for the detection of upper gastrointestinal malignancy or for Barrett's. However, once Barrett's is detected patients are entered into a surveillance programme. The benefits of this are controversial, as the absolute risk of malignant transformation is low, 0.8–1.5% per annum. Some studies have concluded that because of this there is no benefit to surveillance (Watson et al. 2005). Computer modelling has been used to predict an effective balance point for surveillance intervals, and a widely used interval for surveillance endoscopy is 2 years (Watson et al. 2005). Quadrantic random biopsies every 2 cm are part of the standard protocol. The cost per life year saved is around £19,000.

Random biopsies are not ideal for identifying neoplasia within Barrett's. There are a range of techniques available for examining the oesophagus in detail, and can help improve neoplasia pickup rate. Chromo endoscopy can be used to identify areas of Barrett's metaplasia and dysplasia. Several dyes have been used, with most of the research examining methylene blue (MB), indigo carmine (IC) and acetic acid (AA).

Methylene blue 0.5% is an absorptive stain which highlights areas of specialised columnar epithelium (Canto et al. 1996, 2001). Dysplasia and cancer are detected more frequently than with random four quadrant biopsies (Canto et al. 2000). Unfortunately MB is inconvenient to use. It must be left in contact with the mucosa for 3 min followed by vigorous washing to clear away excess dye. As a result the endoscopic appearances are unpredictable and subjective (Dacosta et al. 2002). There have been recent concerns about DNA toxicity with MB so it is falling out of favour. IC 0.4% is not absorbed but accumulates in pits and valleys between cells highlighting the architecture (Hetil 2002) and has been shown to help in the detection of dysplasia (Sharma et al. 2003, 2006).

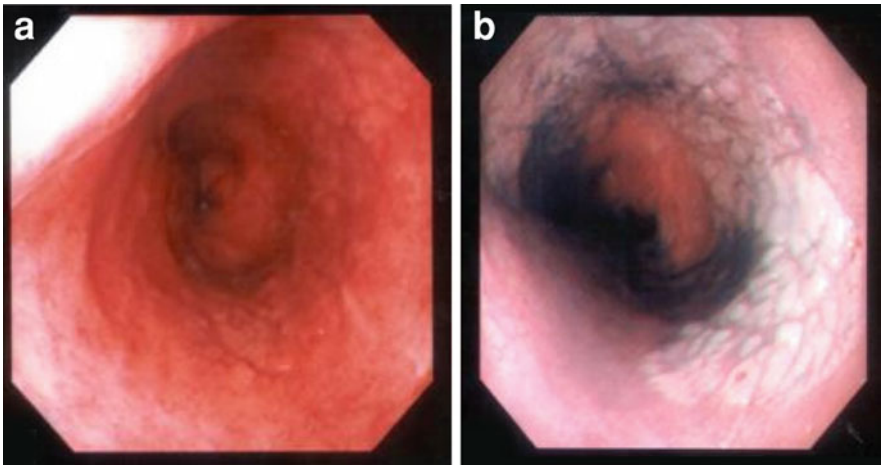

Fig. 1 Oesophagus: **a** routine endoscopic appearances, **b** post acetic acid chromoendoscopy

AA 2.5% when sprayed onto Barrett's mucosa causes a reversible acetylation of nuclear proteins to occur. This leads to an acetowhitening reaction, with increased opacity of the mucosal surface (Lambert et al. 2003). The associated vascular congestion improves visualisation of the surface vasculature (Lambert et al. 2003). It also enhances the surface pattern assessment allowing early recognition of neoplasia (see Fig. 1).

AA has been successfully used in the detection of neoplasia of the cervix during colposcopy (Guelrud and Herrera 1998; Van Le et al. 1993). While no randomised controlled trials for its use in the oesophagus exist, cohort studies have demonstrated effectiveness in the identification of dysplasia. The sensitivity for the identification of neoplasia has been suggested to be 71–100%, with a specificity between 80 and 99% (Vázquez-Iglesias et al. 2007; Gossner et al. 2005; Fortun et al. 2006; Longcroft-Wheaton et al. 2010). We believe that this is the dye of the future in the evaluation of Barrett's. It is cheap, effective and universally available.

Narrow band imaging (NBI) is a form of 'digital virtual chromo endoscopy'. It carries the potential advantage that it can be activated at the press of a button on the endoscope. It highlights mucosal vascular patterns to enhance abnormal dysplastic areas. A prospective cohort study has demonstrated that a sensitivity of 100%, specificity of 98.7% and positive predictive value of 95.3% for HGD could be achieved. Another tandem endoscopy study involving 65 patients compared standard resolution endoscopy to NBI. It found that NBI directed biopsies detected dysplasia in more patients (57%) compared to biopsies taken using standard resolution endoscopy (43%) (Wolfsen et al. 2008). Auto-fluorescence imaging (AFI) is another novel technique which is based on the principle of variable fluorescence between tissues. Normal mucosa when exposed to light emits a green fluorescence as compared to magneta/pink fluorescence in neoplastic areas within the mucosa. This principle is exploited by the technique of AFI to detect early

neoplasia. Early studies without NBI suggested that while detection rates were very good (91%) a 51% false positive rate limited its use without NBI (Kara et al. 2005). AFI is now being used as a 'red flag' technique to highlight points of concern in wide areas followed by detailed scrutinising using NBI (Curvers et al. 2008).

Fujinon has developed a technology for vascular enhancement, known as FICE. Rather than using filters it utilises a post processor technology to digitally reconstruct spectral data to enhance particular wavelengths. When compared with random biopsy in patients with suspected high grade intraepithelial neoplasia or early cancer, a sensitivity of 83% for FICE was achieved (Pohl et al. 2007). There was high grade dysplasia or early cancer in 24/57 patients. Due to its nature as a post processor technology, FICE can utilise a wide range of different frequencies for lesion enhancement. Therefore it has the potential to develop into a very powerful tool for examining Barrett's for neoplasia, perhaps with specific settings for the purpose. This is an area for future research.

All of the 'virtual chromo endoscopy' techniques (NBI, FICE and i-scan) produce different appearances. Although some of the skills are transferrable additional training is normally required to transfer between systems.

Confocal endomicroscopy is a new technology for obtaining true in vivo histology. Studies in Barrett's surveillance have suggested that Barrett's oesophagus with associated neoplasia could be predicted with a sensitivity of 96.4% (Trovato et al. 2008). It has been shown that confocal endomicroscopy improves the yield of neoplasia in apparent Barrett's oesophagus compared to a four quadrant random biopsy protocol (Dunbar et al. 2009). There is limited evidence that it is also effective in the identification of gastric cancers (Yeoh et al. 2005). This technique only works once neoplasia has been identified by the endoscopist using other endoscopic techniques, so it does not help improve detection but can improve the confidence for diagnosis. We believe that this is an excellent experimental technique which has yet to find a major clinical role.

3.5 Treatment

Traditionally the only treatment for high grade dysplasia and intramucosal adenocarcinoma was an oesophagectomy. This is a highly invasive intervention, associated with significant mortality and morbidity, variable according to centre, with high volume units producing better results (Birkmeyer et al. 2002). Postoperative morbidity is accepted to be significant, with rates between 30 and 50%, with a mortality of 2–10% (Chang et al. 2008).

Endoscopic resection and ablation techniques are becoming increasingly popular due to low morbidity and mortality. Endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD) involve removing the mucosal and submucosal layers of the oesophagus. EMR uses either a cap and snare kit from Olympus or Duette banding ligator from Cook to remove the abnormal tissue. This can be taken in one piece (May et al. 2002a, b; Pech et al. 2008) or, for larger areas,

piecemeal excision can be performed. EMR gives a better histological diagnosis as compared to biopsy. However, if a lesion is >1.5 cm then piecemeal resection can make it hard to determine completeness of the lateral resection margins. ESD uses a specialised endoscopic knife to dissect out neoplastic areas of any size in an en-block fashion. It can provide a clear resection margin but carries increased risks, including that of perforation. This can be combined with Argon plasma coagulation (APC), multipolar electrocautery (MPEC), which all aim to destroy any residual abnormal tissue through either ionised argon gas or an electric current.

Ablative photodynamic therapy (PDT) involves the use of a photosensitizing agent which is preferentially taken up by tumour tissue (Panjehpour et al. 2008). After a suitable time period an endoscopy is performed where the abnormal area is exposed to light at an appropriate wavelength. The neoplasia which has preferentially taken up the drug then undergoes cell death. Using this technique a randomised controlled trial has shown 98% efficacy at eliminating low grade dysplasia (Ackroyd et al. 2000). Success has also been demonstrated with HGD and superficial T1 cancers, with successful ablation of HGD as high as 93% in one prospective series (Overholt et al. 1999), although a more recent RCT by the same author has suggested that complete HGD ablation is achieved in 77% of cases over a mean follow up period of 24 months (Overholt et al. 2005). It can cause stricture formation and photosensitivity reactions. Radiofrequency ablation (RFA) is similar in concept. Radiofrequency electrodes deliver thermal energy through a focal device or balloon inflated to make contact with the oesophageal wall. This induces mucosal destruction. The depth of burn is less than with PDT which improves the safety profile of RFA over that of PDT, with a low oesophageal stricture rate of 6%, no deaths and no perforations seen in a large sham-controlled trial (Shaheen et al. 2008). RFA is a useful technique for multifocal dysplasia (Shaheen et al. 2009). It is not suitable for raised nodular areas which should first be removed by EMR. There have been no trials conducted to date which show whether RFA combined with EMR is any better than EMR alone.

4 The Stomach

The incidence of gastric cancer is falling in the west. This is attributed to the declining incidence of *Helicobacter pylori* (*H. pylori*) colonisation. Gastric cancer is therefore considered to develop on a background of inflammation (McNamara and El-Omar 2008; Ernst 1999). Eradication of *H. pylori* is probably effective in reducing risk and possibly cost effective (Parsonnett et al. 1996; Roderick et al. 2003). In practical terms in the west the incidence is falling anyway, so the gain from actively seeking to eradicate in an asymptomatic population may be small.

The most common areas for gastric cancer associated with *H. Pylori* is in the antrum or insura. In helicobacter negative patients it is more prevalent along the greater curve or in the body. It is very important therefore when examining patients to look closely in these areas.

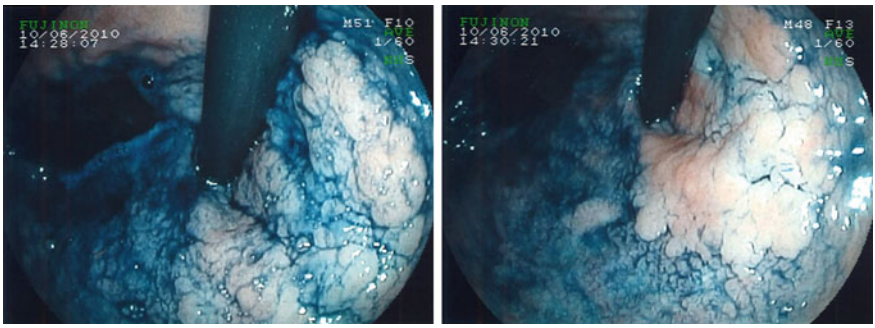

Fig. 2 High grade dysplasia in the gastric cardia after indigo carmine dye spray

Screening for gastric cancer is controversial. A screening programme place is in Japan, which has been successful in reducing mortality. This has not been adopted in the west, where the lower prevalence of the condition makes the usefulness questionable. However, patients with atrophic gastritis are at increased risk, and yearly endoscopic screening has been suggested in this group of patients (Whiting et al. 2002).

The diagnosis of gastric cancer is predominately by endoscopy. A non-healing gastric ulcer is the classical appearance of gastric cancer. This however, typically represents advanced disease. The key problem is in detecting early, potentially treatable lesions. However, the Japanese have demonstrated that not only it is possible to find early gastric cancer, but that it is even possible to accurately stage the depth of lesion invasion in 95% early cancer and 86% for advanced cancer (Yin et al. 2009). This assessment is best made by indigocarmine assisted chromo endoscopy techniques (see Fig. 2). NBI has been utilised with some success. There is very limited evidence available regarding whether FICE is beneficial.

4.1 Treatment

Early gastric cancer in the west has traditionally been treated by gastrectomy. However, in Japan endoscopic resection is the standard treatment. The Japanese gastric cancer association (JGCA) released a series of recommendations for the management of gastric cancer in 2001, (Nakajima 2002) updated in 2008 to reflect the rapid advances in management options available. Endoscopic resection is indicated for lesions <2 cm in size with a differentiated histological type where there is no ulceration present (Tsujitani et al. 1999). Recently the indications have been expanded further to include all intramucosal cancer regardless of size without ulceration, or intramucosal cancer <30 mm with ulceration.

EMR of mucosal cancer is indicated for lesions under 2 cm, where the risk of lymph node metastasis is very low. An enbloc resection should be performed whenever possible, and is important in reducing the risk of recurrence (Eguchi et al. 2003). A problem with both cap and snare and duette cap ligation devices is that the

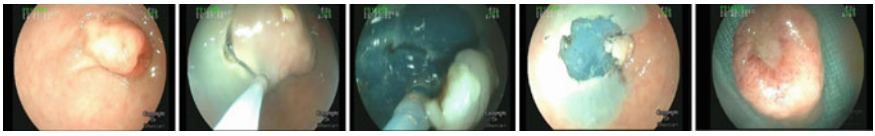

Fig. 3 Dual and IT-2 knives being used to resect a gastric lesion. Note how a circumferential incision is made and the final specimen removed enbloc

maximum size lesion which can be resected in one piece is 15 mm (Ell et al. 2000; Tanabe et al. 2002). Using these techniques recurrence free survival can be achieved in up to 92% of cases (Oda et al. 2006), with the recurrence rate most often quoted in the literature as being between 2 and 32% (Gotoda 2007).

Because of the importance of enbloc resection on recurrence free survival and the inherent limitations imposed by EMR techniques. ESD has become increasingly popular in Japan. This enables a true R0 resection to be achieved in a greater proportion of cases, and allows larger lesions to be removed. It is used for all lesions over 2 cm in diameter or where there is ulceration seen. There are now a wide range of endoscopic knives available for performing ESD, and it is accepted that these techniques are technically challenging (Choi et al. 2005). The risk of serious complications, including bleeding and perforation, is higher than that with EMR, with a risk of delayed bleeding of 6% and perforation around 4% (Oda et al. 2005). It is generally accepted that it is necessary to perform between 30 and 40 resections in a closely supervised environment before becoming competent. However, the potential gain in disease free survival is clear, with a comparative multicentre study demonstrating a significant difference in the disease free survival rates between ESD and EMR (97.6 vs. 92.5%) (Oda et al. 2006). We believe that ESD is an excellent technique for lesions over 2 cm, but is very challenging and expertise outside of Japan in this technique remains limited (see Fig. 3).

5 The Colon

A major risk factor for inflammatory neoplasia in the colon is ulcerative colitis. This is an idiopathic inflammatory condition which affects the large bowel. It has a prevalence of 3–12 per 100,000 in Northern Europe and America, but is much less common in Asia and the Far East, with a prevalence between 1 and 6 per 100,000 (Loftus 2004).

Chronic ulcerative colitis increases the risk of colonic cancer. The cancer risk in patients with ulcerative colitis is dependent on the extent of disease and the frequency of attacks. It is generally accepted that the cumulative risk for patients with extensive disease between 7 and 15% at 20 years. Up to 15 years the risk is very low. Carcinoma is usually preceded by dysplasia. Because of this there is a national screening policy for patients with Ulcerative colitis. It is the general consensus that colonoscopic screening should start at 8–10 years for patients with a pan-colitis or after 15 years for disease restricted to the left side (Riddell 1990), with regular

follow up thereafter. The standard approach has been to take quadrantic biopsies every 10 cm. This is unfortunately time consuming and expensive. There is evidence that more dysplastic lesions are picked up using methylene blue or indigo carmine than with white light alone (Marion et al. 2008; Rutter et al. 2004). The most recent guidelines from the British Society of Gastroenterology are recommending the use of chromo endoscopy where there is appropriate expertise available. Unfortunately to date colonoscopy with NBI has not been shown to be effective (Dekker et al. 2007). Caution needs to be taken when interpreting these findings; however, as the amount of evidence available is very limited.

The aim of chromoendoscopy in ulcerative colitis is to pick up areas of dysplasia associated lesions or mass (DALMS) which could turn into cancer. These abnormalities can be very subtle. As a result it is not possible where pseudo-polyps predominate or there is significant active inflammation. Dye can be applied by a spray catheter or flushed directly down the endoscope, depending on the preferences of the endoscopist. It is important to note that at present the evidence is not there for abandoning random biopsies in these patients. Therefore if dye spray is used and no abnormalities are seen biopsies still need to be taken. This may change with time.

Confocal endomicroscopy has been used in the examination of patients with ulcerative colitis. Early studies have shown some promise, with yields for intraepithelial neoplasia greater in the chromo endoscopically guided biopsies than those targeted by chromo endoscopy alone (Hurlstone et al. 2008; Kiesslich et al. 2007). This suggests that if a targeted area is examined by the confocal endomicroscope it has the potential to prove or disprove whether it needs to be biopsied, in doing so reducing the number of unnecessary biopsies taken.

6 Endoscopic Resection of DALMS

The traditional treatment of high grade dysplasia on a background of ulcerative colitis has been colectomy. However, this strategy was developed on the hypothesis that dysplasia could not be visualised. The traditional view was that colon cancers originating on a background of dysplasia associated lesion or mass (DALMS) do not follow the traditional adenoma-carcinoma sequence, with rapid progression being seen (Blackstone et al. 1981). This view is now being challenged. There have been two studies which have investigated the endoscopic resection of polyp like DALMS (Rubin et al. 1999; Engelsgjerd et al. 1999). The principle behind removal is to distinguish between lesions which are close to turning into cancer from those which are likely to behave more like adenomatous polyps.

An adenomatous DALM is defined as a well-circumscribed, smooth or papillary, non-necrotic sessile or pedunculated lesion (Odze and Robert 2008). Non-adenoma like lesions appear as velvety patches, plaques, irregular bumps, stricturing lesions and broad based masses. A small long term outcome study has investigated the outcome of 34 patients who had DALMs, 28 resected

endoscopically and six by colonic resection. These were compared to 49 non-colitic patients treated for sporadic adenomas. It showed that one patient developed adenocarcinoma 7.5 years after resection. However, she was a high risk patient with sclerosing cholangitis. Twenty patients developed further DALMS. This was no different to the rate of sporadic adenomas in the control group (Hornick et al. 2004).

The current American Gastroenterology Association guidelines are that adenomatous like DALMS can be removed provided they can be completely excised, with an absence of dysplasia at the resection margins, and that there is no flat dysplasia anywhere else in the colon. All patients with flat non-adenoma like dysplasia should be treated with total colectomy (Itzkowitz et al. 2010).

7 Conclusions

Neoplasia in the gastrointestinal tract can develop on a background of chronic inflammation or via a non-inflammatory pathway. These neoplasias can be recognised by an endoscopist at a very early stage. The prerequisites for early diagnosis are adequate luminal preparation, a high resolution endoscope and an endoscopist with an ability to recognise subtle mucosal changes indicative of neoplasia.

Endoscopic resection and ablation techniques can now cure patients with dysplasia or early cancerous lesions without the need for radical resectional surgery and its associated morbidity and mortality. However, the success of these techniques depends on the skills and experience of the endoscopist and we believe that these procedures should only be performed at high volume early cancer centres.

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