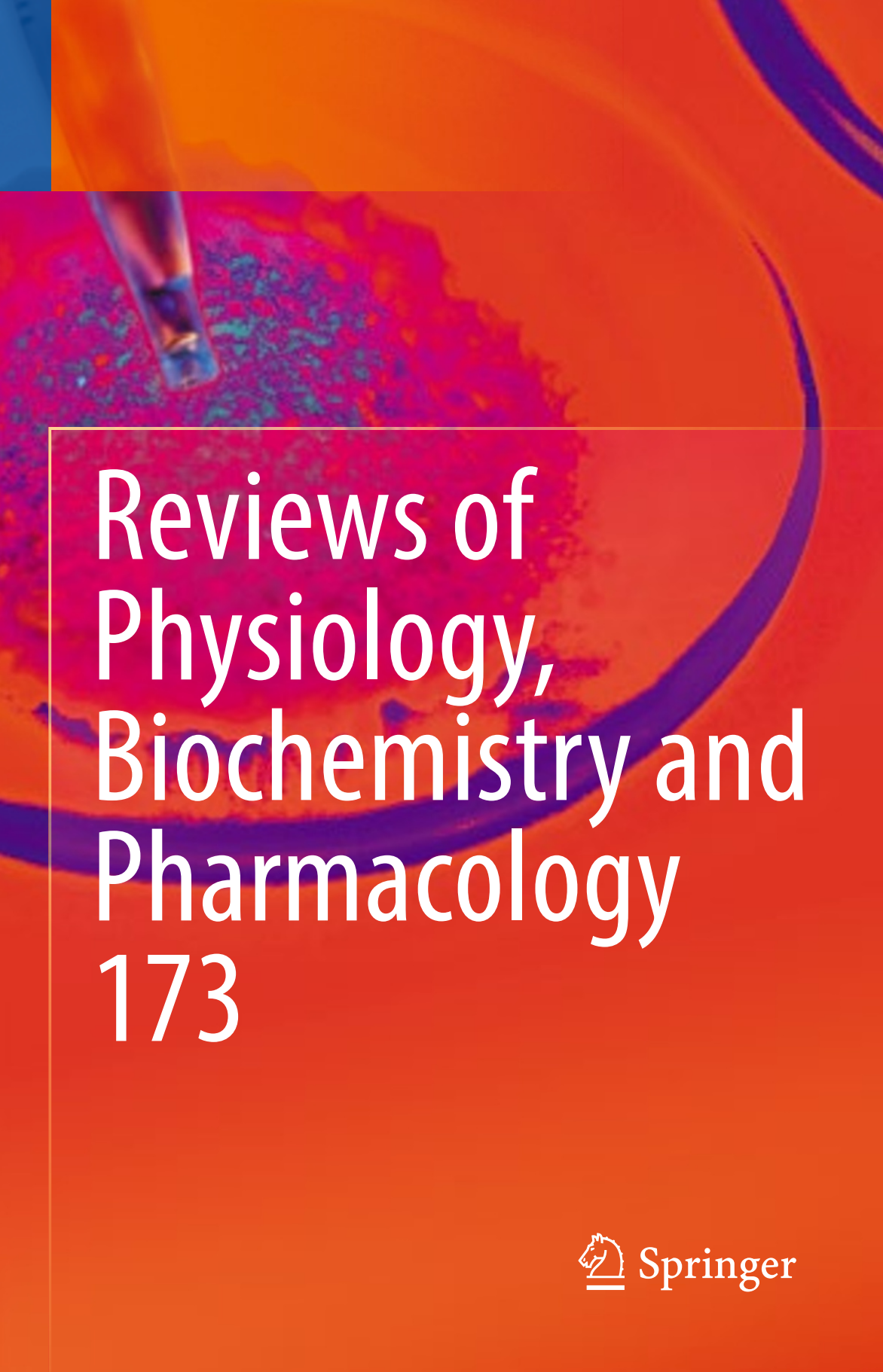
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Catechol-*O*-Methyltransferase (COMT): An Update on Its Role in Cancer, Neurological and Cardiovascular Diseases

Pedro Bastos, Tiago Gomes, and Laura Ribeiro

Abstract Catechol-*O*-methyltransferase (COMT) is an enzyme that catalyses the methylation of catechol substrates, classically in catecholamine metabolism, but also acting upon other substrates such as oestrogen and polyphenols. Although its classical function has been established for more than five decades, an ever expanding COMT role in other pathways and diseases has become a subject of active study in recent years. The most highlighted domains are related with COMT involvement in neuropsychiatric disorders and its role in the neurobiology of cognition, behaviour, emotions, pain processing and perception, sleep regulation, addictive behaviour and neurodegeneration. Nonetheless, great attention is also being devoted to a possible COMT contribution to the development of cardiovascular disorders and hormonally influenced diseases, including cancer. This review aims to update the role of COMT function and its involvement in cardiovascular and neurological disorders.

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Keywords Cancer • Cardiovascular • COMT • Neurodegeneration • Polymorphisms • Psychiatric disorders

Contents

1 Introduction

It was in 1958 that Axelrod et al. first identified and described the properties of the enzyme responsible for the *O*-methylation of catecholamines (CA) (Axelrod and Tomchick 1958). They were prompted by findings of *O*-methylated catechols in the urine of pheochromocytoma patients (Axelrod et al. 1958) and of exogenously administered CA (Axelrod and Tomchick 1958), suggesting another pathway for CA inactivation besides deamination.

Catechol-*O*-methyltransferase (COMT: EC 2.1.1.6) is an enzyme that in the presence of magnesium (Mg^{2+}) catalyses the transference of a methyl group from *S*-adenosyl-L-methionine (SAM) to a catechol substrate with *O*-methylated catechol and *S*-adenosyl-L-homocysteine (SAH) as reaction products (Männistö and Kaakkola 1999).

As a ubiquitous enzyme COMT has been found in most studied organisms across phylogenetic levels, including yeasts, plants, insects, fish, amphibians, birds and all studied mammals (Bonifacio et al. 2007; Lundström et al. 1995), stating its evolutionary and physiological relevance.

In humans, besides its role in CA catabolism COMT has been implicated in the inactivation of catecholoestrogen (CE) (Worda et al. 2003), catechol-containing xenobiotics, such as catechins and bioflavonoids (Zhu et al. 2000, 2001), and indole intermediates from melanin metabolism (Smit and Pavel 1995). COMT tissue distribution and substrate specificity support a putative protective role as an enzymatic barrier against exogenous catechols by preventing their oxidation to *o*-quinones and the formation of electrophiles (Smit and Pavel 1995; Weisz et al. 2000; Zahid et al. 2007). The catechol *O*-methylation seems to decrease the

tendency for redox cycling (Zhu et al. 1994) and to increase lipophilicity facilitating the transmembrane transport out of the cell (Smit and Pavel 1995). In addition, COMT is of great pharmacological significance, being involved in the metabolism of catechol-containing drugs such as L-DOPA, dobutamine, carbidopa, isoprenaline, benserazide, rimiterol, α-methyldopa and apomorphine, used in the treatment of asthma, hypertension and Parkinson's disease (Øverbye and Seglen 2009; Männistö and Kaakkola 1999).

COMT is strictly intracellular with two known isoforms, one soluble in the cytoplasm (soluble COMT: S-COMT) and one associated to membranes (membrane bound COMT: MB-COMT) (Huh and Friedhoff 1979; Ulmanen et al. 1997). Although these isoforms share several biochemical properties, differences are observed regarding molecular weight, cellular location, substrate specificity, kinetics and preferential position of methylation (Lundström et al. 1995). An alternative nomenclature based on the size of the isoforms has been proposed, designating S-COMT as small (S) and MB-COMT as large (L) (Øverbye and Seglen 2009).

In mammals, COMT is coded by a single gene, which in the human species is located in the band q11.21 of chromosome 22 (Tenhunen et al. 1994). The human gene has 6 exons (Fig. 1), being the first two non-coding, and presents two partially overlapping open reading frames of 663 bp and 813 bp for S- and MB-COMT (Tenhunen et al. 1994), respectively. The 5′ distal promoter (P2) regulates the synthesis of a 1.5 kb mRNA and the proximal promoter (P1), positioned between the MB- and S-COMT initiation codons in the third exon, regulates the synthesis of a 1.3 kb transcript (Lundström et al. 1995). Both transcripts share the same termination codon (TGA) in +814 of the sixth exon (Tenhunen et al. 1994). Due to a less favourable location of the MB-COMT AUG initiation codon and the occurrence of a leaky scanning mechanism, the larger mRNA has the ability to originate both COMT isoforms with translation most frequently commencing in the S-COMT AUG initiation codon (Tenhunen et al. 1994; Lundström et al. 1995).

The variable expression of COMT isoforms across different tissues and cell lines suggests that tissue specific transcription factors regulate the expression (Tenhunen

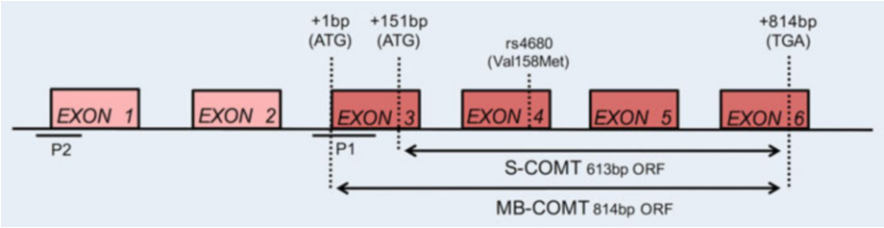

Fig. 1 Scheme of the human COMT gene structure. The human COMT gene has 6 exons, being the two most 5′ non-coding. The expression is controlled by two promoters, the P2 distal promoter regulating the synthesis of a 1.5 kb mRNA and the P1 proximal promoter regulating the synthesis of a 1.3 kb mRNA. The initiation codons for both isoforms are located in the third exon, MB-COMT (+1 bp) and S-COMT (+150 bp). The gene has a single translation termination codon (TGA) in the position +814 of the sixth exon. The two open reading frames (ORF's) of 663 and 813 bp are partially overlapping

et al. 1994; Männistö and Kaakkola 1999). Progesterone was shown to induce a distinct tissue specific effect on COMT expression via activation of one of the two progesterone receptor isoforms PR-A or PR-B (Salama et al. 2007; Jiang et al. 2003).

Both COMT promoters have oestrogen and glucocorticoid response elements that intricately regulate expression. Progesterone and dexamethasone are known to upregulate expression in leiomyoma cells (associated with PR-A) (Salama et al. 2006a) while progesterone and oestrogen downregulate expression in breast cancer cell lines (associated with PR-B) (Salama et al. 2007).

Several sequences upstream to the MB-COMT initiation codon represent potential targets for the Sp1 transcription factor and are probably responsible for the basic activity of the promoter (Tenhunen et al. 1994). In astrocytes, activation of NF-κB and p65 binding seems to downregulate the P2 promoter (Tchivileva et al. 2009; Hartung et al. 2015) and 17-β-estradiol shows a similar action in oestrogen receptor-positive human breast carcinoma (MCF-7) cells, both through the receptor's CCAAT/enhancer binding protein sites (Xie et al. 1999) and via oestrogen response elements (Xie et al. 1999; Jiang et al. 2003). No effects on COMT mRNA levels were observed after treating a glial cell line with 17-β-estradiol, supporting a tissue specific regulation mechanism (Jiang et al. 2003). In male rats, exogenous estradiol did not change COMT protein levels in the liver and frontal cortex, but decreased protein expression in the kidney and prefrontal cortex and increased COMT protein levels in the hippocampus, cerebellum and prostate (Schendzielorz et al. 2011). A positive regulation by insulin, dihydrotestosterone and *trans* retinoic acid was observed for the P1 promoter (Salih et al. 2008a). Due to the presence of a CArG box in the promoter, COMT has been identified as a transcriptional target for myocardin-related transcription factors (MRTFs), with the activation occurring through histone acetyltransferase p300 recruitment by megakaryoblastic leukemia 1 (MKL1) (Liu et al. 2013). Possible targets for p53 and for the transcription factors AP-2, NF-IL6, HNF-4, Ets-1 and NF-D have also been described (Tchivileva et al. 2009; Wang et al. 2001; Tenhunen et al. 1994). Several CpG islets have been found in the 5′ upstream region and their methylation was shown to silence MB-COMT expression (Sasaki et al. 2003). Due to the downregulation exerted by oestrogen, women have around 30% lower COMT activity than men (Harrison and Tunbridge 2008; Tenorio-Laranga et al. 2009).

1.1 Enzyme Structure and Kinetics

The enzyme consists of a typical methyltransferase topology with 8 α-helixes surrounding 7 β-sheets with the sixth sheet in antiparallel sense (Tsuji et al. 2009). The active site is located on the enzyme external surface and comprised of two distinct parts, a deeper region for SAM binding and a catalytic one for substrate binding where the Mg^{2+} ion occupies the central position (Männistö and Kaakkola 1999). In this S_N2 transfer mechanism, SAM binds first, followed by Mg^{2+} and last by the catechol substrate. After the reaction the unmethylated product SAH is the last to dissociate (Männistö and Kaakkola 1999). It is proposed that catechol recognition

depends on coordination of the catechol hydroxyl groups with the Mg^{2+} ion (Lautala et al. 2001). The catechol moiety has an octahedral coordination and the Mg^{2+} ion controls its orientation to two aspartic acid and one asparagine residues, a water molecule and the two catechol hydroxyl groups (Männistö and Kaakkola 1999). Ionization is also facilitated by Mg^{2+} through positioning of one of the hydroxyls in proximity with both the SAM methyl group and the COMT lysine 144 amine group. The latter then accepts the hydroxyl proton and enables the methyl transference (Lautala et al. 2001; Männistö and Kaakkola 1999). The remaining hydroxyl group is connected by hydrogen bonds to the carboxylic oxygen of COMT glutamate 199 (Lautala et al. 2001). The catechol ring is maintained in the correct position by the tryptophan residues 38 and 143 and by the proline residue 174, which form an hydrophobic barrier and define the enzyme selectivity towards the catechol lateral chains (Männistö and Kaakkola 1999).

Despite essential for the catalysis, the Mg^{2+} ion can be exchanged by manganese (Mn^{2+}), cobalt (Co^{2+}), zinc (Zn^{2+}), cadmium (Cd^{2+}), ferrous iron (Fe^{2+}), tin (Sn^{2+}) or nickel (Ni^{2+}), although with changes in the enzyme kinetics (Sparta and Alexandrova 2012). Mn^{2+} and Co^{2+} were found to even increase the enzyme activity (Axelrod and Tomchick 1958). Calcium (Ca^{2+}), ferric iron (Fe^{3+}) and aluminium (Al^{3+}) inhibit the enzyme (Sparta and Alexandrova 2012; Axelrod and Tomchick 1958), as well as SAH, which has a binding affinity close to SAM (Pihlavisto and Reenilä 2002; Bunker et al. 2008).

The human S-COMT has 221 amino acids and a molecular weight of 24.4 kDa, while the MB isoform contains 50 more amino acids and 30 kDa (Männistö and Kaakkola 1999). Both isoforms have identical active site structures, similar affinity for SAM and close optimum pH (7.4 for MB- and 7.8 for S-COMT), but differ in the kinetics (Reenilä and Männistö 2001; Tsunoda et al. 2002). The MB isoform has a 10–100 times higher affinity towards CA, relatively to the S isoform (Ellingson et al. 1999), while S-COMT has a higher affinity for CE and catechol-containing xenobiotics and methylates these compounds two to three times faster than CA (Zhu et al. 2000). These differences suggest that MB-COMT acts preferentially at lower physiologic concentrations (in the μM range), while S-COMT functions mostly under substrate saturating conditions (Bonifacio et al. 2000; Pihlavisto and Reenilä 2002). Accordingly, MB-COMT seems to have a most relevant role in the inactivation of the catecholaminergic neurotransmission, playing S-COMT a higher part in the inactivation of CE and catechol-containing xenobiotics (Pihlavisto and Reenilä 2002). The existence of a second MB-COMT isoform with 39 kDa was proposed (Tunbridge et al. 2006).

The methylation occurs mostly at the meta (3′-hydroxyl) position of the catechol ring, but can also take place at the para (4′-hydroxyl) position, depending on the lateral substrate chains, COMT isoform and experimental conditions (Männistö and Kaakkola 1999). The meta/para ratio is generally higher with MB-COMT (Pihlavisto and Reenilä 2002). In CE's metabolism the methylation of the 2′-hydroxyl or 4′-hydroxyl of the catechol ring is favoured (Nissinen and Männistö 2010).

1.2 Cell Location and Tissue Distribution

COMT is intracellular and prevails in the S isoform (Tenhunen and Ulmanen 1993). The S isoform is mainly cytoplasmic but has also been detected in the nucleus of hepatocytes (Ulmanen et al. 1997), mammary epithelial cells (Weisz et al. 2000) and renal cortex cells (Weisz et al. 1998). The MB-COMT amine terminal region is composed of 50 amino acids, of which 20 are hydrophobic, and acts as a signal sequence that directs the protein to the membrane (Lundström et al. 1995). This restricts MB-COMT to the membranes and mainly to the rough endoplasmic reticulum where it is orientated towards the cytoplasm (Ulmanen et al. 1997).

In mammals, the enzyme is broadly distributed in most tissues and the S isoform usually found at levels more than three times higher than the MB (Karhunen et al. 1994). S-COMT has been detected in the liver, kidneys, lungs, adrenal glands, mammary glands, spleen, stomach epithelium, duodenum, ileum, cerebral cortex, hypothalamus, thalamus, cerebellum, pons and pituitary gland (Karhunen et al. 1994). In the brain it accounts for more than 60% of the dopamine degradation in the prefrontal cortex (PFC), but for less than 15% in the striatum (Dauvilliers et al. 2015). MB-COMT is found in the liver, kidneys, brain, adrenal glands, lungs, intestine, salivary glands and ducts, thyroid gland, retina, ciliated cells of the eye and in the pancreas, especially in β-cells and δ-cells (Karhunen et al. 1994; Meister et al. 1993). COMT activity has also been found in tissues such as epidermis, dermis, placenta, uterus, parotid gland and cell types as keratinocytes, melanocytes, adipocytes, erythrocytes, lymphocytes, macrophages and in the walls of the blood vessels of the dental pulp (Nomura et al. 1996; Inoue et al. 1987, 1991; Inoue and Creveling 1991).

In the brain, immunoreactivity for COMT has been observed in astrocytes (including the terminations surrounding the capillaries), dendritic cells, oligodendrocytes, microglia cells, in the pia and arachnoid mater surface, epithelial cells of the choroid plexus, hippocampus, neuroglia cells of the circumventricular organs, cerebrovascular endothelial cells and, with less intensity, in some postsynaptic spines of the parietal cortex, cerebellum and striate body (Helkamaa et al. 2007; Matsumoto et al. 2003; Reenilä et al. 1997; Myöhänen et al. 2010). Although both isoforms are present in neuronal and non-neuronal cells, S-COMT is more expressed in glial cells and MB-COMT in postsynaptic neurons (Karhunen et al. 1995a, b). Turnbridge et al. detected the presence of 7 mRNA COMT variants expressed in low abundance in the brain, resulting from insertions and deletions in the primary transcript and probably representing splice variants differentially expressed in different brain regions (Tunbridge et al. 2007).

In contrast with most tissues the MB isoform dominates in the brain and in the adrenal gland medulla representing 70% of the COMT total protein content (Männistö and Kaakkola 1999). In the rat and human species, the greatest activity by decreasing order is found in the liver, kidney and brain (Bonifacio et al. 2000).

1.3 Physiological Role

COMT seems to act as a physiological barrier at the blood–brain barrier, gastrointestinal tract and placenta (Männistö and Kaakkola 1999). During the first quarter of pregnancy COMT protects the developing embryo and the placenta from the action of active hydroxyl compounds (Männistö and Kaakkola 1999). In neural tissues, the location of COMT indicates its role as a barrier that prevents dissemination of catechol compounds between compartments (Karhunen et al. 1995b) and in particular from injured areas (Redell and Dash 2007). The enzyme also regulates levels of dopamine (DA) and norepinephrine (NE) in several cerebral regions, inactivates postsynaptic CA (Männistö and Kaakkola 1999) and protects dopaminergic neurons from the free radicals generated by the oxidative metabolism of DA (Matsumoto et al. 2003).

The main and the first described role of COMT (Axelrod and Tomchick 1958) concerns the metabolism of CA, mainly NE and epinephrine (EPI), and most importantly the inactivation of the latter (Eisenhofer et al. 1995). These CA are released by the sympatho-adreno-medullary system in response to several endogenous and exogenous threats to homeostasis and can act locally at the terminals of sympathetic neurons, mainly NE, or via the bloodstream, in the case of EPI (Brede et al. 2003). Catecholamines are short acting bioactive compounds with a very short half-life of 1–2 min (Fung et al. 2008). Among other functions, they generically promote vasoconstriction, sphincter contraction, hepatic glycogenolysis and have ionotropic and chronotropic effects in the heart (Guimarães and Moura 2001). Although vital for normal physiology of cardiovascular dynamics their protracted actions, mainly when in excess, are deleterious and partially responsible for cardiovascular disorders (Adameova et al. 2009).

The major oestrone (E1) and estradiol (E2) metabolites, the CE's 2-hydroxyestrone (estradiol) (2-OHE1(E2)) and mainly the 4-hydroxyestrone (estradiol) (4-OHE1(E2)), have been shown to be carcinogenic in various tissues, including the kidney, liver, uterus and mammary glands (Yager and Davidson 2006). E2 was specifically shown to play a major role in the progression and growth of hormone-dependent breast cancer (Siebert et al. 2011). In addition, these metabolites can be further oxidized to electrophilic CE quinones, namely E2-3,4-quinones and E2-2,3-quinones (Zhang et al. 2007), and generate reactive oxygen species (ROS) and depurating DNA adducts that may lead to cancer-initiating mutations (Zahid et al. 2006; Mailander et al. 2006). In extra hepatic tissues, *O*-methylation by COMT is the main pathway for CE's inactivation, this neutralizes their oestrogen activity and prevents accumulation of oestrogen quinones reducing the risk of DNA damage (Fig. 2) (Yager 2012).

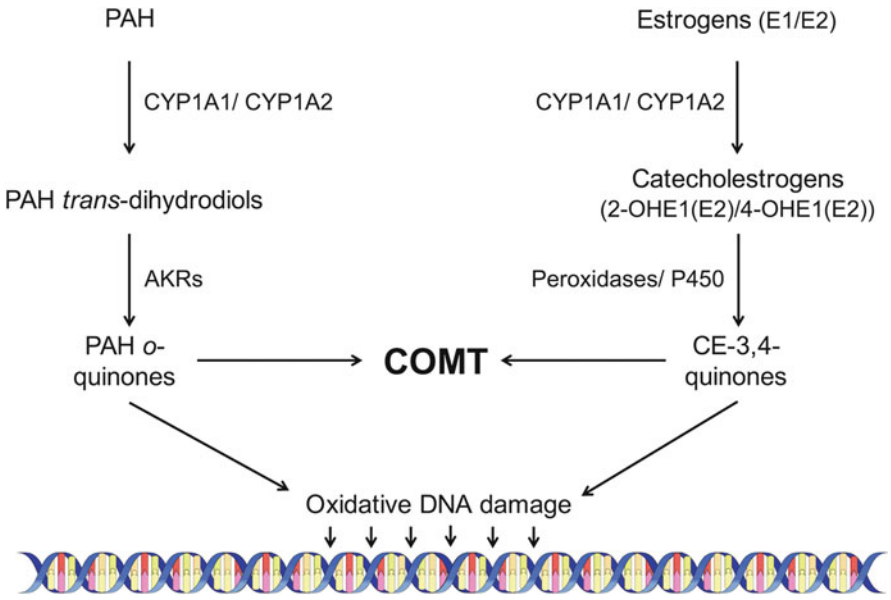

Fig. 2 Scheme of the pathways of polycyclic aromatic hydrocarbons (PAH) and oestrogen metabolism that lead to the formation of PAH *o*-quinones and CE-3,4-quinones, respectively, compounds with the ability to form depurinating DNA adducts and reactive oxygen species and induce mutagenesis. COMT can intercept and prevent the deleterious effects of these compounds. Cytochrome P450 1A1 (CYP1A1), cytochrome P450 1A2 (CYP1A2), aldo-keto reductases (AKRs), cytochrome P450 (P450)

1.4 COMT Polymorphisms and Altered Function

Regarding its critical physiological roles, the regulation of COMT expression and polymorphisms affecting enzyme activity have been gathering attention in face of their potential effects on the pathophysiology and susceptibility to hormonally influenced diseases including neurodegenerative (Lee and Song 2014; Jiménez-Jiménez et al. 2014) and cardiovascular diseases (Chi et al. 2011; Voutilainen et al. 2007), oestrogen induced cancers (Li et al. 2014a; Xiao et al. 2013; Teng et al. 2013) and DA and NE dependent neuropsychiatric disorders (Gatt et al. 2015; Lachman et al. 1996). The Val158Met polymorphism (rs4680) is considered the main cause for COMT activity variation. This single nucleotide polymorphism (SNP), involving a G to A transition at codon 158, results in an amino acid change and makes the enzyme prone to active-site distortion and protein aggregation at physiological temperature, leading to a four times reduction in enzymatic activity when in homozygosity for the Met allele (Rutherford et al. 2008; Bilder et al. 2004). The Val/Val, Val/Met and Met/Met genotypes are associated with high, intermediate and low activity phenotypes of the enzyme, respectively. Individually, the alleles are differently distributed among ethnic groups, being the low activity allele found with a frequency between 54 and 49% in Caucasians, 49% in Southwest

Asians, between 18 and 30% in East Asians, 2.6% in African Americans and between 3.6 and 2.6% in Africans (Ameyaw et al. 2000; McLeod et al. 1994). This difference in activity has also been suggested to occur due to a lower enzyme concentration in the Met/Met genotype, as observed in human liver tissue (Doyle et al. 2004), COS-1 and HEK293 cell lines (Shield et al. 2004), arising either from alterations in transcription or shorter half-life from increased protein degradation.

Nonetheless, the inconsistent association between this polymorphism and some disorders suggests the existence of other functional COMT polymorphisms or of genetic epistaxis with several other genes (Smith et al. 2014b; Wang et al. 2014a; Zhang et al. 2014). Studies involving the Ala22Ser (rs6267) and the Ala52Thr (rs5031015) polymorphisms, respectively, responsible for a 30% lower activity and a lower SAM affinity, have shown an association with some disorders (Rutherford and Dagget 2009). Nackley et al. suggested the existence of 3 COMT haplotypes formed by the combination of 4 SNPs, one positioned in the S-COMT promoter region (rs6269) and three in the S and MB-COMT coding region at codons His62His (rs4633), Leu136Leu (rs4818) and Val158Met (Nackley et al. 2006). The haplotype with the lowest activity showed an enzymatic activity 25 times lower than the haplotype with the highest activity (Nackley et al. 2006). Supporting a possible role for other SNPs, the Val allele was shown to be present in both low and high activity COMT haplotypes suggesting an haplotype-specific secondary mRNA structures change affecting the efficiency of protein synthesis (Diatchenko et al. 2005; Nackley et al. 2006). Curiously, it was observed that transcripts encoding the high activity Val were expressed in lower levels, in the human brain, than those encoding the low activity Met, suggesting a compensatory mechanism (Bray et al. 2003). A similar behaviour was observed by Tsao et al., with average pain sensitivity haplotypes (characterized by lower COMT activity) exhibiting significantly higher protein levels than wild-type low pain sensitivity haplotypes (characterized by a higher COMT activity) (Tsao et al. 2011).

It would be interesting to observe if the inconsistencies commonly observed in studies concerning the association between COMT polymorphisms and health disorders would endure if COMT genetic variants other than Val158Met were considered. This would allow for a more precise evaluation of the effects of a truly high, intermediate or low activity enzyme.

Epigenetic regulation of COMT expression by differential methylation of the S- and MB-COMT promoters is also gaining attention with findings of hypomethylation of the COMT promoters in saliva (Nohesara et al. 2011) and the frontal lobe (Abdolmaleky et al. 2006) of patients with schizophrenia and bipolar disease. Methylation of COMT can be environmentally induced and is an important mechanism of gene expression regulation (Diwadkar et al. 2014). Stress (Ursini et al. 2011) and physical activity (Lott et al. 2013) are among the main environmental factors believed to modulate DNA methylation of COMT and affect protein expression and activity with measurable effects on prefrontal cortex DA levels and cognition. Childhood adversities have also been proposed to promote an epigenetic downregulation of the COMT gene (Green et al. 2014).

2 COMT Role in Disease

2.1 Cancer

It has been hypothesized that a reduced COMT activity may increase the risk of hormone-dependent diseases by enhancing the serum and tissue levels of E2, promoting CE accumulation (Cordts et al. 2014). Postmenopausal women with at least one Met allele (low activity) were shown to have higher E2 serum values 3 h after E2 valerate administration (Worda et al. 2003). Inhibition of COMT activity has shown to increase the amount of oxidative damage and depurating DNA adducts in MCF-10F (Zahid et al. 2007) and MCF-7 cells (Yamazaki et al. 2012). COMT inhibition also leads to the occurrence of cytotoxicity in melanocytes (Smit and Pavel 1995). Furthermore, multiple authors have reported anticarcinogenic (Chang et al. 2012), antimitotic (Kuo et al. 2013), antiangiogenic (Quezada et al. 2013), antiproliferative (Siebert et al. 2011; Wu et al. 2015a), proapoptotic (Salama et al. 2006b; Carothers et al. 2002) and anti-inflammatory (Shand et al. 2011) properties for 2- and 4-methoxyestradiol, both products of CE's methylation by COMT. In addition, S-COMT selective presence in the nucleus may represent a defence mechanism against an excess of CE (Weisz et al. 1998, 2000). COMT also showed evidence of tumour suppression activity in a colorectal cancer cell line, to modulate the PI3K/Akt pathway involved in the onset of human cancers, to potentiate tumour suppressor genes such as p53, p27 and PTEN and to arrest the cell cycle at the G1-phase (Wu et al. 2015a). The modulation of the PI3K/Akt/mTOR pathway was also observed in 3 cell lines models of pancreatic cancer, where COMT overexpression was shown to downregulate p-Akt, mutant p53, cyclin D1, anti-apoptotic p-Bad and upregulate p-GSK3-b, PTEN, proapoptotic Bim and Bax and the invasion suppressor molecule E-cadherin (Wu et al. 2015b). COMT silencing promoted cell growth and invasion, an increase in the S-phase cell ratio and inhibited apoptosis (Wu et al. 2015b). A potential COMT protective effect is suggested by the higher mean survival time of pancreatic cancer patients with a high activity COMT genotype (Wu et al. 2015b). Decreased COMT mRNA and protein levels were also observed in cell lines of renal cell carcinoma (Chang et al. 2012). COMT polymorphisms may also play a role on prostate cancer (Tanaka et al. 2006) and the prostatic regions more prone to cancer development were shown to have decreased metabolization of CE by COMT (Cavalieiri et al. 2002). Sasaki et al. observed the occurrence of methylation in the MB-COMT promoter in 78.3% of the endometrial cancer samples studied, reporting an absence of methylation in normal endometrial samples (Sasaki et al. 2003). Even though a role for a low activity COMT polymorphism in the development of lung cancer was proposed (Stabile et al. 2002), based on expression of oestrogen receptors by lung cancer cell lines, only a probable increase in susceptibility was found by a recent meta-analysis (Tan and Chen 2014).

2.2 Pregnancy and Hormone-Dependent Diseases

COMT is suggested to regulate the uterine oestrogenic environment and oestrogen dependent functions such as the uterine contractility pathways (Harirah et al. 2009). The E2 COMT metabolite, 2-methoxyestradiol (2-ME), is considered to be part of the ovarian physiological apparatus and crucial for proper placental vascular development and maintenance (Salih et al. 2008b). COMT expression increases with the pregnancy progression and peaks at delivery (Harirah et al. 2009) with a 1,000-fold increase in 2-ME levels (Pérez-Sepúlveda et al. 2012). During labour, COMT mRNA levels in the amnion layer suffer a ninefold increase, when compared with non-labouring tissues obtained from elective caesarean section (Harirah et al. 2009). Not only patients with premature ovarian insufficiency present frequently with low COMT activity and 2-ME synthesis (Cordts et al. 2014), but 2-ME levels are also found decreased in the serum of pregnant women suffering from hypertensive disorders of pregnancy as preeclampsia (Shen et al. 2014b). Women who developed preeclampsia were shown to present low 2-ME plasma levels, even during the first trimester of pregnancy (Pérez-Sepúlveda et al. 2012). COMT protein levels and enzyme activity were found to be lower in the placenta of third trimester preeclampsia cases (Zhao et al. 2011). The G675A polymorphism of COMT was shown to associate with hypertensive disorders of pregnancy, particularly in the presence of the MTHFR C677T SNP, and inhibition of COMT in pregnant rats caused arterial hypertension, endothelial dysfunction and increased embryo-to-placenta/decidua ratio (Vazquez-Alaniz et al. 2014; Kanasaki et al. 2008). In comparison to wild-type animals, pregnant COMT knockout mice exhibit preterm delivering, higher fetal wastage, arteriopathy and proteinuria with glomerular disease similar to women with preeclampsia (Kanasaki et al. 2008). Exogenous 2-ME corrects the embryo-to-placenta weight ratio, prevents proteinuria and ultrastructural glomerular lesions and ameliorates the excessive fetal wastage and vascular abnormalities (Kanasaki et al. 2008). Administration of 2-ME to the COMT knockout pregnant mice also decreased the markers of hypoxia, the accumulation of HIF-1α in the placenta, the plasma concentrations of sFLT-1 and restored eNOS expression in the placenta (Kanasaki et al. 2008). Both exogenous 2-ME and COMT overexpression in human uterine leiomyoma cells promote microtubule stabilization, attenuate nuclear receptors signalling, decrease HIF-1α and antagonize the expression of CYP19 induced by TNF-α (Salama et al. 2009). The low activity COMT haplotype was significantly associated with recurrent preeclampsia and higher risk of preterm labour, fetal growth restriction and later life cardiovascular disease (Roten et al. 2011). While the S-COMT promoter was shown to suffer tissue specific unmethylation in the placenta, the methylation status of the two COMT promoters was not observed to be significantly different between normal or preeclamptic pregnancies (Zhao et al. 2011). Abnormal COMT activity and 2-ME levels are thought to contribute to placental pathology by different ways in different stages of the pregnancy (Roten et al. 2011). Women carrying the

Met allele were found to have a 20% higher risk of developing endometriosis and adenomyosis (Tong et al. 2014).

COMT was also implicated in Ca^{2+} homeostasis, namely affecting the expression of several Ca^{2+} transporters in the duodenum, kidney and placenta of pregnant mice, and COMT inhibition produced pathophysiological changes similar to pre-eclampsia (Yang et al. 2015).

Early pubertal Caucasian girls carrying the low activity Met allele presented higher height and skeletal size than girls with the high activity allele, with more pronounced differences when in homozygosity, probably reflecting a more rapid onset of oestrogen-regulated pubertal development (Eriksson et al. 2005).

2.3 Cardiovascular Disease and Related Disorders

CA mediate the cardiovascular effects of the sympathetic nervous system and higher circulatory levels of these amines are associated with cardiovascular diseases through their metabolic effects on the glucose metabolism and cardiovascular physiology (Sun et al. 2012; Hall et al. 2016a). Compared with normotensives, hypertensive subjects have higher levels of norepinephrine (NE) and lower levels of normetanephrine, a COMT methylated product of NE, suggesting a lower COMT inactivation of this CA (Sun et al. 2012). Expectedly, it has been shown that the COMT Val158Met polymorphism effects cardiovascular health (Hall et al. 2014) with a higher risk of acute coronary events (Voutilainen et al. 2007), higher systolic blood pressure (Chi et al. 2011), waist-hip ratio (Annerbrink et al. 2008) and systemic atherosclerotic disease, the latter also in linkage with the rs4633(C>T) variant (Ko et al. 2012). In agreement, the COMT Val allele was associated with lower incidence of cardiovascular disease in women compared with higher incidence among Met carriers (Hall et al. 2014) and Val/Val seems to confer further protection (Hagen et al. 2007). A recent genome wide association study (GWAS) linked the Val158Met SNP to variation on systolic blood pressure (Liu et al. 2016). This association was previously reported by another GWAS, for both the Val158Met and the rs4633 SNPs, showing higher diastolic and systolic pressures in Met/Met individuals (Miyaki et al. 2012). The Val158Met was also significantly linked with hypertension while in linkage disequilibrium with the SNP-1187G>C of COMT (Kamide et al. 2007). Likewise, spontaneously hypertensive rats (SHR) exhibit not only a lower expression and activity of hepatic MB-COMT, but also a decreased ability to methylate CA when compared with Wistar-Kyoto (WKY) rats (Tsunoda et al. 2003). This difference in MB-COMT activity was also observed in the cerebral cortex, being the NE concentrations in the cerebral cortex of SHR rats significantly higher than in WKY rats (Masuda et al. 2006). Cardiac surgery patients homozygous for the low activity Met allele had higher plasma levels of CA as a possible result of lower COMT activity and decreased removal of the EPI and NE released preoperatively (Haase-Fielitz et al. 2009). These patients had a higher incidence of vasodilatory shock, acute kidney injury and prolonged hospital

stay (Haase-Fielitz et al. 2009). During myocardial ischemia, CA accumulate in the myocardial interstitium, especially NE, and COMT was found to be one of the major pathways for CA clearance in this condition (Kuroko et al. 2005). A COMT-dependent relationship between the consumption of coffee and coronary heart disease has also been observed (Happonen et al. 2006). In Met/Met men, heavy coffee intake was associated with higher incidence of acute coronary events when compared with Val allele carriers (Happonen et al. 2006). Caffeine stimulates adrenomedullary secretion of CA and it was hypothesized that a slower COMT would promote CA accumulation and increase coronary risk (Happonen et al. 2006). In addition, caffeic acid directly inhibits COMT and caffeine promotes the formation of SAH, a known COMT inhibitor (Happonen et al. 2006).

COMT activity may also play a role in the inverse relationship between the intake of mineral rich waters and the cardiovascular risk (Catling et al. 2008). In rats, drinking a mineral rich water increased COMT expression and activity in the liver while decreasing expression without affecting activity in the adrenal glands (Bastos et al. 2014). A decrease in the COMT-dependent CA catabolism may also play a role in the inverse relationship between Ω-3 polyunsaturated fatty acids intake and cardiovascular mortality (Gomes et al. 2015).

In contrast, Eriksson et al. observed a lower incidence of myocardial infarction in patients with a low activity (Met/Met) COMT genotype, suggesting a cardio-protective effect for this enzyme, probably attributable to a lower degradation of estradiol, being this effect particularly evident in the older patients, who have lower and even critical estradiol levels (Eriksson et al. 2004).

Considering the CA influence on insulin and glucose metabolism, the Val allele was linked with lower HbA_{1c} in the Women's Genome Health Study (WGHS) and showed a similar but borderline association with HbA_{1c} levels and type 2 diabetes in the Meta-Analyses of Glucose and Insulin-related traits Consortium (Hall et al. 2016a).

COMT activity in the kidney may also contribute to its possible role in the pathogenesis of hypertension (Ooshima et al. 2009). The enzyme activity is in the range of the observed in the liver and besides being the main inactivation mechanism for kidney derived DA, it may also contribute to the inactivation of CA in general and modulation of renin release (Ibarra et al. 2005; Odlind et al. 2001; Zhang et al. 2009). DA formed in the kidney plays an important role in the regulation of Na^+ transport and excretion and decreases Na^+ reabsorption by inhibiting the Na-K-ATPase and Na/H exchanger (Ibarra et al. 2005). COMT knockout mice exhibit resistance to salt induced hypertension and have higher urinary DA (Helkamaa et al. 2003) and NE excretion (Odlind et al. 2002). Comparing to normotensive rats, SHR and malignant stoke prone spontaneously hypertensive rats exhibit a decrease in renal cortex expression of COMT, being lower in the latter (Ooshima et al. 2009). A lower COMT-dependent metabolism of DA was also associated with an increased susceptibility to diabetic kidney disease in rats (Prasad et al. 2008).

To our knowledge, COMT was the first reported modifier gene for polycystic kidney disease (PKD) and its selective inhibitor, tolcapone, was demonstrated to

prevent renal failure in PKD/Mhm COMT-overexpressing rats, animal models of PKD (Boehn et al. 2013).

The variation in COMT activity has also been implicated in the chronic fatigue syndrome, a debilitating condition characterized by unexplained long-lasting fatigue, widespread muscle pain, cognitive impairment and orthostatic intolerance (Hall et al. 2016b). Although the underlying disease mechanisms are still unknown, the hypothalamic pituitary axis has been suggested to play a role in this condition (Light et al. 2009) and this hypothesis is supported by the elevated NE and EPI levels found at rest and an attenuated heart rate variability in these patients (Hall et al. 2016b). Curiously, homozygosity for the Met allele is more prevalent in patients with this syndrome (Löbel et al. 2015).

2.4 Other Peripheral Roles

The COMT *O*-methylation has also been proposed to be an important mechanism for the inactivation of polycyclic aromatic hydrocarbon (PAH) *o*-quinones produced during the redox-cycling process of PAH, known tobacco carcinogens suspected as causative agents in lung cancer (Fig. 2) (Zhang et al. 2011).

COMT polymorphisms and activity may also play an important role in gene–environment interactions. The most abundant tea catechin, (−)-epigallocatechin-3-gallate (EGCG), is an important exogenous COMT inhibitor (IC_{50} between 0.04 and 0.5 μM). It is readily absorbed after intake and reaches plasma concentrations between 0.1 and 0.4 μM, being shown to inhibit the COMT *O*-methylation of CE in human liver samples (Nagai et al. 2004). This effect may be more pronounced in subjects with low activity haplotypes and women whose COMT activity is naturally 30% lower than men.

Although a role for COMT has not yet been proposed, high levels of CE are being implicated in autoimmune diseases, namely rheumatoid arthritis (Khan and Moinuddin 2011) and systemic lupus erythematosus (Khan et al. 2009), leading the DNA damage to the induction of autoantibodies. Curiously, in an experimental model of rheumatoid arthritis, 2-ME has shown to ameliorate the clinical and histopathological signs, attenuating the macrophages and neutrophils infiltration and the production of ROS (Stubelius et al. 2011). S-COMT activity is also reported to be higher in patients with moderate to severe psoriasis (Souteiro et al. 2013), and in psoriatic skin lesions, being the enzyme activity decreased by narrowband UVB phototherapy (Magina et al. 2013).

2.5 Nervous System Physiological Role and Related Diseases

Numerous association studies on COMT polymorphisms, reporting strong, weak and sometimes complex and opposite relations, have been conducted for cognitive

function performance and cognitive disorders (Pap et al. 2012; Mier et al. 2010), emotion processing and related disorders (Kilford et al. 2015; Opmeer et al. 2013), sleep and related disorders (Dauvilliers et al. 2015; Valomon et al. 2014), creativity (Zhang et al. 2014), impulsivity (Soeiro-De-Souza et al. 2013), anxiety and stress related disorders (Papaleo et al. 2008; Kline et al. 2015), pain processing and sensitivity (Lee et al. 2015; Martínez-Jauand et al. 2013), violent behaviours and cognitive function in schizophrenia (Green et al. 2014; Singh et al. 2012), alcoholism (Nedic et al. 2011; Wang et al. 2010) and risk of developing addictive behaviours (Herman et al. 2013; Mione et al. 2015).

2.5.1 Cognition, Behaviour and Related Disorders

In recent years, COMT involvement in the neurobiology of cognition, emotion, personality and related disorders has been actively researched due to its critical role in the degradation of synaptic dopamine (DA) (Fig. 3) (Gatt et al. 2015; Frank and Fossella 2011).

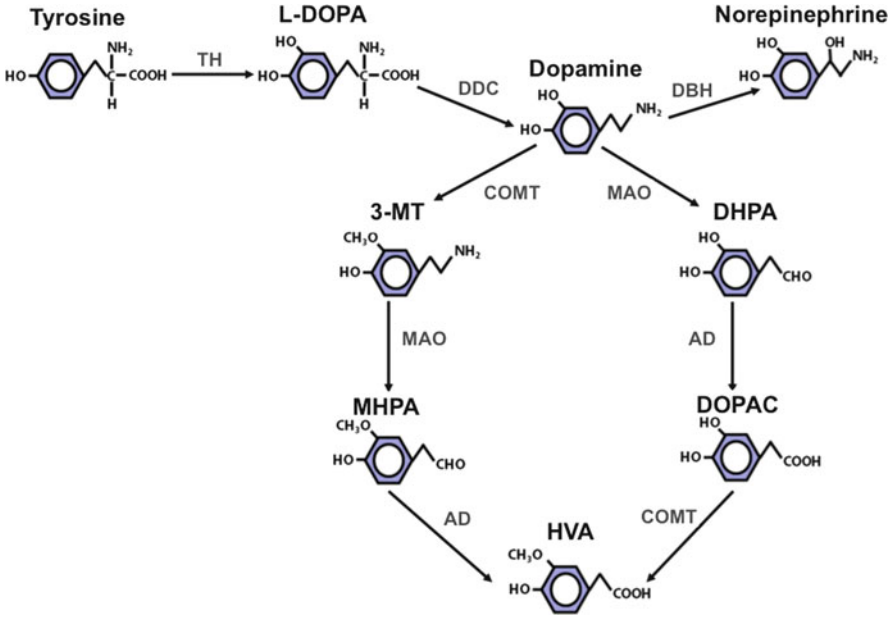

Fig. 3 The synthesis and degradation of DA. Enzymes involved in the synthesis and catabolism of DA are represented in *grey*. Dopamine is synthesized from tyrosine via L-DOPA (the rate-limiting step being the conversion of tyrosine to L-DOPA, mediated by tyrosine hydroxylase (TH)). Dopamine can be converted to norepinephrine, via the action of dopamine β-hydroxylase (DBH) or catabolized by the joint action of the enzymes COMT, monoamine oxidase (MAO) and aldehyde dehydrogenase (AH). Other abbreviations: *DDC* dopamine decarboxylase, *3-MT* 3-methoxytyramine, *DHPA* 3,4 dihydrophenylacetaldehyde, *MHPA* 3-methoxy-4-hydroxyphenylacetaldehyde (Laatikainen et al. 2013)

Controversies apart, the Val158Met polymorphism has been associated with changes in the prefrontal cortex (PFC) function (Ira et al. 2013) and structure (Sannino et al. 2015; Tian et al. 2013), affecting personality traits and the risk or severity of several major mental disorders (Antypa et al. 2013; Harrison and Tunbridge 2008) such as schizophrenia (Walton et al. 2014), psychosis (Collip et al. 2011), anxiety and stress related disorders (Lee and Prescott 2014; Hernaus et al. 2013), mood disorders such as bipolar disorder (Gutiérrez et al. 1997; Soeiro-de-Souza et al. 2012) and depression (Du et al. 2014; Shen et al. 2014a), and attention related disorders such as attention deficit hyperactivity disorder (ADHD) (Lee and Song 2014; Cheuk and Wong 2006). Several studies suggest Val158Met modulates DA signalling in a way that the more active enzyme form (Val allele) decreases synaptic DA levels and impairs PFC function and connectivity efficiency (Jaspar et al. 2014; Tunbridge et al. 2013; Wu et al. 2012). The expression of COMT is considered to display sexual dimorphism, with levels in the PFC shown to be higher in males than females in both human and rodent brains, and to associate with increased susceptibility to Val/Val effects in men (Kilford et al. 2015; White et al. 2014). However, no association between Val158Met and working memory performance was found in a population-based cohort of healthy young adults (Wardle et al. 2013).

In a recent study, Sannino et al. (2015) reported a remarkable interaction between COMT genetics and gender on cortical morphometry and function (Sannino et al. 2015). Overall, reduced COMT activity, either by COMT knockout in mice or the Met allele in humans, was associated with increased cortical thickness in the PFC and postero-parieto-temporal cortex, and with increased neuronal density in the PFC of male healthy adult mice and humans, while no effect on thickness and a decreased neuronal density was observed on females (Sannino et al. 2015). Moreover, COMT genetics displayed divergent effects on PFC-dependent working memory in both mice and humans, with low COMT activity associated with better performance (Sannino et al. 2015). Another recent work concluded that Val/Val male carriers may have lower cognitive control when compared to Met allele male carriers (Val/Met and Met/Met) and women (Mione et al. 2015). In addition, a meta-analysis on the neural substrates of pleiotropic action of COMT genetic variation found that Val/Val subjects seem to have greater activation on PFC for the same level of cognitive performance, implying a somehow lower cognitive efficiency compared to other genotypes (Mier et al. 2010; Tunbridge et al. 2013). Latest neuroimaging studies further confirm these effects on neural substrates of working memory with Met human carriers benefiting from enhanced cognitive stability and efficiency in memory interference paradigms (Jaspar et al. 2014, 2015). Similar results on schizophrenic patients revealed a poorer cognitive performance and reduction of temporal areas in association with the Val allele, and also a reduction of frontal areas among Val/Val schizophrenics at high risk for psychosis (Ira et al. 2013). Therefore, there is evidence for a potentially protective effect of oestrogens on pathologies where the DA level is crucial, such as in schizophrenia (Mione et al. 2015).

As aforementioned, COMT activity effects seem to be beyond the classical PFC function and to involve many other cortical and subcortical areas and neuronal networks, potentially modulating a wide range of behaviours (Frank and Fossella 2011; Dauvilliers et al. 2015). In fact, Val/Val carrying healthy young adults showed greater intrinsic activity on the left para-hippocampal cortex when compared with Met carriers (Zhang et al. 2015), and COMT was reported to modulate functional coupling between PFC and hippocampus during retrieval of episodic memories (Frank and Fossella 2011). Accordingly, tolcapone, a brain penetrating COMT inhibitor, was recently shown to significantly increase DA levels in the ventral hippocampus of rats, without an affect in other regions, nor on norepinephrine on all tested brain regions (Laatikainen et al. 2013). Tolcapone also affected DA brain metabolism as shown by an overall effect in the levels of its metabolites in all studied brain regions, with a greater impact on the PFC of female rats compared to males (Laatikainen et al. 2013). It should be mentioned that COMT activity did not associate with DA levels in the striatum (Huotari et al. 2002; Laatikainen et al. 2013), though its absence in mice related with higher levels of 3,4-dihydroxyphenylacetic acid (DOPAC) in the dorsal striatum (Tammimaki et al. 2010), a DA metabolite associated with increased behavioural activity and stereotypy in rats (Nakazato and Akiyama 2002). Likewise, variations on *O*-methylation of DA and DOPAC in the brain of mice may produce subtle nuances on social behaviours (Tammimaki et al. 2010). There is also evidence of a potential clinically relevant sexual dimorphism on behaviour and response to COMT inhibitors in both rodents and humans (Laatikainen et al. 2013; White et al. 2014). A curious study, comparing HIV-infected with HIV-uninfected women, revealed a significant interaction between serostatus and Val158Met, reporting working memory deficits and altered PFC function only among HIV-infected Val/Val carriers (Sundermann et al. 2015).

Notwithstanding, the predicted higher levels of DA in the PFC of Met allele human carriers, which seems beneficial for working memory stability and reduced distractibility, were associated with reduced cognitive flexibility and lower performance on task-switching and cognitive adaptability when compared to Val/Val carriers (Colzato et al. 2010; Frank and Fossella 2011). In agreement, Met/Met male human carriers, while shown to outperform their Val/Val counterparts on a working memory related task, displayed greater risk aversion, and tolcapone was reported to remarkably reverse these genotype differences (Farrell et al. 2012).

Additionally, another meta-analysis, focusing on neuroimaging studies, concluded that there is a significant association concerning COMT activity haplotypes and PFC functional activation (Mier et al. 2010). Moreover, available data supports a robust opposite effect of the Val158Met on executive cognition paradigms (favouring Met allele carriers) and emotional paradigms (favouring Val allele carriers) (Mier et al. 2010; Frank and Fossella 2011). In opposition, Val158Met was reported to affect emotional information processing on depression, with Met carriers displaying increased activity in limbic areas and PFC, but being also more likely to respond better to antidepressants, compared to the Val carriers (Antypa et al. 2013). In another study, Met/Met carriers were found to outperform Val

carriers on manipulation of self-generated thoughts, being Val male carriers more susceptible to affective distractors, compared to females (Kilford et al. 2015). Furthermore, Val158Met was shown to modulate the functional connectivity of the executive control network (head of the caudate, anterior cingulate and frontal cortical areas), with Val/Val carriers showing an increased resting connectivity within the left ventrolateral PFC (Tunbridge et al. 2013). In opposition, lower DA function was associated with decreased baseline activity in frontal regions implicated in salience attribution (orbitofrontal cortex) and inhibitory control (anterior cingulate gyrus), and functional disruption of these areas are known to result in compulsivity and impulsivity (Volkow et al. 2011).

Despite some contradictory results, more recent data support a non-linear inverted-U model of COMT activity and/or DA levels impact on executive performance, PFC function and efficiency of connectivity of related neuronal networks (Htun et al. 2014; Mier et al. 2010). In this model, intermediate levels of COMT activity and/or DA signalling produce an optimized executive function, stable enough to maintain optimal cognitive coherence, yet flexible enough to allow optimal cognitive adaptability (Smith et al. 2014a; Farrell et al. 2012).

Expectedly, there is epistaxis between COMT polymorphisms and several other genes related to cognition and behaviour. For instance, Val158Met interacts with the methylenetetrahydrofolate reductase (MTHFR) gene Ala222Val polymorphisms on cognitive function, with the COMT Met/Met and MTHFR Ala/Ala genotype associating with a better performance (Wang et al. 2014a). Another study found a non-linear inverted-U association between cognitive performance and COMT Val158Met haplotypes ranked by predicted enzymatic activity, but only in the presence of the MTHFR Ala/Ala and not found among MTHFR Val carriers regardless of COMT haplotype (Htun et al. 2014). Also, a logistic regression model on major depression among humans, adjusted for age and gender, found a significant COMT-MTHFR epistaxis, so that the combination of COMT Val/Met and MTHFR Ala/Val caused the highest probability of suffering from depression (Shen et al. 2014a).

The DA receptor D2 (DRD2) gene is another candidate for epistaxis with COMT. The TaqIA polymorphism, located downstream of the actual gene, modulates DRD2 density in the striatum in a way that the C/C carriers (or A2/A2) benefit from higher striatal DA levels and better performance on working memory tasks and error avoidance when compared with T-allele (or A1) carriers (Berryhill et al. 2013). However, here too, the COMT Val158Met only seems to affect cognitive function when associated with the DRD2 C-allele (Berryhill et al. 2013). Taken together, these findings link better working memory performance with slower dopaminergic metabolism in the PFC and greater density of DA receptors in the striatum.

Epistaxis has also been reported between COMT and the protein phosphatase 1, regulatory (inhibitor) subunit 1B (PPP1R1B) gene, which modulates signalling through the DA receptor D1 (DRD1), the dominant DA receptor in the frontal cortex (Smith et al. 2014a). Data suggests that the T allele of PPP1R1B, which associates with increased dopaminergic signalling, may correct the relative

cognitive performance impairment associated with the presumable low frontal DA levels of COMT Val carriers, while somehow impairing it among Met carriers, which have putatively higher frontal DA levels (Smith et al. 2014a).

Finally, the metabotropic glutamate 3 receptor (GRM3) gene, namely its rs6465084 intron polymorphism, interacts with COMT Val158Met to modulate the efficiency of cortical activation in PFC and its connectivity with the parietal cortex (Tan et al. 2007; Xia et al. 2012). The GRM3 A/A or T allele carriers are associated with reduced MRI markers of synaptic activity and tissue glutamate levels (measured by *N*-acetylaspartate), mainly in the PFC, in association with decreased executive function, while the G allele seems to be protective for cognition (Xia et al. 2012). The GRM3 A/A genotype associated with greater (therefore inefficient) cortical activation and decreased cortical network connectivity when combined with COMT Val/Val, but not Met/Met genotype (Tan et al. 2007).

Variation in the COMT expression has been extensively investigated in relation to clinical phenotypes of depression, in parallel with neurocognitive processes (Antypa et al. 2013). In fact, the Met allele has been associated with greater risk for depression, in line with depressed patients showing a blunted reactivity to both positive and negative stimuli, possibly, as a sign of anhedonia (Antypa et al. 2013). Furthermore, Met/Met carriers were reported to be more prone to cognitive impairment caused by acute stressors, which suggests that Met may be a risk allele for depression under stress (Antypa et al. 2013). Accordingly, subjective stress responses may be smaller in Val/Val, compared to Met carriers, which may be more vulnerable to psychosocial stressors (Hernaus et al. 2013) and psychosis (van Winkel et al. 2008). In mice, greater COMT activity leads to cortical cognitive dysfunction and protects against stressful conditions, whereas lower COMT activity enhances working memory processes at the cost of exaggerated stress reactivity (Papaleo et al. 2008). In human populations, Met carriers were reported to be less resilient to negative affective states, such as pain, to show a stronger response to metabolic or psychosocial stress, and to be prone to anxiety and reduced extraversion or novelty seeking (van Winkel et al. 2008). Even so, other studies support the hypothesis of a hypodopaminergic PFC state on Val/Val carriers, which would increase the risk of stress-induced psychotic experiences (van Winkel et al. 2008). Additionally, COMT polymorphisms are proposed to contribute to inadequate endocrine and psychological responses towards stress (Kvetnansky et al. 2009), being lower levels of COMT mRNA also observed in high stressed pigs (Oster et al. 2014) and aggressive mice (Ginsberg et al. 2011).

Likewise, a recent meta-analysis of 27 studies ($N = 15{,}979$) found an association between COMT Val158Met and anxiety traits with ethnic and sex-specific effects on phenotype variation (Ji et al. 2015). Compared to Met/Met men, Val carriers had higher levels of neuroticism related anxiety in Caucasians and harm avoidance in Asians, not seen in women (Ji et al. 2015). The Val allele was common across schizophrenia and anxiety disorders (panic disorder in Caucasians), whereas the Met allele was in common across bipolar disorder and anxiety disorders (obsessive compulsive disorder in particular) (Gatt et al. 2015). However, there were some

studies with conflicting null findings suggesting a possible impact of gender and ethnicity (Gatt et al. 2015).

Gene epistaxis studies shed light on these inconsistences, with reactivity to environmental stress and risk of psychosis associated with COMT Val158Met, depending on other gene polymorphisms (Collip et al. 2011). For instance, COMT Val158Met stress susceptibility phenotypes were only expressed in the presence of the Val allele of the MTHFR Ala677Val (Peerbooms et al. 2012). Besides stress, gender seems to be an important modulator of COMT expression and its effects on neuropsychological, behavioural and clinical phenotypes (Antypa et al. 2013). Available data support COMT as an environmentally modulated subtract for genetic susceptibility to stress and neurocognitive vulnerability to psychiatric disorders such as depression (Antypa et al. 2013; Gatt et al. 2015). As aforesaid, COMT contribution to such disorders depends on genetic epistaxis between several other dopaminergic and non-dopaminergic polymorphisms (Gatt et al. 2015; Antypa et al. 2013).

Overall, the available literature points to an important role of COMT polymorphisms on cognitive control processes with complex and sometimes opposite effects when pharmacologically manipulated (Smith et al. 2014a; Laatikainen et al. 2013; Farrell et al. 2012; Htun et al. 2014).

2.5.2 Pain Processing and Related Disorders

As abovementioned, functional genetic variants affecting COMT activity modulate several psychological traits which influence the perception of pain in humans (Diatchenko et al. 2007). Overall data supports an association between low COMT activity with increased pain perception and higher risk of pain-related disorders (Tammimäki and Männistö 2012; Karling et al. 2011; Kambur and Männistö 2010).

In agreement, haplotypes coding for low COMT activity were shown to contribute to gender specific pain behaviours in mice and pain ratings in humans, as revealed by increased capsaicin-induced pain behaviour in female mice and pain perception in women, with no effect on male mice or men (Belfer et al. 2013). In accordance, two recent meta-analysis concluded that lower COMT activity, as coded by the Met allele from the COMT Val158Met polymorphism, associates with increased risk and/or severity of fibromyalgia (Lee et al. 2015) and other chronic widespread pains (Tammimäki and Männistö 2012), especially when comparing Met/Met with the Val/Val carriers (Lee et al. 2015). However, no association was found with migrainous headache (Tammimäki and Männistö 2012) and a recent study reported no influence of Val158Met and another SNP on migraine susceptibility or phenotype (De Marchis et al. 2015). Nonetheless, Val158Met may influence non-migrainous headache, especially among women, with the Met allele as a risk factor (Hagen et al. 2006). Though without controversy (Tammimäki and Männistö 2012), the Met allele has also been associated with

increased risk of temporomandibular and other musculoskeletal pains (Smith et al. 2014b).

On the other hand, anxiety, conscientiousness and gastrointestinal increased perception seem to be favoured by the Val/Val genotype among patients with irritable bowel syndrome (IBS) (Karling et al. 2011; Hall et al. 2015b). On a different approach, IBS Met/Met patients were found to be more responsive to placebo treatment that Val carriers, with similar results reported for another closely linked SNP (Hall et al. 2012). Accordingly, the Met/Met genotype was associated with higher levels of DA in the PFC, a region implicated in the placebo response pathway (Hall et al. 2015a). Additionally, Met/Met human carriers shown stronger pain-related functional MRI signals than Val/Val in several brain structures related to pain processing after repeated high intensity pain stimuli (Loggia et al. 2011). Finally, the prevalence of the Met allele was found to be higher among elder IBS patients compared to controls, suggesting it is a risk factor, while Val may be protective (Wang et al. 2014b). Curiously, in a seemingly unrelated study on schizophrenia treatment, Val/Val carriers improved with tolcapone, while Met carriers responded better when treated with placebo (Hall et al. 2015a).

It is suggested that reduced COMT activity increases catecholamines in the peripheral and CNS which promotes pain by stimulation of β-adrenergic receptors (β-AR) (Kline et al. 2015; Nackley et al. 2007). A recent paper concluded that COMT inhibitors acutely increase anxiety and pain-related behaviours induced by nociceptive stimuli of deferent modalities and localizations, an affect that could be blocked by the non-selective antagonist of β-AR, propranolol (Kline et al. 2015). Also, there is evidence that the nuclear factor-kappa B (NF-κB) inhibits COMT expression in the peripheral and CNS, including forebrain and midbrain structures, suggesting a COMT role in inflammatory pain in rodents (Tchivileva et al. 2009; Hartung et al. 2015).

The effects of COMT polymorphisms on pain are bound to be complex and polygenic, and several genes have been shown to modulate the phenotype of COMT in a way that the Val158Met may produce unexpectedly opposite effects (Smith et al. 2014b). For instance, polymorphisms of the guanosine-5-triphosphate cyclohydrolase 1 (GCH1) may modulate the Val158Met phenotype by normalizing COMT activity and increasing mechanical pain thresholds on Met carriers (namely, the GCH1 rs10483639 minor G allele in homozygosity) (Smith et al. 2014b). On the other hand, oestrogen receptor 1 (ESR1) polymorphisms may impair COMT activity, increase bodily pain and produce poorer self-reported health among Val carriers (namely the ESR1 rs3020377 minor A allele) (Smith et al. 2014b). Such data suggests that the ability to predict the downstream effects of genetic variation on COMT activity may be critical to understanding the molecular basis of chronic pain conditions.

Finally, COMT Val158Met may also affect the response to analgesic medication on patients with chronic low back pain and migraine, depending on the mechanism of action (Cargnin et al. 2013). The Met allele was found to be an independent predictor for lower risk of poor analgesic response to intrathecal morphine in chronic low back pain and for a higher risk of poor analgesic response to triptans

in migraine without aura (Cargnin et al. 2013). Accordingly, low COMT activity seems to increase opioid receptors and enhance opioid analgesia and side effects in cancer related pain (Tammimäki and Männistö 2012). Rodent models also show that COMT inhibitors seem to be pro-nociceptive, except for neuropathic pain models (Tammimäki and Männistö 2012). Moreover, the Met allele (Ahlers et al. 2013) and low activity alleles from other polymorphisms (rs6269, rs4633 and rs4818) (Sadhasivam et al. 2014) have also been associated with increased overall pain, postoperative pain perception and postoperative morphine requirements. However, other authors studying all four polymorphisms found a strong association between the Met allele and lower postoperative morphine requirements (De Gregori et al. 2013). Moreover, there is good evidence suggesting that Val158Met Met/Met carriers are more likely to respond to morphine than Val/Val (Rakvåg et al. 2008; Reyes-Gibby et al. 2007). In accordance, the low activity and dominant Ser allele of the COMT Ala72Ser SNP was also associated with increased pain, motor symptoms and depression among patients with Parkinson's disease (Li et al. 2014b).

2.5.3 Sleep and Related Disorders

Sleep and sleep disorders are complex and highly variable phenotypes regulated by many genes and environmental factors. As a major enzyme of catecholamines catabolism, COMT is a logic candidate to modulate PFC activity, sleep–wake cycle regulation and the phenotype of sleep pathologies (Dauvilliers et al. 2015). Considering COMT Val158Met, Val/Val human carriers, who presumably have higher COMT activity, showed lower DA signalling in the PFC when compared to carriers of the Met allele (Dauvilliers et al. 2015). A similar effect is observed on electroencephalography (EEG) alpha oscillations after partial sleep deprivation; and response to stimulate drugs, such as modafinil, with Val/Val healthy carriers showing lower alpha oscillations power, compared with Met/Met, in both wakefulness and sleep (Dauvilliers et al. 2015). Accordingly, COMT genotype may predict inter-individual susceptibility to sleep rebound resulting from partial sleep deprivation in healthy individuals (Dauvilliers et al. 2015). Also, faster alpha-peak frequency and higher upper alpha-band power have been associated with better cognitive performance and independently associated with the Met/Met genotype (Dauvilliers et al. 2015).

Sparse evidence exists suggesting that Val/Val and Met/Met carriers may prolong sleep when allowed (resting days), whereas heterozygotes do not (Valomon et al. 2014). This is consistent with an inverted U-shaped relationship between COMT genotype-dependent differences in DA levels in the PFC and build-up of a sleep debt during the workdays (Dauvilliers et al. 2015). Curiously, COMT also showed an association with body mass index (BMI), such that Val/Met individuals had lower BMI than the homozygous (Valomon et al. 2014).

An interesting study found that narcoleptic women with Met/Met and Val/Met genotypes had a multiple sleep latency test (MSLT) score twice as long as those with the Val/Val genotype, while the opposite was observed for men (Dauvilliers

et al. 2001). Independently from gender, COMT genotype also significantly affected the presence or absence of sleep paralysis, sleep latency at night and the number of sleep-onset REM periods during the MSLT (Dauvilliers et al. 2001). In another approach, evidence exists that COMT genotype may modulate the subjects response to stimulant drugs (Dauvilliers et al. 2015). As an example, modafinil was shown to have a greater positive effect on vigilance and executive function during sleep deprivation in healthy Val/Val carriers compared with other genotypes (Dauvilliers et al. 2015). On the other hand, narcoleptics with presumably higher DA function (Met/Met carriers) may have a similar response to modafinil as other genotypes, but at a lower dosage (Dauvilliers et al. 2015). COMT has also been suggested to affect the response to other psychostimulants, such as amphetamine and methylphenidate, modulating the amplitude and direction of their effect, and the pattern of their side effects (Dauvilliers et al. 2015).

2.5.4 Addictive Behaviour and Related Disorders

COMT has been implicated in addictive behaviours as DA plays a central role in motivation and reward, while withdrawal from chronic drug abuse is believed to associate with hypodopaminergia in the human mesocorticolimbic system (Jasinska et al. 2014). Furthermore, the PFC plays an important role on addictive behaviour (Volkow et al. 2011) and has been shown to be one of the brain areas where COMT affects DA metabolism the most (Laatikainen et al. 2013).

In fact, COMT Val158Met associates with abstinence-induced alterations in working memory performance and brain activity in executive control regions, including the PFC (Ashare et al. 2013; Loughead et al. 2009). For instance, abstinent Val/Val smokers, but not Met/Met, exhibit less suppression in task-negative brain regions, such as the posterior cingulate cortex, suggesting abstinence-related working memory deficits and possible higher susceptibility to nicotine dependency and smoking relapse (Loughead et al. 2009; Nedic et al. 2010). However, other studies found that Met carriers could be at a higher risk of becoming cigarette smokers (Suriyaprom et al. 2013). Notwithstanding, Val/Val smokers, compared to Met carriers, experienced more severe withdrawal symptoms, with Val/Val female smokers reporting greater concentration difficulties and irritability than men carrying Val/Val or Met (Herman et al. 2013). Interestingly, the Val/Val genotype also associated with better performance on the math task and greater systolic blood pressure (Herman et al. 2013). Male (but not female) alcoholic suicide attempters, compared to male non-attempters, had a higher prevalence of the Met/Met genotype or Met allele, also displaying higher aggression and depression (Nedic et al. 2011). These results support the rationale of pharmacologically inhibiting COMT to aid with smoking cessation among Val/Val genotype smokers. Studies on rats suggest that COMT inhibitors, like tolcapone, may be capable of alleviating the extremely motivating or salient nature of stimuli associated with alcohol and suppress high seeking/drinking phenotypes (McCane et al. 2014; Loughead et al. 2009). Also, recent studies suggest that pharmacological

cognitive enhancement may be a candidate strategy for treating drug addictions (Sofuoglu et al. 2013).

2.5.5 Neurodegeneration and Related Disorders

To prevent the immediate peripheral inactivation of the L-DOPA (a DA precursor) used to control the motor symptoms of Parkinson's disease (PD), COMT inhibitors are used as adjuvant drugs (Sozio et al. 2012), increasing its bioavailability, half-life and efficacy, by allowing for an higher penetration through the blood–brain barrier (Rivest et al. 1999). For similar motor severity, patients carrying the low COMT activity haplotypes display greater nigrostriatal denervation, compared to subjects with higher COMT activity, suggesting that the first may better compensate for the neuronal loss by increasing DA bioavailability in the striatum (Muellner et al. 2015). Accordingly, the polymorphism Val158Met seems to be a genetic modifier of the age of onset of PD, so that the COMT high activity haplotype associates with an earlier onset, especially among men with idiopathic PD, compared to women (Klebe et al. 2013). Additionally, the COMT low activity haplotype was also associated with greater risk of dyskinesia induced by in PD (de Lau et al. 2012). Interestingly, the high activity of COMT Val/Val genotype was associated with an increased risk of wearing-off, displaying significant epistaxis with monoamine oxidase B (MAO-B), with no association with dyskinesia risk (Hao et al. 2014). Finally, it should be stressed that a recent meta-analysis concluded that COMT polymorphisms, and not only Val158Met, may not be major determinants for the risk of developing PD, nor of its clinical, pharmacological and neurochemical features (Jiménez-Jiménez et al. 2014).

As for other neurodegenerative diseases, an extensive review concluded that the COMT genotype is an unlikely independent risk factor for Alzheimer disease (AD) (Xing et al. 2013). Nevertheless, the high activity Val allele, from the COMT Val158Met SNP, may have a synergistic effect with the apolipoprotein E (ApoE) ε4 allele on increasing the risk for cognitive decline and AD, especially among women (Xing et al. 2013). Accordingly, a recent meta-analysis concluded that the low activity Met allele may be protective against AD in Asians, but not in Europeans (Lee and Song 2014).

3 Conclusion

Although the COMT function has been established for more than 50 years, new roles in human physiology have been proposed in recent years and the COMT impact in the neurobiology of neuropsychiatric disorders and in the genesis of hormonally influenced diseases are currently domains of active study. Although not yet established, the COMT function has also been gaining attention as a possible contributor to the development of cardiovascular disorders. The COMT gene is

probably not a gene for a particular disease but might have some critical effects on prefrontal cognitive performance, behavioural profile, sleep architecture, sleep EEG, vulnerability to sleep loss and response to stimulant treatment. Nevertheless, it should be emphasized the importance of taking into account the combined effect of gender and genetics on pharmacogenetic studies, diagnostic and therapeutic strategies.

References

Abdolmaleky HM, Cheng KH, Faraone SV, Wilcox M, Glatt SJ, Gao F, Smith CL, Shafa R, Aeali B, Carnevale J, Pan H, Papageorgis P, Ponte JF, Sivaraman V, Tsuang MT, Thiagalingam S (2006) Hypomethylation of MB-COMT promoter is a major risk factor for schizophrenia and bipolar disorder. Hum Mol Genet 15(21):3132–3145

Adameova A, Abdellatif Y, Dhalla N (2009) Role of the excessive amounts of circulating catecholamines and glucocorticoids in stress-induced heart disease. Can J Physiol Pharmacol 87(7):493–514

Ahlers SJ, Elens LL, van Gulik L, van Schaik RH, van Dongen EP, Bruins P, Tibboel D, Knibbe CA (2013) The Val158Met polymorphism of the COMT gene is associated with increased pain sensitivity in morphine-treated patients undergoing a painful procedure after cardiac surgery. Br J Clin Pharmacol 75(6):1506–1515. doi:10.1111/bcp.12052

Ameyaw MM, Syvänen AC, Ulmanen I, Ofori-Adjei D, McLeod HL (2000) Pharmacogenetics of catechol-O-methyltransferase: frequency of low activity allele in a Ghanaian population. Hum Mutat 16(5):445–446. doi:10.1002/1098-1004(200011)16:5<445::AID-HUMU13>3.0.CO;2-3

Annerbrink K, Westberg L, Nilsson S, Rosmond R, Holm G, Eriksson E (2008) Catechol *O*-methyltransferase val158-met polymorphism is associated with abdominal obesity and blood pressure in men. Metabolism 57(5):708–711

Antypa N, Drago A, Serretti A (2013) The role of COMT gene variants in depression: bridging neuropsychological, behavioral and clinical phenotypes. Neurosci Biobehav Rev 37 (8):1597–1610

Ashare RL, Valdez JN, Ruparel K, Albelda B, Hopson RD, Keefe JR, Loughead J, Lerman C (2013) Association of abstinence-induced alterations in working memory function and COMT genotype in smokers. Psychopharmacology 230(4):653–662. doi:10.1007/s00213-013-3197-3

Axelrod J, Tomchick R (1958) Enzymatic *O*-methylation of epinephrine and other catechols. J Biol Chem 233(3):702–705

Axelrod J, Senoh S, Wiktop B (1958) *O*-methylation of catechol amines *in vivo*. J Biol Chem 233 (3):697–701

Bastos P, Araújo JR, Azevedo I, Martins MJ, Ribeiro L (2014) Effect of a natural mineral-rich water on catechol-O-methyltransferase function. Magnes Res 27(3):131–141

Belfer I, Segall SK, Lariviere WR, Smith SB, Dai F, Slade GD, Rashid NU, Mogil JS, Campbell CM, Edwards RR, Liu Q, Bair E, Maixner W, Diatchenko L (2013) Pain modality- and sex-specific effects of COMT genetic functional variants. Pain 154(8):1368–1376. doi:10.1016/j.pain.2013.04.028

Berryhill ME, Wiener M, Stephens JA, Lohoff FW, Coslett HB (2013) COMT and ANKK1-Taq-Ia genetic polymorphisms influence visual working memory. PLoS One 8(1):e55862. doi:10.1371/journal.pone.0055862

Bilder RM, Volavka J, Lachman HM, Grace AA (2004) The cathecol-*O*-methyltransferase polymorphism: relations to the tonic-phasic dopamine hypothesis and neuropsychiatric phenotypes. Neuropsychopharmacology 29(11):1943–1961

Boehn SNE, Spahn S, Neudecker S, Keppler A, Bihoreau M-T, Kränzlin B, Pandey P, Hoffmann SC, Li L, Torres VE, Gröne H-J, Gretz N (2013) Inhibition of Comt with tolcapone slows progression of polycystic kidney disease in the more severely affected PKD/Mhm (cy/+) substrain of the Hannover Sprague-Dawley rat. Nephrol Dial Transplant 28(8):2045–2058

Bonifacio MJ, Vieira-Coelho MA, Borges N, Soares-da-Silva P (2000) Kinectics of rat brain and liver solubilized membrane-bound catechol-*O*-methyltransferase. Arch Biochem Biophys 384 (2):361–367

Bonifacio MJ, Palma PN, Almeida L, Soares-da-Silva P (2007) Catechol-*O*-methyltransferase and its inhibitors in Parkinson's disease. CNS Drug Rev 13(3):352–379

Bray NJ, Buckland PR, Williams NM, Williams HJ, Norton N, Owen MJ, O'Donovan MC (2003) A haplotype implicated in schizophrenia susceptibility is associated with reduced COMT expression in human brain. Am J Hum Genet 73(1):152–161

Brede M, Nagy G, Philipp M, Sorensen J, Lohse M, Heinz L (2003) Differential control of adrenal and sympathetic catecholamine release by α2-adrenoceptor subtypes. Mol Endocrinol 17 (8):1640–1646

Bunker A, Männistö PT, St. Pierre J, Rog T, Pomorski P, Stimson L, Karttunen M (2008) Molecular dynamics simulations of the enzyme catechol-*O*-methyltransferase: methodological issues. SAR QSAR Environ Res 19(1-2):179–189

Cargnin S, Magnani F, Viana M, Tassorelli C, Mittino D, Cantello R, Sances G, Nappi G, Canonico PL, Genazzani AA, Raffaeli W, Terrazzino S (2013) An opposite-direction modulation of the COMT Val158Met polymorphism on the clinical response to intrathecal morphine and triptans. J Pain 14(10):1097–1106. doi:10.1016/j.jpain.2013.04.006

Carothers AM, Hughes SA, Ortega D, Bertagnolli MM (2002) 2-Methoxyestradiol induces p53-associated apoptosis of colorectal cancer cells. Cancer Lett 187(1-2):77–86

Catling L, Abubakar I, Lake I, Swift L, Hunter P (2008) A systematic review of analytical observational studies investigating the association between cardiovascular disease and drinking water hardness. J Water Health 6(4):433–442

Cavalieiri EL, Devanesan P, Bosland MC, Badawi AF, Rogan EG (2002) Catechol estrogen metabolites and conjugates in different regions of the prostate of noble rats treated with 4-hydroxyestradiol: implications for estrogen-induced initiation of prostate cancer. Carcinogenesis 23(2):329–333

Chang I, Liu J, Majid S, Saini S, Zaman MS, Yamamura S, Shahryari V, Chiyomaru T, Deng G, Dahiya R, Tanaka Y (2012) Catechol-*O*-methyltransferase-mediated metabolism of 4-hydroxyestradiol inhibits the growth of human renal cancer cells through the apoptotic pathway. Carcinogenesis 33(2):420–426

Cheuk DK, Wong V (2006) Meta-analysis of association between a catechol-O-methyltransferase gene polymorphism and attention deficit hyperactivity disorder. Behav Genet 36(5):651–659

Chi HN, Miyaki K, Song Y, Ikeda S, Shimbo T, Muramatsu M (2011) Association of the catechol-*O*-methyltransferase gene Val158Met polymorphism with blood pressure and prevalence of hypertension: interaction with dietary energy intake. Am J Hypertens 29(9):1022–1026

Collip D, van Winkel R, Peerbooms O, Lataster T, Thewissen V, Lardinois M, Drukker M, Rutten BP, Van Os J, Myin-Germeys I (2011) COMT Val158Met-stress interaction in psychosis: role of background psychosis risk. CNS Neurosci Ther 17(6):612–619

Colzato LS, Waszak F, Nieuwenhuis S, Posthuma D, Hommel B (2010) The flexible mind is associated with the catechol-O-methyltransferase (COMT) Val158Met polymorphism: evidence for a role of dopamine in the control of task-switching. Neuropsychologia 48 (9):2764–2768. doi:10.1016/j.neuropsychologia.2010.04.023

Cordts EB, Santos MC, Peluso C, Kayaki EA, Bianco B, Barbosa CP, Christofolini DM (2014) COMT polymorphism influences decrease of ovarian follicles and emerges as a predictive factor for premature ovarian insufficiency. J Ovarian Res 7:47. doi:10.1186/1757-2215-7-47

Dauvilliers Y, Neidhart E, Lecendreux M, Billiard M, Tafti M (2001) MAO-A and COMT polymorphisms and gene effects in narcolepsy. Mol Psychiatry 6(4):367–372. doi:10.1038/sj.mp.4000911

Dauvilliers Y, Tafti M, Landolt HP (2015) Catechol-O-methyltransferase, dopamine, and sleep-wake regulation. Sleep Med Rev 22:47–53

De Gregori M, Garbin G, De Gregori S, Minella CE, Bugada D, Lisa A, Govoni S, Regazzi M, Allegri M, Ranzani GN (2013) Genetic variability at COMT but not at OPRM1 and UGT2B7 loci modulates morphine analgesic response in acute postoperative pain. Eur J Clin Pharmacol 69(9):1651–1658. doi:10.1007/s00228-013-1523-7

de Lau LM, Verbaan D, Marinus J, Heutink P, van Hilten JJ (2012) Catechol-O-methyltransferase Val158Met and the risk of dyskinesias in Parkinson's disease. Mov Disord 27(1):132–135. doi:10.1002/mds.23805

De Marchis ML, Barbanti P, Palmirotta R, Egeo G, Aurilia C, Fofi L, Piroso S, Ialongo C, Della-Morte D, D'Andrea G, Ferroni P, Guadagni F (2015) Look beyond Catechol-O-Methyltransferase genotype for cathecolamines derangement in migraine: the BioBIM rs4818 and rs4680 polymorphisms study. J Headache Pain 16:520. doi:10.1186/s10194-015-0520-x

Diatchenko L, Slade GD, Nackley AG, Bhalang K, Sigurdsson A, Belfer I, Goldman D, Xu K, Shabalina SA, Shagin D, Max MB, Makarov SS, Maixner W (2005) Genetic basis for individual variations in pain perception and the development of a chronic pain condition. Hum Mol Genet 14(1):135–143. doi:10.1093/hmg/ddi013

Diatchenko L, Nackley AG, Tchivileva IE, Shabalina SA, Maixner W (2007) Genetic architecture of human pain perception. Trends Genet 23(12):605–613. doi:10.1016/j.tig.2007.09.004

Diwadkar VA, Bustamante A, Rai H, Uddin M (2014) Epigenetics, stress, and their potential impact on brain network function: a focus on the schizophrenia diatheses. Front Psychiatry 5:71

Doyle AE, Goodman JE, Silber PM, Yager JD (2004) Catechol-O-methyltransferase low activity genotype (COMTLL) is associated with low levels of COMT protein in human hepatocytes. Cancer Lett 214(2):189–195

Du L, Merali Z, Poulter MO, Palkovits M, Faludi G, Anisman H (2014) Catechol-O-methyltransferase Val158Met polymorphism and altered COMT gene expression in the prefrontal cortex of suicide brains. Prog Neuro-Psychopharmacol Biol Psychiatry 50:178–183. doi:10.1016/j.pnpbp.2013.12.016

Eisenhofer G, Rundquist B, Aneman A, Friberg P, Dakak N, Kopin I, Jacobs M, Lenders J (1995) Regional release and removal of catecolamines and extraneuronal metabolism to metanephrines. J Clin Endocrinol Metab 80(10):3009–3017

Ellingson T, Duddempudi S, Greenberg BD, Hooper D, Eisenhofer G (1999) Determination of differential activities of soluble and membrane bound catechol-*O*-methyltransferase in tissues and erythrocytes. J Chromatogr B Biomed Sci Appl 729(1–2):347–353

Eriksson AL, Skrtic S, Niklason A, Hultén LM, Wiklund O, Hedner T, Ohlsson C (2004) Association between the low activity genotype of catechol-O-methyltransferase and myocardial infarction in a hypertensive population. Eur Heart J 25(5):386–391

Eriksson AL, Suuriniemi M, Mahonen A, Cheng S, Ohlsson C (2005) The COMT val158met polymorphism is associated with early pubertal development, height and cortical bone mass in girls. Pediatr Res 58(1):71–77

Farrell SM, Tunbridge EM, Braeutigam S, Harrison PJ (2012) COMT Val(158)Met genotype determines the direction of cognitive effects produced by catechol-O-methyltransferase inhibition. Biol Psychiatry 71(6):538–544. doi:10.1016/j.biopsych.2011.12.023

Frank MJ, Fossella JA (2011) Neurogenetics and pharmacology of learning, motivation, and cognition. Neuropsychopharmacology 36(1):133–152. doi:10.1038/npp.2010.96

Fung M, Viveros O, O'Connor D (2008) Diseases of the adrenal medulla. Acta Physiol (Oxford) 192(2):325–335

Gatt JM, Burton KL, Williams LM, Schofield PR (2015) Specific and common genes implicated across major mental disorders: a review of meta-analysis studies. J Psychiatr Res 60:1–13

Ginsberg SD, Che S, Hashim A, Zavadil J, Cancro R, Lee SH, Petkova E, Sershen HW, Volavka J (2011) Differential regulation of catechol-*O*-methyltransferase expression in a mouse model of aggression. Brain Struct Funct 216(4):347–356

Gomes A, Correia G, Coelho M, Araújo JR, Pinho MJ, Teixeira AL, Medeiros R, Ribeiro L (2015) Dietary unsaturated fatty acids differently affect catecholamine handling by adrenal chromaffin cells. J Nutr Biochem 26(5):563–570

Green MJ, Chia TY, Cairns MJ, Wu J, Tooney PA, Scoot RJ, Carr VJ (2014) Catechol-*O*-methyltransferase (COMT) genotype moderates the effects of childhood trauma on cognition and symptoms in schizophrenia. J Psychiatr Res 49:43–50

Guimarães S, Moura D (2001) Vascular adrenoceptors: an update. Pharmacol Rev 53(2):319–356

Gutiérrez B, Bertranpetit J, Guillamat R, Vallès V, Arranz MJ, Kerwin R, Fañanás L (1997) Association analysis of the catechol O-methyltransferase gene and bipolar affective disorder. Am J Psychiatry 154(1):113–115

Haase-Fielitz A, Haase M, Bellomo R, Lambert G, Matalanis G, Story D, Doolan L, Buxton B, Gutteridge G, Luft FC, Schunck WH, Dragun D (2009) Decreased catecholamine degradation associates with shock and kidney injury after cardiac surgery. J Am Soc Nephrol 20 (6):1393–1403

Hagen K, Pettersen E, Stovner LJ, Skorpen F, Zwart JA (2006) The association between headache and Val158Met polymorphism in the catechol-O-methyltransferase gene: the HUNT study. J Headache Pain 7(2):70–74. doi:10.1007/s10194-006-0281-7

Hagen K, Pettersen E, Stovner LJ, Skorpen F, Holmen J, Zwart JA (2007) High systolic blood pressure is associated with Val/Val genotype in the catechol-O-methyltransferase gene. The Nord-Trøndelag Health Study (HUNT). Am J Hypertens 20(1):21–26

Hall KT, Lembo AJ, Kirsch I, Ziogas DC, Douaiher J, Jensen KB, Conboy LA, Kelley JM, Kokkotou E, Kaptchuk TJ (2012) Catechol-O-methyltransferase val158met polymorphism predicts placebo effect in irritable bowel syndrome. PLoS One 7(10):e48135. doi:10.1371/journal.pone.0048135

Hall KT, Nelson CP, Davis RB, Buring JE, Kirsch I, Mittleman MA, Loscalzo J, Samani NJ, Ridker PM, Kaptchuk TJ, Chasman DI (2014) Polymorphisms in catechol-O-methyltransferase modify treatment effects of aspirin on risk of cardiovascular disease. Arterioscler Thromb Vasc Biol 34(9):2160–2167

Hall KT, Loscalzo J, Kaptchuk TJ (2015a) Genetics and the placebo effect: the placebome. Trends Mol Med 21(5):285–294. doi:10.1016/j.molmed.2015.02.009

Hall KT, Tolkin BR, Chinn GM, Kirsch I, Kelley JM, Lembo AJ, Kaptchuk TJ, Kokkotou E, Davis RB, Conboy LA (2015b) Conscientiousness is modified by genetic variation in catechol-O-methyltransferase to reduce symptom complaints in IBS patients. Brain Behav 5(1):39–44. doi:10.1002/brb3.294

Hall KT, Jablonski KA, Chen L, Harden M, Tolkin BR, Kaptchuk TJ, Bray GA, Ridker PM, Florez JC, Mukamal KJ, Chasman DI (2016a) Catechol-O-methyltransferase association with hemoglobin A1c. Metab Clin Exp 65(7):961–967

Hall KT, Kossowsky J, Oberlander TF, Kaptchuk TJ, Saul JP, Wyller VB, Fagermoen E, Sulheim D, Gjerstad J, Winger A, Mukamal KJ (2016b) Genetic variation in catechol-O-methyltransferase modifies effects of clonidine treatment in chronic fatigue syndrome. Pharmacogenomics J 16(5):454–460. doi:10.1038/tpj.2016.53

Hao H, Shao M, An J, Chen C, Feng X, Xie S, Gu Z, Chan P, Chinese Parkinson Study G (2014) Association of Catechol-O-Methyltransferase and monoamine oxidase B gene polymorphisms with motor complications in Parkinson's disease in a Chinese population. Parkinsonism Relat Disord 20(10):1041–1045. doi:10.1016/j.parkreldis.2014.06.021

Happonen P, Voutilainen S, Tuomainen TP, Salonen JT (2006) Catechol-O-methyltransferase gene polymorphism modifies the effect of coffee intake on incidence of acute coronary events. PLoS One 1:e117

Harirah H, Thota C, Wentz MJ, Zaman W, Al-Hendy A (2009) Elevated expression of catechol-O-methyltransferase is associated with labor and increased prostaglandin E(2) production by human fetal membranes. Am J Obstet Gynecol 201(5):496.e491–496.e497

Harrison P, Tunbridge E (2008) Catechol-*O*-methyltransferase (COMT): a gene contributing to sex differences in brain function, and to sexual dimorphism in the predisposition to psychiatric disorders. Neuropsychopharmacology 33(13):3037–3045

Hartung JE, Eskew O, Wong T, Tchivileva IE, Oladosu FA, O'Buckley SC, Nackley AG (2015) Nuclear factor-kappa B regulates pain and COMT expression in a rodent model of inflammation. Brain Behav Immun 50:196–202. doi:10.1016/j.bbi.2015.07.014

Helkamaa T, Mannisto PT, Rauhala P, Cheng ZJ, Fickenberg P, Huotari M, Gogos JA, Karayiorgou M, Mervaala EMA (2003) Resistance to salt-induced hypertension in catechol-*O*-methyltransferase-gene disrupted mice. J Hypertens 21(12):2365–2374

Helkamaa T, Reenilä I, Tuominen E, Soinila S, Väänänen A, Tilgmann C, Rauhala P (2007) Increased catechol-*O*-methyltransferase activity and protein expression in OX-42-positive cells in the substantia nigra after lipopolysaccharide microinfusion. Neurochem Int 51 (6–7):412–423

Herman AI, Jatlow PI, Gelernter J, Listman JB, Sofuoglu M (2013) COMT Val158Met modulates subjective responses to intravenous nicotine and cognitive performance in abstinent smokers. Pharmacogenomics J 13(6):490–497

Hernaus D, Collip D, Lataster J, Ceccarini J, Kenis G, Booij L, Pruessner J, Van Laere K, van Winkel R, van Os J, Myin-Germeys I (2013) COMT Val158Met genotype selectively alters prefrontal [18F]fallypride displacement and subjective feelings of stress in response to a psychosocial stress challenge. PLoS One 8(6):e65662

Htun NC, Miyaki K, Zhao C, Muramatsu M, Sato N (2014) Epistasis effects of COMT and MTHFR on inter-individual differences in mental health: under the inverted U-shaped prefrontal dopamine model. Biochem Biophys Res Commun 451(4):574–579. doi:10.1016/j.bbrc.2014.08.023

Huh MM-O, Friedhoff AJ (1979) Multiple molecular forms of catechol-*O*-methyltransferase. J Biol Chem 254(2):299–308

Huotari M, Santha M, Lucas LR, Karayiorgou M, Gogos JA, Männistö PT (2002) Effect of dopamine uptake inhibition on brain catecholamine levels and locomotion in catechol-O-methyltransferase-disrupted mice. J Pharmacol Exp Ther 303:1309–1316. doi:10.1124/jpet.102.043042

Ibarra FR, Armando I, Nowicki S, Carranza A, De Luca Sarobe V, Arrizurieta EE, Barontini M (2005) Dopamine is metabolised by different enzymes along the rat nephron. Pflugers Arch 450(3):185–191

Inoue K, Creveling CR (1991) Induction of catechol-O-methyltransferase in the luminal epithelium of rat uterus by progesterone. J Histochem Cytochem 39(6):823–828

Inoue K, Yoshizawa I, Creveling CR (1987) Immunocytochemical evidence for the coexistence of catecholestrogen and catechol-*O*-methyltransferase in the rat parotid gland. J Dent Res 66 (11):1627–1629

Inoue K, Nishino T, Creveling CR (1991) Immunocytochemical evidence for the site of *O*-methylation in rat dental pulp. J Dent Res 70(6):966–969

Ira E, Zanoni M, Ruggeri M, Dazzan P, Tosato S (2013) COMT, neuropsychological function and brain structure in schizophrenia: a systematic review and neurobiological interpretation. J Psychiatry Neurosci 38(6):366–380

Jasinska AJ, Stein EA, Kaiser J, Naumer MJ, Yalachkov Y (2014) Factors modulating neural reactivity to drug cues in addiction: a survey of human neuroimaging studies. Neurosci Biobehav Rev 38:1–16. doi:10.1016/j.neubiorev.2013.10.013

Jaspar M, Manard M, Dideberg V, Bours V, Maquet P, Collette F (2014) Influence of COMT genotype on antero-posterior cortical functional connectivity underlying interference resolution. Cereb Cortex 26:498–509. doi:10.1093/cercor/bhu188

Jaspar M, Dideberg V, Bours V, Maquet P, Collette F (2015) Modulating effect of COMT Val158Met polymorphism on interference resolution during a working memory task. Brain Cogn 95:7–18

Ji W, Li N, Ju K, Zheng H, Yang C, Xu P, Chen S, Cao A, Chen X, Guo L (2015) Association of Catechol-O-methyltransferase val/met polymorphism with cognitive function in Gilles de la Tourette syndrome patients. Neurol Sci 36(4):561–570. doi:10.1007/s10072-014-1992-1

Jiang H, Xie T, Ramsden DB, Ho SL (2003) Human catechol-O-methyltransferase down-regulation by estradiol. Neuropharmacology 45(7):1011–1018

Jiménez-Jiménez FJ, Alonso-Navarro H, García-Martín E, Agúndez JA (2014) COMT gene and risk for Parkinson's disease: a systematic review and meta-analysis. Pharmacogenet Genomics 24(7):331–339

Kambur O, Männistö PT (2010) Catechol-*O*-methyltransferase and pain. Int Rev Neurobiol 95:227–279

Kamide K, Kokubo Y, Yang J, Matayoshi T, Inamoto N, Takiuchi S, Horio T, Miwa Y, Yoshii M, Tomoike H, Tanaka C, Banno M, Okuda T, Kawano Y, Miyata T (2007) Association of genetic polymorphisms of ACADSB and COMT with human hypertension. J Hypertens 25 (1):103–110

Kanasaki K, Palmsten K, Sugimoto H, Ahmad S, Hamano Y, Xie L, Parry S, Augustin HG, Gattone VH, Folkman J, Strauss JF, Kalluri R (2008) Deficiency in catechol-O-methyltransferase and 2-methoxyoestradiol is associated with pre-eclampsia. Nature 453(7198):1117–1121

Karhunen T, Tilgmann C, Ulmanen I, Julkunen I, Panula P (1994) Distribution of catechol-*O*-methyltransferase enzyme in rat tissues. J Histochem Cytochem 42(8):1079–1090

Karhunen T, Tilgmann C, Ulmanen I, Panula P (1995a) Catechol-*O*-methyltransferase (COMT) in rat brain: immunoelectron microscopic study with an antiserum against rat recombinant COMT protein. Neurosci Lett 187(1):57–60

Karhunen T, Tilgmann C, Ulmanen I, Panula P (1995b) Neuronal and non-neuronal catechol-*O*-methyltransferase in primary cultures of rat brain cells. Int J Dev Neurosci 13(8):825–834

Karling P, Danielsson Å, Wikgren M, Söderström I, Del-Favero J, Adolfsson R, Norrback KF (2011) The relationship between the val158met catechol-O-methyltransferase (COMT) polymorphism and irritable bowel syndrome. PLoS One 6(3):e18035. doi:10.1371/journal.pone.0018035

Khan WA, Moinuddin AAS (2011) Immunochemical studies on catechol-estrogen modified plasmid: possible role in rheumatoid arthritis. J Clin Immunol 31(1):22–29

Khan WA, Uddin M, Khan MWA, Chabbra H (2009) Catecholestrogens: possible role in systemic lupus erythematosus. Rheumatology 48(11):1345–1351

Kilford EJ, Dumontheil I, Wood NW, Blakemore SJ (2015) Influence of COMT genotype and affective distractors on the processing of self-generated thought. Cognit Affect Neurosci 10 (6):777–782

Klebe S, Golmard JL, Nalls MA, Saad M, Singleton AB, Bras JM, Hardy J, Simon-Sanchez J, Heutink P, Kuhlenbaumer G, Charfi R, Klein C, Hagenah J, Gasser T, Wurster I, Lesage S, Lorenz D, Deuschl G, Durif F, Pollak P, Damier P, Tison F, Durr A, Amouyel P, Lambert JC, Tzourio C, Maubaret C, Charbonnier-Beaupel F, Tahiri K, Vidailhet M, Martinez M, Brice A, Corvol JC, French Parkinson's Disease Genetics Study G, International Parkinson's Disease Genomics C (2013) The Val158Met COMT polymorphism is a modifier of the age at onset in Parkinson's disease with a sexual dimorphism. J Neurol Neurosurg Psychiatry 84(6):666–673. doi:10.1136/jnnp-2012-304475

Kline RH, Exposto FG, O'Buckley SC, Westlund KN, Nackley AG (2015) Catechol-O-methyltransferase inhibition alters pain and anxiety-related volitional behaviors through activation of β-adrenergic receptors in the rat. Neuroscience 290:561–569

Ko MKC, Ikeda S, Mieno-Naka M, Arai T, Zaidi SAH, Sato N, Muramatsu M, Sawabe M (2012) Association of COMT gene polymorphisms with systemic atherosclerosis in elderly Japanese. J Atheroscler Thromb 19(6):552–558

Kuo KL, Lin WC, Ho IL, Chang HC, Lee PY, Chung YT, Hsieh JT, Pu YS, Shi CS, Huang KH (2013) 2-Methoxyestradiol induces mitotic arrest, apoptosis, and synergistic cytotoxicity with arsenic trioxide in human urothelial carcinoma cells. PLoS One 8(8):e68703

Kuroko Y, Fujii T, Yamazaki T, Akiyama T, Ishino K, Sano S, Mori H (2005) Contribution of catechol O-methyltransferase to the removal of accumulated interstitial catecholamines evoked by myocardial ischemia. Neurosci Lett 388(2):61–64

Kvetnansky R, Sabban EL, Palkovits M (2009) Catecholaminergic systems in stress: structural and molecular genetic approaches. Physiol Rev 89(2):535–606

Laatikainen LM, Sharp T, Harrison PJ, Tunbridge EM (2013) Sexually dimorphic effects of catechol-O-methyltransferase (COMT) inhibition on dopamine metabolism in multiple brain regions. PLoS One 8(4):e61839. doi:10.1371/journal.pone.0061839

Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM (1996) Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics 6(3):243–250

Lautala P, Ulmanen I, Taskinen J (2001) Molecular mechanisms controlling the rate and specificity of catechol *O*-methylation by human soluble catechol *O*-methyltransferase. Mol Pharmacol 59(2):393–402

Lee LO, Prescott CA (2014) Association of the catechol-O-methyltransferase val158met polymorphism and anxiety-related traits: a meta-analysis. Psychiatr Genet 24(2):52–69

Lee YH, Song GG (2014) COMT Val158Met and PPARγ Pro12Ala polymorphisms and susceptibility to Alzheimer's disease: a meta-analysis. Neurol Sci 35(5):643–651

Lee YH, Kim JH, Song GG (2015) Association between the COMT Val158Met polymorphism and fibromyalgia susceptibility and fibromyalgia impact questionnaire score: a meta-analysis. Rheumatol Int 35(1):159–166

Li K, Li W, Zou H (2014a) Catechol-*O*-methyltransferase Val158Met polymorphism and breast cancer risk in Asian population. Tumour Biol 35(3):2343–2350

Li W, Chen Y, Yin B, Zhang L (2014b) Pain in Parkinson's disease associated with COMT gene polymorphisms. Behav Neurol 2014:304203. doi:10.1155/2014/304203

Light AR, White AT, Hughen RW, Light KC (2009) Moderate exercise increases expression for sensory, adrenergic and immune genes in chronic fatigue syndrome patients, but not in normal subjects. J Pain 10(10):1099–1112. doi:10.1016/j.jpain.2009.06.003

Liu Z, Luo X, Liu L, Zhao W, Guo S, Guo Y, Wang N, He H, Liao X, Ma W, Zhou H, Zhang T (2013) Histone acetyltransferase p300 promotes MKL1-mediated transactivation of catechol-*O*-methyltransferase gene. Acta Biochim Biophys Sin Shanghai 45(12):1002–1010

Liu C, Kraja AT, Smith JA, Brody JA, Franceschini N, Bis JC, Rice K, Morrison AC, Lu Y, Weiss S, Guo X, Palmas W, Martin LW, Chen Y-DI, Surendran P, Drenos F, Cook JP, Auer PL, Chu AY, Giri A, Zhao W, Jakobsdottir J, Lin L-A, Stafford JM, Amin N, Mei H, Yao J, Voorman A, Consortium CHDE, Exome BPC, Go TDC, Consortium TDG, Larson MG, Grove ML, Smith AV, Hwang S-J, Chen H, Huan T, Kosova G, Stitziel NO, Kathiresan S, Samani N, Schunkert H, Deloukas P, Myocardial Infarction G, Consortia CAE, Li M, Fuchsberger C, Pattaro C, Gorski M, Consortium CK, Kooperberg C, Papanicolaou GJ, Rossouw JE, Faul JD, Kardia SLR, Bouchard C, Raffel LJ, Uitterlinden AG, Franco OH, Vasan RS, O'Donnell CJ, Taylor KD, Liu K, Bottinger EP, Gottesman O, Daw EW, Giulianini F, Ganesh S, Salfati E, Harris TB, Launer LJ, Dorr M, Felix SB, Rettig R, Volzke H, Kim E, Lee W-J, Lee IT, Sheu WHH, Tsosie KS, Edwards DRV, Liu Y, Correa A, Weir DR, Volker U, Ridker PM, Boerwinkle E, Gudnason V, Reiner AP, van Duijn CM, Borecki IB, Edwards TL, Chakravarti A, Rotter JI, Psaty BM, Loos RJF, Fornage M, Ehret GB, Newton-Cheh C, Levy D, Chasman DI (2016) Meta-analysis identifies common and rare variants influencing blood pressure and overlapping with metabolic trait loci. Nat Genet 48(10):1162–1170. doi:10.1038/ng.3660

Löbel M, Mooslechner AA, Bauer S, Günther S, Letsch A, Hanitsch LG, Grabowski P, Meisel C, Volk H-D, Scheibenbogen C (2015) Polymorphism in COMT is associated with IgG3 subclass level and susceptibility to infection in patients with chronic fatigue syndrome. J Transl Med 13 (1):264. doi:10.1186/s12967-015-0628-4

Loggia ML, Jensen K, Gollub RL, Wasan AD, Edwards RR, Kong J (2011) The catechol-O-methyltransferase (COMT) val158met polymorphism affects brain responses to repeated painful stimuli. PLoS One 6(11):e27764. doi:10.1371/journal.pone.0027764

Lott SA, Burghardt PR, Burghardt KJ, Bly MJ, Grove TB, Ellingrod VL (2013) The influence of metabolic syndrome, physical activity and genotype on catechol-*O*-methyl transferase promoter-region methylation in schizophrenia. Pharmacogenomics J 13(3):264–271

Loughead J, Wileyto EP, Valdez JN, Sanborn P, Tang K, Strasser AA, Ruparel K, Ray R, Gur RC, Lerman C (2009) Effect of abstinence challenge on brain function and cognition in smokers differs by COMT genotype. Mol Psychiatry 14(8):820–826. doi:10.1038/mp.2008.132

Lundström K, Tenhunen J, Tilgmann C, Karhunen T, Panula P, Ulmanen I (1995) Cloning, expression and structure of catechol-*O*-methyltransferase. Biochim Biophys Acta 1251 (1):1–10

Magina S, Moura E, Serrão MP, Moura D, Vieira-Coelho MA (2013) Catechol-*O*-methyltransferase activity is higher in psoriasis patients and is down–regulated by narrowband ultraviolet B treatment. Eur J Dermatol 23(1):49–52

Mailander PC, Meza JL, Higginbotham S, Chakravarti D (2006) Induction of A·T to G·C mutations by erroneous repair of depurinated DNA following estrogen treatment of the mammary gland of ACI rats. J Steroid Biochem Mol Biol 101(4–5):204–215

Männistö PT, Kaakkola S (1999) Catechol-*O*-methyltransferase (COMT): biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. Pharmacol Rev 51(4):593–628

Martínez-Jauand M, Sitges C, Rodríguez V, Picornell A, Ramon M, Buskila D, Montoya P (2013) Pain sensitivity in fibromyalgia is associated with catechol-*O*-methyltransferase (COMT) gene. Eur J Pain 17(1):16–27

Masuda M, Tsunoda M, Imai K (2006) Low catechol-O-methyltransferase activity in the brain and blood pressure regulation. Biol Pharm Bull 29(2):202–205

Matsumoto M, Weickert CS, Akil M, Lipska B, Hyde T, Herman M, Kleinman J, Weinberger D (2003) Catechol *O*-methyltransferase mRNA expression in human and rat brain: evidence for a role in cortical neuronal function. Neuroscience 116(1):127–137

McCane AM, Czachowski CL, Lapish CC (2014) Tolcapone suppresses ethanol intake in alcohol-preferring rats performing a novel cued access protocol. Alcohol Clin Exp Res 38 (9):2468–2478. doi:10.1111/acer.12515

McLeod HL, Fang L, Luo X, Scott EP, Evans WE (1994) Ethnic differences in erythrocyte catechol-O-methyltransferase activity in black and white Americans. J Pharmacol Exp Ther 270(1):26

Meister B, Bean AJ, Aperia A (1993) Catechol-*O*-methyltransferase mRNA in the kidney and its appearance during ontogeny. Kidney Int 44:726–733

Mier D, Kirsch P, Meyer-Lindenberg A (2010) Neural substrates of pleiotropic action of genetic variation in COMT: a meta-analysis. Mol Psychiatry 15(9):918–927

Mione V, Canterini S, Brunamonti E, Pani P, Donno F, Fiorenza MT, Ferraina S (2015) Both the COMT Val158Met single-nucleotide polymorphism and sex-dependent differences influence response inhibition. Front Behav Neurosci 9:127. doi:10.3389/fnbeh.2015.00127

Miyaki K, Htun NC, Song Y, Ikeda S, Muramatsu M, Shimbo T (2012) The combined impact of 12 common variants on hypertension in Japanese men, considering GWAS results. J Hum Hypertens 26(7):430–436

Muellner J, Gharrad I, Habert MO, Kas A, Martini JB, Cormier-Dequaire F, Tahiri K, Vidailhet M, Meier N, Brice A, Schuepbach M, Mallet A, Hartmann A, Corvol JC (2015) Dopaminergic denervation severity depends on COMT Val158Met polymorphism in Parkinson's disease. Parkinsonism Relat Disord 21(5):471–476. doi:10.1016/j.parkreldis.2015.02.009

Myöhänen TT, Schendzielorz N, Männistö PT (2010) Distribution of catechol-*O*-methyltransferase (COMT) proteins and enzymatic activities in wild-type and soluble COMT deficient mice. J Neurochem 113(6):1632–1643

Nackley AG, Shabalina SA, Tchivileva IE, Satterfield SK, Korchynskyi O, Makarov S, Maixner W, Diatchenko LB (2006) Human catechol-*O*-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure. Science 314(5807):1930–1933

Nackley AG, Tan KS, Fecho K, Flood P, Diatchenko L, Maixner W (2007) Catechol-O-methyltransferase inhibition increases pain sensitivity through activation of both beta2- and beta3-adrenergic receptors. Pain 128(3):199–208

Nagai M, Conney AH, Zhu BT (2004) Strong inhibitory effects of common tea catechins and bioflavonoids on the *O*-methylation of catechol estrogens catalyzed by human liver cytosolic catechol-*O*-methyltransferase. Drug Metab Dispos 32(5):497–504

Nakazato T, Akiyama A (2002) Behavioral activity and stereotypy in rats induced by L-DOPA metabolites: a possible role in the adverse effects of chronic L-DOPA treatment of Parkinson's disease. Brain Res 930(1–2):134–142

Nedic G, Nikolac M, Borovecki F, Hajnsek S, Muck-Seler D, Pivac N (2010) Association study of a functional catechol-O-methyltransferase polymorphism and smoking in healthy Caucasian subjects. Neurosci Lett 473(3):216–219. doi:10.1016/j.neulet.2010.02.050

Nedic G, Nikolac M, Sviglin KN, Muck-Seler D, Borovecki F, Pivac N (2011) Association study of a functional catechol-O-methyltransferase (COMT) Val108/158Met polymorphism and suicide attempts in patients with alcohol dependence. Int J Neuropsychopharmacol 14 (3):377–388. doi:10.1017/S1461145710001057

Nissinen E, Männistö PT (2010) Biochemistry and pharmacology of catechol-*O*-methyltransferase inhibitors. Int Rev Neurobiol 95:73–118

Nohesara S, Ghadirivasfi M, Mostafavi S, Eskandari MR, Ahmadkhaniha H, Thiagalingam S, Abdolmaleky HM (2011) DNA hypomethylation of MB-COMT promoter in the DNA derived from saliva in schizophrenia and bipolar disorder. J Psychiatr Res 45(11):1432–1438

Nomura T, Inoue K, Creveling CR, Komatsu F, Ohta N, Chino T, Karasawa N, Nagatsu I (1996) Immunocytochemical localization of aromatic L-amino acid decarboxylase and catechol-*O*-methyltransferase in blood vessel wall of the human dental pulp. Brain Res 735(2):314–316

Odlind C, Reenilä I, Männistö PT, Ekblom J, Hansell P (2001) The role of dopamine-metabolizing enzymes in the regulation of renal sodium excretion in the rat. Pflugers Arch 442(4):505–510

Odlind C, Reenilä I, Männistö PT, Juvonen R, Uhlén S, Gogos JA, Karayiorgou M, Hansell P (2002) Reduced natriuretic response to acute sodium loading in COMT gene deleted mice. BMC Physiol 2:14. doi:10.1186/1472-6793-2-14

Ooshima K, Ozaki S, Tabuchi M, Higashino H, Honda E, Park A, Arima S, Munakata H (2009) Decreased expression of catechol-*O*-methyltransferase in the renal cortex of malignant spontaneously hypertensive rats. Tohoku J Exp Med 219(4):331–336

Opmeer EM, Kortekaas R, van Tol MJ, van der Wee NJ, Woudstra S, van Buchem MA, Penninx BW, Veltman DJ, Aleman A (2013) Influence of COMT val158met genotype on the depressed brain during emotional processing and working memory. PLoS One 8(9):e73290

Oster M, Muráni E, Ponsuksili S, D'Eath RB, Turner SP, Evans G, Thölking L, Kurt E, Klont R, Foury A, Mormède P, Wimmers K (2014) Hepatic expression patterns in psychosocially high-stressed pigs suggest mechanisms following allostatic principles. Physiol Behav 10 (128):159–165

Øverbye A, Seglen PO (2009) Phosphorylated and non-phosphorylated forms of catechol *O*-methyltransferases in rat liver, brain and other tissues. Biochem J 417(2):535–545

Pap D, Gonda X, Molnar E, Lazary J, Benko A, Downey D, Thomas E, Chase D, Toth ZG, Mekli Z, Platt H, Payton A, Elliot R, Anderson IM, Deakin JF, Bagdy G, Juhasz G (2012) Genetic variants in the catechol-*O*-methyltransferase gene are associated with impulsivity and executive function: relevance for major depression. Am J Med Genet B 159B(8):928–940

Papaleo F, Crawley JN, Song J, Lipska BK, Pickel J, Weinberger DR, Chen J (2008) Genetic dissection of the role of catechol-O-methyltransferase in cognition and stress reactivity in mice. J Neurosci 28(35):8709–8723

Peerbooms O, Rutten BP, Collip D, Lardinois M, Lataster T, Thewissen V, Rad SM, Drukker M, Kenis G, van Os J, Myin-Germeys I, van Winkel R (2012) Evidence that interactive effects of COMT and MTHFR moderate psychotic response to environmental stress. Acta Psychiatr Scand 125(3):247–256. doi:10.1111/j.1600-0447.2011.01806.x

Pérez-Sepúlveda A, Torres MJ, Valenzuela FJ, Larraín R, Figueroa-Diesel H, Galaz J, Nien JK, Serra R, Michea L, Illanes SE (2012) Low 2-methoxyestradiol levels at the first trimester of pregnancy are associated with the development of pre-eclampsia. Prenat Diagn 32(11):1053–1058

Pihlavisto P, Reenilä I (2002) Separation methods for catechol *O*-methyltransferase activity assay; physiological and pathophysiological relevance. J Chromatogr B 781(1–2):359–372

Prasad P, Kumar KM, Ammini AC, Gupta A, Gupta R, Thelma BK (2008) Association of dopaminergic pathway gene polymorphisms with chronic renal insufficiency among Asian Indians with type-2 diabetes. BMC Genet 9:26. doi:10.1186/1471-2156-9-26

Quezada M, Alvarez M, Peña OA, Henríquez S, d'Alençon CA, Lange S, Oliva B, Owen GI, Allende MI (2013) Antiangiogenic, antimigratory and antiinflammatory effects of 2-methoxyestradiol in zebrafish larvae. Comp Biochem Physiol 157(2):141–149

Rakvåg TT, Ross JR, Sato H, Skorpen F, Kaasa S, Klepstad P (2008) Genetic variation in the catechol-O-methyltransferase (COMT) gene and morphine requirements in cancer patients with pain. Mol Pain 4:64. doi:10.1186/1744-8069-4-64

Redell JB, Dash PK (2007) Traumatic brain injury stimulates hippocampal catechol-*O*-methyltransferase expression in microglia. Neurosci Lett 413(1):36–41

Reenilä I, Männistö PT (2001) Catecholamine metabolism in the brain by membrane-bound and soluble catechol-*O*-methyltransferase (COMT) estimated by enzyme kinectic values. Med Hypotheses 57(5):628–632

Reenilä I, Tuomainen P, Soinila S, Männistö PT (1997) Increase of catechol-*O*-methyltransferase activity in rat brain microglia after intrastriatal infusion of luorocitrate, a glial toxin. Neurosci Lett 230(3):155–158

Reyes-Gibby CC, Shete S, Rakvåg T, Bhat SV, Skorpen F, Bruera E, Kaasa S, Klepstad P (2007) Exploring joint effects of genes and the clinical efficacy of morphine for cancer pain: OPRM1 and COMT gene. Pain 130(1–2):25–30

Rivest J, Barclay CL, Suchowersky O (1999) COMT inhibitors in Parkinson's disease. Can J Neurol Sci 26:S34–S38

Roten LT, Fenstad MH, Forsmo S, Johnson MP, Moses EK, Austgulen R, Skorpen F (2011) A low COMT activity haplotype is associated with recurrent preeclampsia in a Norwegian population cohort (HUNT2). Mol Hum Reprod 17(7):439–446

Rutherford KJ, Dagget V (2009) A hotspot of inactivation: the A22S and V108M polymorphisms individually destabilize the active site structure of catechol *O*-methyltransferase. Biochemistry 48(27):6450–6460

Rutherford KJ, Alphandéry E, McMillan A, Dagget V, Parson WW (2008) The V108M mutation decreases the structural stability of catechol-*O*-methyltransferase. Biochim Biophys Acta 1784 (7-8):1098–1105

Sadhasivam S, Chidambaran V, Olbrecht VA, Esslinger HR, Zhang K, Zhang X, Martin LJ (2014) Genetics of pain perception, COMT and postoperative pain management in children. Pharmacogenomics 15(3):277–284. doi:10.2217/pgs.13.248

Salama S, Ho S-L, Wang H-Q, Tenhunen J, Tilgmann C, Al-Hendy A (2006a) Hormonal regulation of catechol-*O*-methyl transferase activity in women with uterine leiomyomas. Fertil Steril 86(1):259–262

Salama SA, Nasr AB, Dubey RK, Al-Hendy A (2006b) Estrogen metabolite 2-methoxyestradiol induces apoptosis and inhibits cell proliferation and collagen production in rat and human leiomyoma cells: a potential medicinal treatment for uterine fibroids. J Soc Gynecol Investig 13(8):542–550

Salama SA, Jamaluddin M, Kumar R, Hassan MH, Al-Hendy A (2007) Progesterone regulates catechol-*O*-methyl transferase gene expression in breast cancer cells: distinct effect of progesterone receptor isoforms. J Steroid Biochem Mol Biol 107(3–5):253–261

Salama SA, Kamel MW, Botting S, Salih SM, Borahay MA, Hamed AA, Kilic GS, Saeed M, Williams MY, Diaz-Arrastia CR (2009) Catechol-O-methyltransferase expression and 2-methoxyestradiol affect microtubule dynamics and modify steroid receptor signaling in leiomyoma cells. PLoS One 4(10):e7356

Salih SM, Jamaluddin M, Salama S, Fadl AA, Nagamani M, Al-Hendy A (2008a) Regulation of catechol *O*-methyltransferase expression in granulosa cells: a potential role for follicular arrest in polycistic ovary syndrome. Fertil Steril 89(3):1414–1421

Salih SM, Salama SA, Fadl AA, Nagamani M, Al-Hendy A (2008b) Expression and cyclic variations of catechol-O-methyl transferase in human endometrial stroma. Fertil Steril 90 (3):789–797

Sannino S, Gozzi A, Cerasa A, Piras F, Scheggia D, Managò F, Damiano M, Galbusera A, Erickson LC, De Pietri Tonelli D, Bifone A, Tsaftaris SA, Caltagirone C, Weinberger DR, Spalletta G, Papaleo F (2015) COMT genetic reduction produces sexually divergent effects on cortical anatomy and working memory in mice and humans. Cereb Cortex 25(9):2529–2541

Sasaki M, Kaneuchi M, Sakuragi N, Dahiya R (2003) Multiple promoters of catechol-*O*-methyltransferase gene are selectively inactivated by CpG hypermethylation in endometrial cancer. Cancer Res 63(12):3101–3106

Schendzielorz N, Rysa A, Reenilä I, Raasmaja A, Mannisto PT (2011) Complex estrogenic regulation of catechol-*O*-methyltransferase (COMT) in rats. J Physiol Pharmacol 62(4):483–490

Shand FHW, Langenbach SY, Keenan CR, Ma SP, Wheaton BJ, Schuliga MJ, Ziogas J, Stewart AG (2011) In vitro and in vivo evidence for anti-inflammatory properties of 2-methoxyestradiol. J Pharmacol Exp Ther 336(3):962–972

Shen X, Wu Y, Guan T, Wang X, Qian M, Lin M, Shen Z, Sun J, Zhong H, Yang J, Li L, Yuan Y (2014a) Association analysis of COMT/MTHFR polymorphisms and major depressive disorder in Chinese Han population. J Affect Disord 161:73–78. doi:10.1016/j.jad.2014.03.008

Shen Z, Wu Y, Chen X, Chang X, Zhou Q, Zhou J, Ying H, Zheng J, Duan T, Wang K (2014b) Decreased maternal serum 2-methoxyestradiol levels are associated with the development of preeclampsia. Cell Physiol Biochem 34(6):2189–2199

Shield AJ, Thomae BA, Eckloff BW, Wieben ED, Weinshilboum RM (2004) Human catechol O-methyltransferase genetic variation: gene resequencing and functional characterization of variant allozymes. Mol Psychiatry 9(2):151–160

Siebert AE, Sanchez AL, Dinda S, Moudgil VK (2011) Effects of estrogen metabolite 2-methoxyestradiol on tumor suppressor protein p53 and proliferation of breast cancer cells. Syst Biol Reprod Med 57(6):279–287

Singh JP, Volavka J, Czobor P, Van Dorn RA (2012) A meta-analysis of the Val158Met COMT polymorphism and violent behavior in schizophrenia. PLoS One 7(8):e43423

Smit NPM, Pavel S (1995) Induction of cytotoxicity in melanoma cells through inhibition of catechol-*O*-methyltransferase. Biochem Pharmacol 50(12):1955–1962

Smith CT, Swift-Scanlan T, Boettiger CA (2014a) Genetic polymorphisms regulating dopamine signaling in the frontal cortex interact to affect target detection under high working memory load. J Cogn Neurosci 26(2):395–407

Smith SB, Reenilä I, Männistö PT, Slade GD, Maixner W, Diatchenko L, Nackley AG (2014b) Epistasis between polymorphisms in COMT, ESR1, and GCH1 influences COMT enzyme activity and pain. Pain 155(11):2390–2399

Soeiro-de-Souza MG, Bio DS, David DP, Rodrigues dos Santos DJ, Kerr DS, Gattaz WF, Machado-Vieira R, Moreno RA (2012) COMT Met (158) modulates facial emotion recognition in bipolar I disorder mood episodes. J Affect Disord 136(3):370–376

Soeiro-De-Souza MG, Stanford MS, Bio DS, Machado-Vieira R, Moreno RA (2013) Association of the COMT Met158 allele with trait impulsivity in healthy young adults. Mol Med Rep 7 (4):1067–1072

Sofuoglu M, DeVito EE, Waters AJ, Carroll KM (2013) Cognitive enhancement as a treatment for drug addictions. Neuropharmacology 64:452–463. doi:10.1016/j.neuropharm.2012.06.021

Souteiro P, Vieira-Coelho MA, Serrão MP, Magina S (2013) Catechol-O-methyltransferase activity in psoriasis patients treated with psoralen plus ultraviolet A therapy. Photodermatol Photoimmunol Photomed 29(5):227–232

Sozio P, Cerasa LS, Abbadessa A, Di Stefano A (2012) Designing prodrugs for the treatment of Parkinson's disease. Expert Opin Drug Discovery 7(5):385–406. doi:10.1517/17460441.2012.677025

Sparta M, Alexandrova AN (2012) How metal substitution affects the enzymatic activity of catechol-*O*-methyltransferase. PLoS One 7(10):e47172. doi:10.1371/journal.pone.0047172

Stabile LP, Davis AL, Gubish CT, Hopkins TM, Luketich JD, Christie N, Finkelstein S, Siegfried JM (2002) Human non-small cell lung tumors and cells derived from normal lung express both estrogen receptor alpha and beta and show biological responses to estrogen. Cancer Res 62(7):2141–2150

Stubelius A, Andréasson E, Karlsson A, Ohlsson C, Tivesten A, Islander U, Carlsten H (2011) Role of 2-methoxyestradiol as inhibitor of arthritis and osteoporosis in a model of postmenopausal rheumatoid arthritis. Clin Immunol 140(1):37–46

Sun WP, Li D, Lun YZ, Gong XJ, Sun SX, Guo M, Jing LX, Zhang LB, Xiao FC, Zhou SS (2012) Excess nicotinamide inhibits methylation-mediated degradation of catecholamines in normotensives and hypertensives. Hypertens Res 35(2):180–185

Sundermann EE, Bishop JR, Rubin LH, Little DM, Meyer VJ, Martin E, Weber K, Cohen M, Maki PM (2015) Genetic predictor of working memory and prefrontal function in women with HIV. J Neurovirol 21(1):81–91. doi:10.1007/s13365-014-0305-z

Suriyaprom K, Tungtrongchitr R, Harnroongroj T (2013) Impact of COMT Val 108/158 Met and DRD2 Taq1B gene polymorphisms on vulnerability to cigarette smoking of Thai males. J Mol Neurosci 49(3):544–549. doi:10.1007/s12031-012-9844-z

Tammimäki A, Männistö PT (2012) Catechol-O-methyltransferase gene polymorphism and chronic human pain: a systematic review and meta-analysis. Pharmacogenet Genomics 22(9):673–691. doi:10.1097/FPC.0b013e3283560c46

Tammimaki A, Kaenmaki M, Kambur O, Kulesskaya N, Keisala T, Karvonen E, Garcia-Horsman JA, Rauvala H, Mannisto PT (2010) Effect of S-COMT deficiency on behavior and extracellular brain dopamine concentrations in mice. Psychopharmacology 211(4):389–401. doi:10.1007/s00213-010-1944-2

Tan X, Chen M (2014) Association between catechol-O-methyltransferase rs4680 (G>A) polymorphism and lung cancer risk. Diagn Pathol 9:192. doi:10.1186/s13000-014-0192-x

Tan HY, Chen Q, Sust S, Buckholtz JW, Meyers JD, Egan MF, Mattay VS, Meyer-Lindenberg A, Weinberger DR, Callicott JH (2007) Epistasis between catechol-O-methyltransferase and type II metabotropic glutamate receptor 3 genes on working memory brain function. Proc Natl Acad Sci U S A 104(30):12536–12541. doi:10.1073/pnas.0610125104

Tanaka Y, Sasaki M, Shiina H, Tokizane T, Deguchi M, Hirata H, Hinoda Y, Okayama N, Suehiro Y, Urakami S, Kawakami T, Kaneuchi M, Pookot D, Igawa M, Okuyama A, Ishii N, Dahiya R (2006) Catechol-*O*-methyltransferase gene polymorphisms in benign prostatic hyperplasia and sporadic prostate cancer. Cancer Epidemiol Biomark Prev 15(2):238–244

Tchivileva IE, Nackley AG, Qian L, Wentworth S, Conrad M, Diatchenko LB (2009) Characterization of NF-kB-mediated inhibition of catechol-*O*-methyltransferase. Mol Pain 5(13):13. doi:10.1186/1744-8069-5-13

Teng Y, He C, Zuo X, Li X (2013) Catechol-*O*-methyltransferase and cytochrome P-450 1B1 polymorphisms and endometrial cancer risk: a meta-analysis. Int J Gynecol Cancer 23(3):422–430

Tenhunen J, Ulmanen I (1993) Production of rat soluble and membrane-bound catechol *O*-methyltransferase isoforms from bifunctional mRNAs. Biochem J 296(3):595–600

Tenhunen J, Salminen M, Lundstrom K, Kiviluoto T, Savolainen R, Ulmanen I (1994) Genomic organization of the human catechol *O*-methyltransferase gene and its expression from two distinct promoters. Eur J Biochem 223(3):1049–1059

Tenorio-Laranga J, Männistö PT, Karayiorgou M, Gogos J, García-Horsman J (2009) Sex-dependent compensated oxidative stress in the mouse liver upon deletion of catechol *O*-methyltransferase. Biochem Pharmacol 77(9):1541–1552

Tian T, Qin W, Liu B, Wang D, Wang J, Jiang T, Yu C (2013) Catechol-*O*-methyltransferase Val158Met polymorphism modulates gray matter volume and functional connectivity of the default mode network. PLoS One 8(10):e78697

Tong X, Li Z, Wu Y, Fu X, Zhang Y, Fan H (2014) COMT 158G/A and CYP1B1 432C/G polymorphisms increase the risk of endometriosis and adenomyosis: a meta-analysis. Eur J Obstet Gynecol Reprod Biol 179:17–21

Tsao D, Shabalina SA, Gauthier J, Dokholyan NV, Diatchenko L (2011) Disruptive mRNA folding increases translational efficiency of catechol-O-methyltransferase variant. Nucleic Acids Res 39(14):6201–6212

Tsuji E, Okazaji K, Isaji M, Takeda K (2009) Crystal structures of the apo and holo form of rat catechol-*O*-methyltransferase. J Struct Biol 165(3):133–139

Tsunoda M, Takezawa K, Masuda M, Imai K (2002) Rat liver and kidney catechol-*O*-methyltransferase activity measured by high-performance liquid chromatography with fluorescence detection. Biomed Chromatogr 16(8):536–541

Tsunoda M, Tenhunen J, Tilgmann C, Arai H, Imai K (2003) Reduced membrane-bound catechol-*O*-methyltransferase in the liver of spontaneosly hypertensive rats. Hypertens Res 26 (11):923–927

Tunbridge EM, Weinberger DR, Harrison PJ (2006) A novel protein isoform of catechol O-methyltransferase (COMT): brain expression analysis in schizophrenia and bipolar disorder and effect of Val158Met genotype. Mol Psychiatry 11(2):116–117

Tunbridge EM, Lane TA, Harrison PJ (2007) Expression of multiple catechol-o-methyltransferase (COMT) mRNA variants in human brain. Am J Med Genet B Neuropsychiatr Genet 144B (6):834–839

Tunbridge EM, Farrell SM, Harrison PJ, Mackay CE (2013) Catechol-*O*-methyltransferase (COMT) influences the connectivity of the prefrontal cortex at rest. NeuroImage 68(C):49–54

Ulmanen I, Peränen J, Tenhunen J, Tilgmann C, Karhunen T, Panula P, Bernasconi L, Aubry J-P, Lundstrom K (1997) Expression and intracellular localization of catechol *O*-methyltransferase in transfected mammalian cells. Eur J Biochem 243(1–2):452–459

Ursini G, Bollati V, Fazio L, Porcelli A, Iacovelli L, Catalani A, Sinibaldi L, Gelao B, Romano R, Rampino A, Taurisano P, Mancini M, Di Giorgio A, Popolizio T, Baccarelli A, De Blasi A, Blasi G, Bertolino A (2011) Stress-related methylation of the catechol-*O*-methyltransferase Val 158 allele predicts human prefrontal cognition and activity. J Neurosci 31(18):6692–6698

Valomon A, Holst SC, Bachmann V, Viola AU, Schmidt C, Zürcher J, Berger W, Cajochen C, Landolt HP (2014) Genetic polymorphisms of DAT1 and COMT differentially associate with actigraphy-derived sleep-wake cycles in young adults. Chronobiol Int 31(5):705–714

van Winkel R, Stefanis NC, Myin-Germeys I (2008) Psychosocial stress and psychosis. A review of the neurobiological mechanisms and the evidence for gene-stress interaction. Schizophr Bull 34(6):1095–1105. doi:10.1093/schbul/sbn101

Vazquez-Alaniz F, Lumbreras-Márquez MI, Sandoval-Carrillo AA, Aguilar-Durá M, Méndez-Hernández EM, Barraza-Salas M, Castellanos-Juárez FX, Salas-Pacheco JM (2014) Association of COMT G675A and MTHFR C677T polymorphisms with hypertensive disorders of pregnancy in Mexican mestizo population. Pregnancy Hypertens 4(1):59–64

Volkow ND, Wang GJ, Fowler JS, Tomasi D, Telang F (2011) Addiction: beyond dopamine reward circuitry. Proc Natl Acad Sci U S A 108(37):15037–15042. doi:10.1073/pnas.1010654108

Voutilainen S, Tuomainen TP, Korhonen M, Mursu J, Virtanen JK, Happonen P, Alfthan G, Erlund I, North KE, Mosher MJ, Kauhanen J, Tiihonen J, Kaplan GA, Salonen JT (2007)

Functional COMT Val158Met polymorphism, risk of acute coronary events and serum homocysteine: the Kuopio ischaemic heart disease risk factor study. PLoS One 2(1):e181
Walton E, Liu J, Hass J, White T, Scholz M, Roessner V, Gollub R, Calhoun VD, Ehrlich S (2014) MB-COMT promoter DNA methylation is associated with working-memory processing in schizophrenia patients and healthy controls. Epigenetics 9:8
Wang L, Wu Q, Qiu P, Mirza A, McGuirk M, Kirschmeier P, Greene JR, Wang Y, Pickett CB, Liu S (2001) Analyses of p53 target genes in the human genome by bioinformatic and microarray approaches. J Biol Chem 276(47):43604–43610
Wang Y, Fang Y, Shen Y, Xu Q (2010) Analysis of association between the catechol-O-methyltransferase (COMT) gene and negative symptoms in chronic schizophrenia. Psychiatry Res 179(2):147–150. doi:10.1016/j.psychres.2009.03.029
Wang X, Wang Z, Wu Y, Yuan Y, Hou Z, Hou G (2014a) Association analysis of the catechol-*O*-methyltransferase/methylenetetrahydrofolate reductase genes and cognition in late-onset depression. Psychiatry Clin Neurosci 68(5):344–352
Wang Y, Wu Z, Qiao H, Zhang Y (2014b) A genetic association study of single nucleotide polymorphisms in GNbeta3 and COMT in elderly patients with irritable bowel syndrome. Med Sci Monit 20:1246–1254. doi:10.12659/MSM.890315
Wardle MC, de Wit H, Penton-Voak I, Lewis G, Munafo MR (2013) Lack of association between COMT and working memory in a population-based cohort of healthy young adults. Neuropsychopharmacology 38(7):1253–1263. doi:10.1038/npp.2013.24
Weisz J, Fritz-Wolz G, Clawson GA, Benedict CM, Abendroth C, Creveling CR (1998) Induction of nuclear catechol-O-methyltransferase by estrogens in hamster kidney: implications for estrogen-induced cancer. Carcinogenesis 19(7):1307–1312
Weisz J, Fritz-Wolz G, Gestl S, Clawson GA, Creveling CR, Liehr JG, Dabbs D (2000) Nuclear localization of catechol-*O*-methyltransferase in neoplastic and nonneoplastic mammary epithelial cells. Am J Pathol 156(6):1841–1848
White TP, Loth E, Rubia K, Krabbendam L, Whelan R, Banaschewski T, Barker GJ, Bokde AL, Büchel C, Conrod P, Fauth-Bühler M, Flor H, Frouin V, Gallinat J, Garavan H, Gowland P, Heinz A, Ittermann B, Lawrence C, Mann K, Paillère ML, Nees F, Paus T, Pausova Z, Rietschel M, Robbins T, Smolka MN, Shergil LSS, Schumann G (2014) Sex differences in COMT polymorphism effects on prefrontal inhibitory control in adolescence. Neuropsychopharmacology 39(11):2560–2569. doi:10.1038/npp.2014.107
Worda C, Sator MO, Schneeberger C, Jantschev T, Ferlitsch K, Huber JC (2003) Influence of the catechol-O-methyltransferase (COMT) codon 158 polymorphism on estrogen levels in women. Hum Reprod 18(2):262–266
Wu K, O'Keeffe D, Politis M, O'Keeffe G, Robbins T, Bose S, Brooks D, Piccini P, Barker R (2012) The catechol-*O*-methyltransferase Val(158)Met polymorphism modulates fronto-cortical dopamine turnover in early Parkinson's disease: a PET study. Brain 135(Pt 8):2449–2457
Wu W, Wu Q, Hong X, Xiong G, Xiao Y, Zhou J, Wang W, Wu H, Zhou L, Song W, Dai H, Qiu H, Zhao Y (2015a) Catechol-O-methyltransferase inhibits colorectal cancer cell proliferation and invasion. Arch Med Res 46(1):17–23
Wu W, Wu Q, Hong X, Zhou L, Zhang J, You L, Wang W, Wu H, Dai H, Zhao Y (2015b) Catechol-O-methyltransferase, a new target for pancreatic cancer therapy. Cancer Sci 106 (5):576–583
Xia Y, Ma D, Hu J, Tang C, Wu Z, Liu L, Xin F (2012) Effect of metabotropic glutamate receptor 3 genotype on N-acetylaspartate levels and neurocognition in non-smoking, active alcoholics. Behav Brain Funct 8:42. doi:10.1186/1744-9081-8-42
Xiao L, Tong M, Jin Y, Huang W, Li Z (2013) The l58Val/Met polymorphism of catechol-*O*-methyl transferase gene and prostate cancer risk: a meta-analysis. Mol Biol Rep 40 (2):1835–1841
Xie T, Ho S-L, Ramsden D (1999) Characterization and implications of estrogenic down-regulation of human catechol-*O*-methyltransferase gene transcription. Mol Pharmacol 56 (1):31–38

Xing Y, Jia JP, Ji XJ, Tian T (2013) Estrogen associated gene polymorphisms and their interactions in the progress of Alzheimer's disease. Prog Neurobiol 111:53–74. doi:10.1016/j.pneurobio.2013.09.006

Yager JD (2012) Catechol-*O*-methyltransferase: characteristics, polymorphisms and role in breast cancer. Drug Discov Today Dis Mech 9(1–2):41–46

Yager JD, Davidson NE (2006) Estrogen carcinogenesis in breast cancer. N Engl J Med 354 (3):270–282

Yamazaki S, Sakakibara H, Takemura H, Shimoi K (2012) 4-Hydroxyestradiol induces γ-H2AX in the presence of an inhibitor of catechol-*O*-methyltransferase in human breast cancer MCF-7 cells. Genes Environ 34(3):129–135

Yang H, Ahn C, Jeung EB (2015) Differential expression of calcium transport genes caused by COMT inhibition in the duodenum, kidney and placenta of pregnant mice. Mol Cell Endocrinol 401:45–55. doi:10.1016/j.mce.2014.11.020

Zahid M, Kohli E, Saeed M, Rogan EG, Cavalieiri EL (2006) The greater reactivity of estradiol-3,4-quinone vs estradiol-2,3-quinone with DNA in the formation of depurinating adducts: implications for tumor-initiating activity. Chem Res Toxicol 19(1):164–172

Zahid M, Saeed M, Lu F, Gaikwad N, Rogan EG, Cavalieiri EL (2007) Inhibition of catechol-*O*-methyltransferase increases estrogen-DNA adduct formation. Free Radic Biol Med 43 (11):1534–1540

Zhang Y, Gaikwad N, Olson K, Zahid M, Cavalieiri EL, Rogan EG (2007) Cytochrome P450 isoforms catalyze formation of catechol estrogen quinones that react with DNA. Metab Clin Exp 56(7):887–894

Zhang MZ, Yao B, Fang X, Wang S, Smith JP, Harris RC (2009) Intrarenal dopaminergic system regulates renin expression. Hypertension 53(3):564–570

Zhang L, Jin Y, Chen M, Huang M, Harvey RG, Blair IA, Penning TM (2011) Detoxication of structurally diverse polycyclic aromatic hydrocarbon (PAH) *o*-quinones by human recombinant catechol-*O*-methyltransferase (COMT) via *O*-methylation of PAH cathecols. J Biol Chem 286(29):25644–25654

Zhang S, Zhang M, Zhang J (2014) Association of COMT and COMT-DRD2 interaction with creative potential. Front Hum Neurosci 8:216

Zhang X, Li J, Qin W, Yu C, Liu B, Jiang T (2015) The catechol-o-methyltransferase Val(158)Met polymorphism modulates the intrinsic functional network centrality of the parahippocampal cortex in healthy subjects. Sci Rep 5:10105. doi:10.1038/srep10105

Zhao A, Cheng Y, Li X, Li Q, Wang L, Xu J, Xiang Y, Xing Q, He L, Zhao X (2011) Promoter hypomethylation of COMT in human placenta is not associated with the development of pre-eclampsia. Mol Hum Reprod 17(3):199–206

Zhu BT, Ezell E, Liehr JG (1994) Catechol-*O*-methyltransferase catalyzed rapid *O*-methylation of mutagenic flavonoids. J Biol Chem 269(1):292–299

Zhu BT, Patel UK, Cai XC, Conney AH (2000) *O*-methylation of tea polyphenols catalyzed by human placental cytosolic catechol-*O*-methyltransferase. Drug Metab Dispos 28(9):1024–1030

Zhu BT, Patel UK, Cai XM, Lee AJ, Conney AH (2001) Rapid conversion of tea catechins to monomethylated products by rat liver cytosolic catechol-*O*-methyltransferase. Xenobiotica 31 (21):879–890

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Origin, Function, and Fate of Metallothionein in Human Blood

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Abstract Toxic heavy metals, toxic organic compounds, reactive oxygen species (ROS), infections, and temperature are well-known metallothionein (MT) inducers in human blood. The current review aims to summarize synthesis, function, and fate of human blood MT in response to the known MT inducers. Part of the MTs that are synthesized in different organs such as the liver, kidney, and spleen is transported and stored in different blood cells and in plasma. Cells of the circulatory system also synthesize MT. From the circulation, MT returns to the kidney where the metal-bound MTs are degraded to release the metal ion that in turn induces MT expression therein. The blood MTs play important roles in metal detoxification, transportation, and storage. By neutralizing ROS, MTs protect blood cells from oxidative stress-induced cytotoxicity and genotoxicity. Arguably, MTs are also involved in immune suppression. Given the permeating distribution of blood MT throughout the body as well as its diverse role in the protection against harmful environmental factors and in metal homeostasis, MT could be better recognized as a major public health protein.

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Contents

1 Introduction

Metallothioneins (MTs) are a group of cysteine-rich, low molecular weight metal-binding proteins (Hamer 1986; Kagi 1991). During the investigation of the role of cadmium (Cd), this protein was first identified in the equine renal cortex in 1957 (Margoshes and Vallee 1957). Later, MT was detected in microorganisms, plants, and animals (Bagheri et al. 2009; Butt and Ecker 1987; Das et al. 2006; Domènech et al. 2006; Leszczyszyn et al. 2013; Oh et al. 1999; Robinson et al. 2001; Robinson 2008b). To date, four major isoforms, namely, MT-1, MT-2, MT-3, and MT-4, have been identified in humans. MT-1 and MT-2 were detected in all organs (Coyle et al. 2002; Moffatt and Denizeau 1997), whereas MT-3 was detected in the brain, lungs, kidneys, and reproductive organs (Garrett et al. 1999; Moffatt and Seguin 1998; Neal et al. 1996; Suzuki et al. 1994; Uchida et al. 1991; Werynska et al. 2013), and MT-4 was found in differentiating stratified squamous epithelial cells (Quaife et al. 1994). Until to date, eight functional MT-1 isogenes (MT_{ISO}), namely, MT-1A, MT-1B, MT-1E, MT-1F, MT-1G, MT-1H, MT-1M, and MT-1X, have been identified in various organs in humans (Mao et al. 2012; Moleirinho et al. 2011).

MT expression in humans is linked with stress and various inducers (or initiators) such as heavy metals, endotoxins, cytokines, glucocorticoids (GC), reactive oxygen species (ROS), and toxic organic compounds (Chang et al. 2009; Karin and Herschman 1980; Karin et al. 1985; Nourani et al. 2011; Phillippi et al. 2009; Yamada and Koizumi 1991, 2001). Expression of MT in human tissues is also induced during different pathological conditions (Boonprasert et al. 2012;

Chang et al. 2009). Therefore, MTs are primarily involved in homeostasis (storage) and transportation of essential metals such as zinc (Zn) and copper (Cu) and in the detoxification of toxic metals, such as Cd and mercury (Hg) (Carpene et al. 2007; Nordberg and Nordberg 2000; Rigby and Stillman 2006; Vasak 2005). MTs also play important roles in neutralizing ROS, hence inhibiting oxidative stress-induced cytotoxicity and genotoxicity. Arguably, MTs are also involved in immune suppression. Notably, blood provides the avenue for MT, similar to many other biomolecules, in order to perform its roles in storage and transportation of essential metals, defense against toxic substances, as well as in immune responses. However, a comprehensive review on the origin, function, and fate of MT in human blood is yet to appear. Therefore, the current review summarizes the synthesis, function, and fate of MT in human blood. MTs that are synthesized in other organs, such as the liver and kidneys, are eventually transported into the blood and are also discussed in the review. Induced and upregulated expression of MT in nucleated blood cells in response to various MT inducers is also summarized.

2 Sources of MT in Blood

Liver is considered to be the primary source of MT in blood, while plasma-MT may have its origin both in the liver and in cells involved in the circulatory system (Bremner and Beattie 1990; Zalups and Ahmad 2003). Necrosis or apoptosis of hepatocytes caused by Cd toxicity results in Cd-MT mobilization from the liver to plasma (Formigari et al. 2007; Zalups and Ahmad 2003). However, the precise mechanism of the mobilization of Cu/Zn-MT from liver or other tissues to plasma remained unexplained (Bremner et al. 1986).

Hidalgo et al. (1988) reported the permissive role of GC in mobilizing MT from tissues to serum. Hence, MT mobilization from the tissue to plasma in response to GC has been thought to be another major source of extracellular MT (Bremner and Beattie 1990; Hidalgo et al. 1988; Sato et al. 1984). In nucleated blood cells, such as PBL and monocytes, MTs are synthesized within the cells (Chang et al. 2006; Pauwels et al. 1994; Vandeghinste et al. 2000). However, MTs that are detected in anucleated cells, such as erythrocytes and thrombocytes, could be synthesized in their nucleated precursors (Bagheri et al. 2009; Rahman et al. 2000; Rahman and De Ley 2001, 2008; Tanaka et al. 1985) (Fig. 1).

Nakazato et al. (2014) reported that serum MT-1 plus MT-2 (MT-1/MT-2) concentration in healthy human blood ($n = 200$) could be as low as 10 ng/mL and as high as >90 ng/mL. Earlier, it was reported that the MT-1/MT-2 concentration in human serum could be in the range of 10–30 ng/mL (Nagamine and Nakajima 2013) with an average of 23 ± 4.6 ng/mL (Nakajima et al. 2010). However, an increased level of MT-1/MT-2 was detected in various liver diseases such as chronic hepatitis, leading to liver cirrhosis and hepatocellular carcinoma, and Wilson's and Menkes diseases (Nakazato et al. 2014).

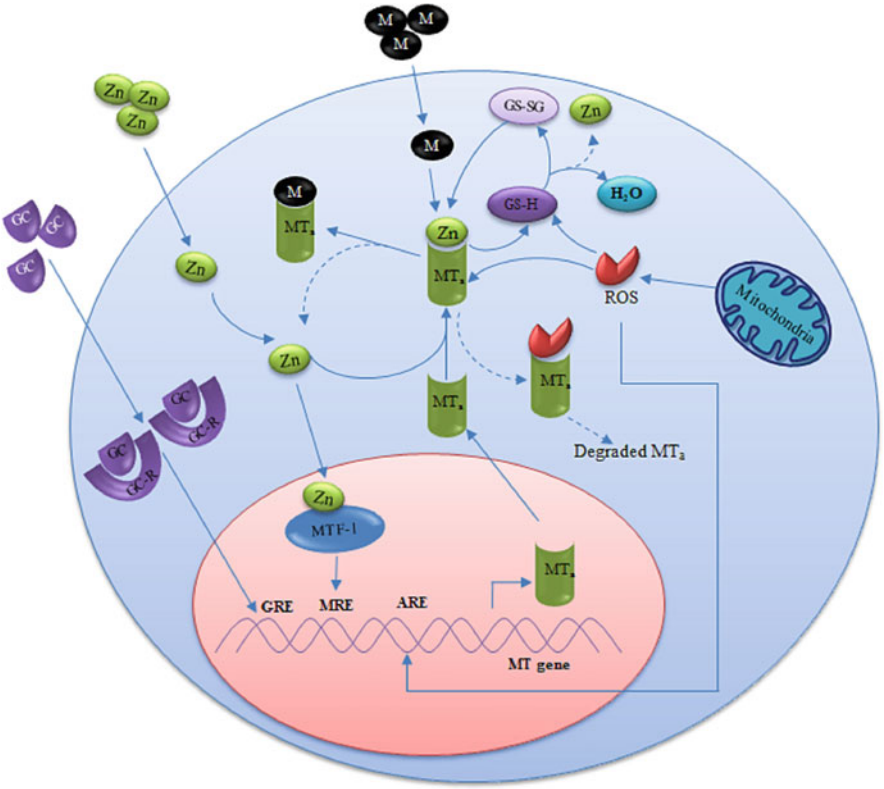

Fig. 1 Metallothionein (MT) expression in nucleated blood cells. Metallothionein (MT) gene contains metal (MRE), antioxidant (ARE), and glucocorticoid response elements (GRE) within its promoter region. Stress induces the production of glucocorticoids (GC), causing it to bind to its receptor, resulting in upregulated or induced expression of MT. MRE is activated by Zn-bound MRE-binding transcription factor MTF-1. Other metals (M) such as Cu and Cd are also capable of inducing MT expression. It is hypothesized that the M could replace Zn from Zn-bound MT (Zn-MT) and produce respective metal-bound MT (M-MT). The free Zn then regulates MT production with the help of MTF-1. Due to its antioxidant property, Zn-MT neutralizes reactive oxygen species (ROS) by binding to them and releases Zn that regulates the expression of MT. Meanwhile Zn unbound oxidized MT is degraded in the cytoplasm (Davis and Cousins 2000; Ruttkay-Nedecky et al. 2013). Reactive oxygen species (ROS) such as superoxide ($O_2^{\cdot-}$) and hydroxyl ($HO^{\cdot}$) radicals can be directly neutralized by MT. At the same time, MT may be involved in activation of the antioxidant agent glutathione (GSH). GSH degrades harmful hydrogen peroxide (H_2O_2) into water (H_2O). During this process GSX is converted to its inactive oxidized form, glutathione disulfide (GS-SG), that returns to its activation state (GSX) in the presence of MT. ROS induce the expression of MT through regulation of ARE as well

3 MT in Blood: Induced and Upregulated Expression

3.1 In Response to Metals

Human beings are often exposed to a number of metals at toxic level through various industrial and environmental sources. Industries, such as jewelry, metal plating, Zn and Pb refining, Cd and Pb smelting, and Ni-Cd batteries, remain the major sources of human exposure to these metals at toxic levels (Wittman and Hu 2002; Qu et al. 2012). The common sources of Cd exposure also include foods contaminated with Cd (Satarug et al. 2010; Wittman and Hu 2002) and tobacco (Edwards et al. 2015; Sánchez-Rodríguez et al. 2015). Among the environmental sources, dust and drinking water comprise the major sources of human exposure to various toxic metals. For example, Cd, Pb, Co, Mn, and Cr through dust (Mohmand et al. 2015) and arsenic (As) through drinking water are the major routes of exposure of those metals into the human body (Carlin et al. 2016). Irrespective of the source, many of these metals such as Cd, Zn, and As can induce MT biosynthesis in blood circulation and in other organs, such as the liver and kidneys, which then enter the circulation (Klaassen et al. 2009; Sabolic et al. 2010). These metals effect MT biosynthesis in blood in different ways, as summarized below.

Transcription of MT (i.e., expression of MT mRNA) in PBLs was found positively correlated with the Cd concentration both in blood (Cd_B) and urine (Cd_U) of occupationally Cd-exposed workers (Lu et al. 2001). At the same time, in vitro Cd treatment of PBLs harvested from the Cd-exposed individuals having a lower level of urinary-acetyl-β-D-glucosaminidase, a marker of renal dysfunction, showed an induced expression of MT mRNA (Lu et al. 2001, 2005). The level of Cd_B was also correlated with the isogene-specific expression of MT mRNA of MT-IE, MT-IF, and MT-IX in PBL obtained from the occupationally Cd-exposed individuals (Chang et al. 2009). Similarly, the expression of MT-IA mRNA in PBL was also found to be positively correlated with increased Cd_U level (Chang et al. 2009).

Dietary Zn supplement has shown to upregulate MT expression in human blood cells (Table 1). A significant increase in monocyte MT expression was observed on day 6 with a 50 mg consumption of dietary Zn supplement per day (Sullivan and Cousins 1997). Similar increase in MT transcription in monocytes (on day 2) and translation in erythrocytes (day 8) was also observed upon 50 mg of dietary Zn supplement on a daily basis (Sullivan et al. 1998). A significant increase in MT expression was also reported in monocytes and peripheral blood mononuclear cells of human volunteers given 15 mg Zn supplement per day (Cao and Cousins 2000). Notably, MT expression in human blood cells continues to increase as long as the dietary Zn supplement continues. For example, erythrocyte MTs increase by sevenfold at day 63 with the continuation of the dietary Zn supplement (50 mg Zn/day); however, it reduces by 61% on day 14 after the withdrawal of the supplement (Grider et al. 1990). In a recent review, Hennigar et al. (2016) reported that human leukocyte MT decreases with Zn depletion (−39% change from

Table 1 In vitro transcription of MT isogenes in blood cells

Cell types/cell lines	Treatment	MT expression		Reference
In vitro studies		*Upregulated transcription*	*Induced transcription*	
CD 61+ megakaryocytes	Zn 100 μM, 48 h	1A, 1B, 1E, 1G, 1H, 1X, 2A	–	Rahman and De Ley (2008)
K562 (megakaryocytic cell line)	Zn 50 μM, 24 h Zn 75 μM, 24 h	1A, 1B, 1E, 1F, 1G, 1H, 1X, 2A	–	Bagheri et al. (2009)
DAMI (megakaryocytic cell line)		1A, 1F, 1H, 2A	1B, 1E, 1G, 1X	
MEG-01 (megakaryocytic cell line)		1A, 1F, 1G, 1H, 1X, 2A	1B, 1E	
ELF-153 (megakaryocytic cell line)		1A, 1F, 1G, 1X, 2A	–	
Blast-forming unit-erythroid (BFU-E)	Zn 60 μM, 48 h	2A	1E, 1G, 1X	Rahman and De Ley (2001)
	Zn 100 μM, 48 h	2A	1A, 1B, 1E, 1G, 1H, 1X	Rahman et al. (2000) and Rahman and De Ley (2001)
	Zn 100 μM, 96 h	1E, 1G, 1X, 2A	1A, 1B, 1H	Rahman and De Ley (2001)
Glycoprotein A+ cells	Zn 100 μM, 48 h	1A, 1B, 1E, 1G 1H, 1X, 2A, 4	–	Rahman et al. (2000) and Rahman and De Ley (2001)
CD71+ cells			–	
Monocytes	Zn 200 μM, 16 h	1E, 1G, 2A	1H	Pauwels et al. (1994)
Lymphocytes		1A, 1E, 1F, 1G 1H, 1X, 2A, 3	–	Vandeghinste et al. (2000)
	Cd 10 μM, 4/12/24 h Cd 20 μM, 4/12/24 h Cd 40 μM, 4/12/24 h Cd 80 μM, 4/12/24 h	1A, 1E, 1F, 1G, 1H, 1X	–	Chang et al. (2006)

(continued)

Table 1 (continued)

Cell types/cell lines	Treatment	MT expression	Reference
In vivo studies		*Upregulated expression*	
Monocytes	50 mg Zn/day	Significantly increased total MT-mRNAs detected on day 6	Sullivan and Cousins (1997)
		Significantly increased total MT-mRNAs detected on day 2	Sullivan et al. (1998)
	15 mg Zn/day		Cao and Cousins (2000)
Erythrocytes	15 mg Zn/day	Significantly increased total MT proteins detected on day 8	Cao and Cousins (2000)
	50 mg Zn/day		Sullivan et al. (1998)
		Sevenfold increase in total MT proteins detected on day 7	Grider et al. (1990)
	0.46 mg Zn/day	Significant reduction in total MT proteins detected on day 7	

baseline, <5 mg Zn/day) and increases with Zn supplementation in a dose-dependent manner (35%, 15–22 human beings are often exposed to a number of milligram Zn/day, $n = 7$ studies; 267%, 50 mg Zn/day). However, erythrocyte MT remains unchanged in regard to the Zn status (Hennigar et al. 2016).

Furthermore, in response to metal inducers, in vitro expression of MT, both at transcriptional and translational level, can either be upregulated or induced in various blood cell populations, such as erythrocyte and thrombocyte precursors, lymphocytes, and monocytes (Table 1). In vitro MT expression is mostly observed in a dose-dependent (Bagheri et al. 2009; Chang et al. 2006; Jonai et al. 1992) and time-dependent (Chang et al. 2006) manner. In the absence of any inducer, the MT expression (at basal level) is restricted in the nucleus and scarcely in the cytoplasm of hematopoietic precursor cells, including immature and mature megakaryocytes, while the induced level of MTs was mostly detected in the cytoplasm (Bagheri et al. 2009; Rahman et al. 2000; Rahman and De Ley 2001, 2008). Notably, MT-I is more abundantly expressed, compared to MT-II in induced cultures of lymphocytes (Jonai et al. 1992).

Studies on the changes in MT expression in response to As in human tissues are scanty. In vivo and in vitro studies on the As inducible MT expressions in mammals that are closely related to humans are mostly available using rodent animal models. For example, Ronchetti et al. (2016) demonstrated increased expression of MT-1 in the pituitary gland of mice given inorganic As. Induced MT expression in response to As has been studied in various human cell lines such as (Falnoga et al. 2012; Slusser et al. 2015; Zhou et al. 2005, 2006). Furthermore, in vitro upregulated expression of MT at the cytoplasmic membrane of human peripheral blood leukocytes was observed in response to As (Rahman and De Ley 2016).

3.2 *In Response to Intracellular ROS*

Intracellular ROS are known to induce MT biosynthesis in different cells (Krężel et al. 2007; Yang and Chitambar 2008). Factors that contribute to the generation of intracellular ROS include radiation (ultraviolet, X-ray, and γ-ray), ozone, toxic emission from automobiles, industry (Gracy et al. 1999), smoking (Barua et al. 2003), alcoholic drinks (Ignatowicz et al. 2013), and excessive exercise (Shi et al. 2007). Physiological sources of ROS include mitochondria, NADPH oxidases, other enzymes, and phagocyte superoxide bursts (Droge 2002; Holmstrom and Finkel 2014; Kang 1999; Sato and Bremner 1993). Thus, once exposed to these ROS inducers, intracellular ROS in the blood cells can induce MT therein (Fig. 1).

3.3 *In Response to Hormones and Cytokines*

Blood harbors a large number of hormones and cytokines. Synthesis, distribution, and concentration of these circulatory molecules vary depending on the physiological status of the individual. Among these circulatory molecules, GC – a stress responsive hormone – was extensively studied for its potential role in MT biosynthesis. In response to different types of psychological stress, the level of GC increases in blood (Michel et al. 2007; Kavushansky et al. 2009; Mahbub et al. 2011) that in turn induces MT transcription in different cells (Haq et al. 2003; Kelly et al. 1997; Quaife et al. 1986) including the GC receptors containing PBL (Bartholome et al. 2004; Griese et al. 1988). At the cellular level, GC dissociates heat shock proteins that are bound to the cytoplasmic GC receptors, resulting in the formation of active GC receptor homodimers, which then translocate into the nucleus and bind to glucocorticoid response elements (GREs) in the MT gene regulatory region. Thus GC induces MT-1/MT-2 gene transcription (Collingwood et al. 1999; Di Croce et al. 1999).

Among the hematopoietic cytokines, erythropoietin (EPO) and IL-3 were shown to induce transcription of MT in erythrocyte progenitors (Abdel-Mageed et al. 2003; Rahman and De Ley 2001). Similar cytokines, including EPO, IL-3, IL-6, and stem cell factor (SCF), are shown to induce MT biosynthesis in ex vivo expanded erythrocyte progenitors (Fig. 2).

4 MT Inducer in Blood: Interplay of Zn

In animals, Zn-specific MTF-1 is the only transcription factor which binds to MRE directly. Therefore, dietary Zn has a direct impact on the MT induction (as discussed above). Induction of MT by Cd and Cu (Chang et al. 2006; Jonai et al. 1992; Selvaraj et al. 2005; Smirnova et al. 2000) is also dependent on Zn

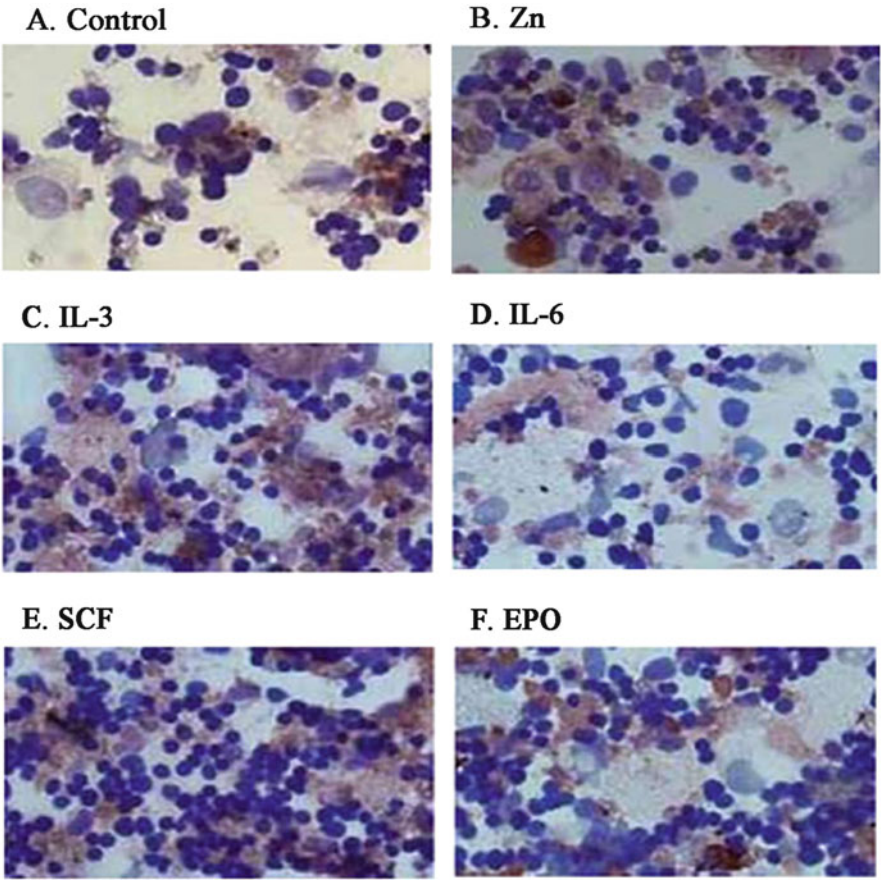

Fig. 2 Metallothionein (MT) expression in response to hematopoietic cytokines. Ex vivo expanded erythrocyte precursors were treated with either different hematopoietic cytokines (**c**–**f**) or with 50 μM Zn (**b**). Parallel control cultures were maintained without any cytokine or additional Zn (**a**). MT expression in the cells was detected by immunohistochemical staining using monoclonal anti-mouse MT (E9, Dako, Tielt, Belgium) after 48 h. The presence of MT expression is revealed by the *red color* in the cytoplasmic area. An increased expression of MT was observed in the cells treated with 5 ng/mL of IL-3 (**c**), 5 ng/mL of SCF (**e**), or 3 U/mL of EPO (**f**) in comparison to that in the parallel control cultures, treated with none of the cytokines or additional Zn (**a**). Compared to the Zn-added cultures (**b**), MT expression seems to be similar to that in IL-3, SCF, and EPO supplemented without any Zn addition. However, MT expression was considerably lower than in the IL-6-supplemented culture (**d**) compared to that in the Zn-added cultures (**b**). These observations indicated the MT-inducing capability of the IL-3, SCF, and EPO in the human erythroid precursors. Erythroid precursors were separated using the same materials and stained as described earlier (Rahman et al. 2000) at the Laboratory of Biochemistry, Department of Chemistry, Katholieke Universiteit Leuven, Belgium

levels in blood. It was hypothesized that Cd and/or Cu displaces Zn from the Zn-containing protein which in turn allows the free Zn to induce MT expression by binding to the Zn finger of MTF-1 (Waldron et al. 2009; Zhang et al. 2003). The

reduction of Cd toxicity on any cell types including blood cells depends on the level of pre-synthesized MT within the cells. Pre-synthesized MT from Cd-MT complex in the cytosol consequently reduces the amount of free Cd to other cellular organelles (Klaassen et al. 2009).

Zn also mediates protection of blood cells from the adverse effect of ROS. Blood cells such as erythrocytes are most likely to be exposed to ROS during the transportation of oxygen (Tsantes et al. 2006), while in PBL, ROS is produced during respiratory burst as a self-defense mechanism (Bulua et al. 2011; Robinson 2008a; Santos et al. 2012). ROS have cytotoxic and genotoxic effects that could shorten the life span of erythrocytes and are also known for causing aging and DNA breakdown within the PBL (Ghaffari 2008). Erythrocytes cannot produce protein; hence they rely on the antioxidant proteins synthesized in their precursors for the protection against ROS (Hattangadi and Lodish 2007). Zinc-induced pre-synthesized MTs in erythrocyte precursors (Huber and Cousins 1993b) also prevent their preterm death by neutralizing the ROS, whereas in PBL, pre-synthesized MTs from their precursors or freshly synthesized MT induced by the dietary Zn (Chang et al. 2006; Pauwels et al. 1994; Vandeghinste et al. 2000) provide protection against apoptosis, necrosis, or DNA breakdown caused by ROS. Zn supplementation may also help to prevent oxidative damage of DNA due to As exposure by induction of MT expression (Qu and Waalkes 2015; Rahman and De Ley 2016).

Several reports have shown the humoral immune suppression by exo-MT (Lynes et al. 1990, 1993; Youn et al. 1995; Youn and Lynes 1999) originating from the necrotic and apoptotic hepatocytes resulting from Cd toxicity (Zalups and Ahmad 2003). Dietary Zn prevents the Cd-induced necrosis by producing more MT (El-Refaiy and Eissa 2012; Souza et al. 2004) and consequently may maintain homeostasis of humoral immunity. However, higher expressions of endo-MT do not ensure the T cell regulatory capability of tolerogenic DC (Spiering et al. 2012). Transportation of MT to the cell membrane is necessary for their immunoregulatory properties, where Zn is involved in transporting MT to the cell membrane and regulating T cell (Spiering et al. 2014).

5 Regulation of MT Expression in Blood Cells

In blood, regulatory elements of MT genes such as metal (MRE), antioxidant (ARE), and glucocorticoid (GRE) response elements in nucleated cells are induced by the divalent heavy metals (e.g., Cd, Cu, Zn), ROS, and stress hormones such as GC, respectively (Fig. 1) (Davis and Cousins 2000; Ruttkay-Nedecky et al. 2013).

Zn-bound MRE-binding transcription factors (MTF) activate MRE. After Zn occupancy, MTF-1 binds specifically to the MRE sequence to initiate transcription. Due to Zn specificity toward MTF-1, the dietary Zn pool has a direct influence on the MT gene expression (Waldron et al. 2009). However, in vitro induction of the MTF-1 dependent MT expression was detected in the presence of Cu or Cd

(Selvaraj et al. 2005; Smirnova et al. 2000). The mechanism of induction remains elusive though. One possible mechanism of MT induction in response to Cu or Cd might be linked to the presence of Zn in fetal bovine serum used for in vitro culture (Chang et al. 2006; Jonai et al. 1992; Selvaraj et al. 2005; Smirnova et al. 2000). The requirement of additional Zn for the binding of the MTF-1 with its promoter in cell-free system attests the definitive role of Zn in MT biosynthesis (Zhang et al. 2003).

A number of steps might be involved in Cu- and Cd-induced expression of MT genes. Firstly, Cd and Cu may displace Zn from the binding sites of Zn-containing metalloproteins including MT (Fig. 1). Subsequently, free Zn may bind to the Zn finger of MTF-1 and regulate the expression of MT gene (Waldron et al. 2009; Zhang et al. 2003). GRE within the promoter region of the MT gene can act independently to induce MT transcription in the presence of glucocorticoids, a stress hormone (Haq et al. 2003; Kelly et al. 1997; Quaife et al. 1986). ARE also play an important role in the induced expression of MT in response to ROS, such as hydrogen peroxide (Dalton et al. 1994; Haq et al. 2003).

6 Functions of MT in Blood

6.1 Protection Against Toxic Metals

A number of factors can cause human exposure to toxic metals such as Cd and As which eventually are transported into the systemic circulation through the lungs or intestines (Fig. 2). In blood, Cd mostly binds to albumin and is transported to the liver where it induces MT synthesis (Zalups and Ahmad 2003). Erythrocytes also take up a significant amount of circulatory Cd (Nordberg and Kjellström 1979; Zalups and Ahmad 2003) (Fig. 2). Mature erythrocytes rely on the pre-synthesized MT derived from their progenitor cells to detoxify Cd (Min et al. 1995). Notably, Cd-MT complex in the hematopoietic progenitors including the erythrocyte progenitors in the spleen (Min et al. 1995) and bone marrow (Oda et al. 2001) was detected following the subcutaneous administration of $CdCl_2$.

Qu and Waalkes (2015) recently reported on the role of MT in the prevention of As-induced oxidative damage of DNA using transgenic mice embryonic cell lines. Increased expression of MT in several cell lines, such as glioblastoma, skin cancer, bladder cancer, as well as hematopoietic cell lines derived from multiple myeloma in the presence of As, indicates the role of MT in acquiring resistance to As-mediated cytotoxicity (Falnoga et al. 2012; Slusser et al. 2015; Zhou et al. 2005, 2006). Furthermore, the potential of MT to bind after neutralizing As also has been reported by several studies (Irvine et al. 2013; Rahman and De Ley 2016; Vašák and Meloni 2011). However, significantly lower levels of MT-1A and MT-2A expression in the blood of arsenicosis patients compared to healthy subjects indicate that less MT expression in human peripheral blood might increase As

susceptibility (Liu et al. 2007). Nonetheless, whether MT provides protection against As remains controversial as MT was not found to sequester inorganic As especially in the condition of acute As toxicity (Garla et al. 2016).

6.2 MT Neutralizes ROS

The ROS scavenging properties of MT make it capable to protect cells from the detrimental effect of ROS (Fig. 1). Several lines of evidence confirm the ability of MT to scavenge free radicals, such as superoxides and hydroxyls, as well as organic radicals (Irato et al. 2001; Thornalley and Vašák 1985). Thornalley and Vašák (1985) elucidated the superoxide and hydroxyl scavenging mechanism of MT in cell-free conditions. Free radicals that are produced by the in vitro xanthine/xanthine-oxidase reactions, i.e., in cell-free conditions, target thiolate ligands of MT and neutralize the reactive radical species by causing metal loss from protein and thiolate oxidation (Thornalley and Vašák 1985).

Besides ROS scavenging capability, MT was shown to activate intracellular antioxidant agents such as superoxide dismutase (SOD) and glutathione (GSH). This notion was evident by the transfer of Zn or other divalent metal from MT to SOD, while addition of apo-MT was shown to inactivate SOD (Koh and Kim 2001). Glutathione peroxidase reduces H_2O_2 into H_2O by oxidizing GSH into glutathione disulfide (GS-SG), which in turn returns to its activation state in the presence of MT (Jiang et al. 1998; Maret 1994). MT in blood cells, such as macrophages (Irato et al. 2001) and NIH 3T3 cells transfected with murine MT-1 (Schwarz et al. 1994), was shown to exert direct ROS scavenging capability.

Thus MT is expected to protect blood cells from the ROS-mediated cytotoxicity and genotoxicity. In the terminally differentiated anucleated blood cells, such as in erythrocytes and thrombocytes, the MT synthesized in their precursors might play a crucial role in ROS neutralization. However, in terminally differentiated nucleated blood cells, such as in lymphocytes and monocytes, both the MTs synthesized in their precursors and in mature cells (Chang et al. 2006; Pauwels et al. 1994; Vandeghinste et al. 2000) could be involved in protecting them against ROS-induced apoptosis, necrosis, or DNA breakdown.

6.3 Immune Regulation by MT

Both endogenous (endo-) and exogenous (exo-) MT have immunomodulatory properties. In vitro studies showed that exo-MT can induce moderate lymphocyte proliferation. Furthermore, in the presence of concanavalin A or lipopolysaccharides, MT inducible lymphocyte proliferation can be augmented (Lynes et al. 1990, 1993). An augmented proliferation of cytotoxic T lymphocytes (CTL) was also observed in mixed lymphocyte reactions (MLR) in the presence of exo-MT (Youn

and Lynes 1999). However, the exo-MT was known to suppress the proliferation of CTL and expression of major histocompatibility complex Class I and CD8 by the CTL (Youn and Lynes 1999). Increased proliferation of lymphocytes in MLR and reduced proliferation of CTL treated with exo-MT indicated that exo-MT might facilitate the proliferation of immature T cells but suppress their terminal differentiation (Youn and Lynes 1999).

Macrophages treated with the in vitro exo-MT produces superoxide through respiratory burst to destroy antigen (Lynes et al. 1993). At the same time, in vivo antigen-specific IgG synthesis by the lymphocytes was reported to be decreased by the exo-MT (Lynes et al. 1993). Again, a reduced proliferation of antigen-primed T cell indicated the interference of exo-MT in the macrophage-T cell interaction resulting in the decreased antigen-specific humoral immune responsiveness of B cell in vivo (Lynes et al. 1993; Youn et al. 1995).

Higher expression of MT-1 protein has been seen in tolerogenic dendritic cells (DCs) produced by treating mouse bone marrow DCs with dexamethasone (Spiering et al. 2014) or carvacrol and thermal stress (Spiering et al. 2012). Tollerogenic DCs were involved in the induction of regulatory T cells and suppression of pathogenic T cell responses (Spiering et al. 2012). Thus, the immunomodulatory potential of endo-MT induction in tolerogenic DCs could be considered as a possible therapeutic tool in the treatment of autoimmune diseases (Spiering et al. 2012).

6.4 MT in Hematopoiesis

Whether MT is directly involved in hematopoiesis is not fully understood. Nevertheless, a number of observations might lead to hypothesize the potential involvement of MT in hematopoiesis. An upregulated expression of MT both at transcriptional (Rahman and De Ley 2001) and at translational (Fig. 1) level in response to hematopoietic cytokines such as IL-3, SCF, and EPO indicates cross talk between hematopoiesis and MT biosynthesis. Again, Zn supplementation to phenylhydrazine-induced anemic rats showed a higher concentration of marrow MT compared to that of non-anemic control rats. Furthermore, induction of marrow MT by Zn in non-anemic rats required prior treatment with EPO, while the marrow MT was mostly abundant in erythroblasts (Huber and Cousins 1993a). These experiments suggest that MT synthesis occurs in EPO-sensitive hematopoietic precursors in the marrow in response to increased Zn accessibility.

7 Fate of Blood MT

The Cd-MT containing mature erythrocytes ends their life cycle in the liver and spleen, where the MT of the Cd-MT complex will be degraded (Tanaka et al. 1986, 1987) (Fig. 3). The resulting free Cd will either form Cd-MT complex in new

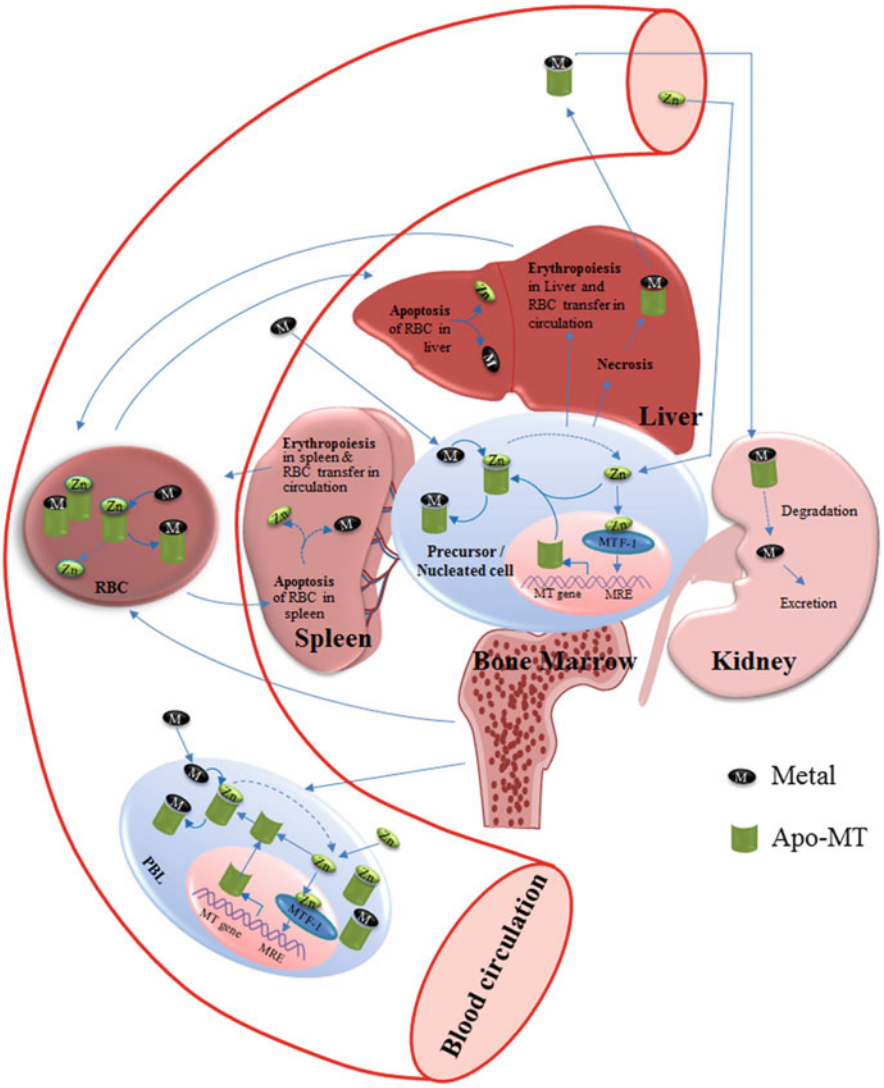

Fig. 3 Role of blood MT in metal detoxification. Toxic heavy metals (M) such as Cd transported to blood from lung or intestine following inhalation or ingestion. After that, a large portion of M is transported to the liver or taken up by blood cells. In erythrocytes (RBC), Cd binds to the MT that originates from their precursors, and in peripheral blood leukocytes (PBL), Cd binds to the MT coming from their precursors and/or newly produced MT following Cd induction. Elderly RBC are decomposed in the liver and the spleen at the end of their life cycle and release Cd-MT complex. The protein part of the Cd-MT complex is degraded there and Cd is released. From the spleen Cd is recirculated through newly produced RBC, whereas in the liver Cd induces the MT synthesis and forms Cd-MT complex. After that Cd-MT complex, which is less toxic compared to inorganic Cd, is released from the liver to the plasma and transported to the kidney. Hemolysis also contributes a portion of the plasma Cd-MT. In the kidney Cd-MT complex filtered through the glomeruli and accumulates in the tubule cells throughout lifetime because of its longer half-life. Cells shown in the center represent differentiated nucleated cells of the respective organ

erythrocytes (in the spleen) or induce MT synthesis in hepatocytes (in the liver). Consequently, Cd-MT complex of the hepatocytes will be released into the plasma mainly from the necrotic or apoptotic hepatocytes (Zalups and Ahmad 2003). Hemolysis of erythrocytes also contributes a small amount of Cd-MT to plasma. Usually, the plasma Cd-MT is not transported back to the spleen but predominantly transported to the kidneys (Min et al. 1995; Tanaka et al. 1981). In the kidney it is efficiently filtered through the glomeruli and taken up by the tubule cells (Dorian et al. 1992; Sabolic et al. 2010). Within the tubule cells, the protein component of Cd-MT degrades rapidly and releases Cd which will then be accumulated throughout the lifetime (Järup and Åkesson 2009; Nordberg and Kjellström 1979; Zalups and Ahmad 2003) and could eventually cause renal toxicity (Dorian et al. 1992; Sabolic et al. 2010). Notably, the Cd-MT complex that is transported from plasma to the kidneys is less toxic than inorganic Cd (Groten et al. 1992, 1994; Prozialeck et al. 1993). Thus the MT from the blood cells and plasma provides active and passive protection to Cd-induced toxicity by taking up Cd and forming less toxic Cd-MT complex and transporting Cd-MT to the kidneys (Fig. 2).

8 Conclusion

MT synthesized in the kidney, liver, bone marrow, and nucleated blood cells can be transported in blood plasma. Blood MT provides primary protection to Cd toxicity. At the same time, MT prevents apoptosis of blood cells by neutralizing ROS. In blood, MT is also involved in immune regulation. Such diversified origin, distribution, and function of MT attest the importance of MT in blood, which can be maintained by Zn supplementation in case of Zn deficiency. Given the permeating distribution of blood MT throughout the body, it's diverse role in the protection against harmful factors, and homeostasis of essential elements, MT could be better recognized as a major public health protein.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

Abdel-Mageed AB, Zhao F, Rider BJ, Agrawal KC (2003) Erythropoietin-induced metallothionein gene expression: role in proliferation of K562 cells. Exp Biol Med (Maywood) 228:1033–1039

Bagheri PM, Rahman MT, Van Soest S, De Ley M (2009) Differential quantitative zinc-induced expression of human metallothionein isogenes in haematopoietic precursor cell lines. J Trace Elem Med Biol 23:124–131

Bartholome B, Spies CM, Gaber T, Schuchmann S, Berki T, Kunkel D et al (2004) Membrane glucocorticoid receptors (MGCR) are expressed in normal human peripheral blood mononuclear cells and up-regulated after in vitro stimulation and in patients with rheumatoid arthritis. FASEB J 18:70–80

Barua RS, Ambrose JA, Srivastava S, DeVoe MC, Eales-Reynolds LJ (2003) Reactive oxygen species are involved in smoking-induced dysfunction of nitric oxide biosynthesis and upregulation of endothelial nitric oxide synthase: an in vitro demonstration in human coronary artery endothelial cells. Circulation 107:2342–2347

Boonprasert K, Ruengweerayut R, Aunpad R, Satarug S, Na-Bangchang K (2012) Expression of metallothionein isoforms in peripheral blood leukocytes from Thai population residing in cadmium-contaminated areas. Environ Toxicol Pharmacol 34:935–940

Bremner I, Beattie JH (1990) Metallothionein and the trace minerals. Annu Rev Nutr 10:63–83

Bremner I, Mehra RK, Morrison JN, Wood AM (1986) Effects of dietary copper supplementation of rats on the occurrence of metallothionein-I in liver and its secretion into blood, bile and urine. Biochem J 235:735–739

Bulua AC, Simon A, Maddipati R, Pelletier M, Park H, Kim K-Y et al (2011) Mitochondrial reactive oxygen species promote production of proinflammatory cytokines and are elevated in TNFR1-associated periodic syndrome (TRAPS). J Exp Med 208:519–533

Butt TR, Ecker DJ (1987) Yeast metallothionein and applications in biotechnology. Microbiol Rev 51:351–364

Cao J, Cousins RJ (2000) Metallothionein mRNA in monocytes and peripheral blood mononuclear cells and in cells from dried blood spots increases after zinc supplementation of men. J Nutr 130:2180–2187

Carlin DJ, Naujokas MF, Bradham KD, Cowden J, Heacock M, Henry HF et al (2016) Arsenic and environmental health: state of the science and future research opportunities. Environ Health Perspect 124:890–899

Carpene E, Andreani G, Isani G (2007) Metallothionein functions and structural characteristics. J Trace Elem Med Biol 21(Suppl 1):35–39

Chang XL, Jin TY, Zhou YF (2006) Metallothionein 1 isoform gene expression induced by cadmium in human peripheral blood lymphocytes. Biomed Environ Sci 19:104–109

Chang X, Jin T, Chen L, Nordberg M, Lei L (2009) Metallothionein I isoform mRNA expression in peripheral lymphocytes as a biomarker for occupational cadmium exposure. Exp Biol Med 234:666–672

Collingwood TN, Urnov FD, Wolffe AP (1999) Nuclear receptors: coactivators, corepressors and chromatin remodeling in the control of transcription. J Mol Endocrinol 23:255–275

Coyle P, Philcox JC, Carey LC, Rofe AM (2002) Metallothionein: the multipurpose protein. Cell Mol Life Sci 59:627–647

Dalton T, Palmiter RD, Andrews GK (1994) Transcriptional induction of the mouse metallothionein-I gene in hydrogen peroxide-treated Hepa cells involves a composite major late transcription factor/antioxidant response element and metal response promoter elements. Nucleic Acids Res 22:5016–5023

Das K, De Groof A, Jauniaux T, Bouquegneau JM (2006) Zn, Cu, Cd and Hg binding to metallothioneins in harbour porpoises *Phocoena phocoena* from the Southern North Sea. BMC Ecol 6:2

Davis SR, Cousins RJ (2000) Metallothionein expression in animals: a physiological perspective on function. J Nutr 130:1085–1088
Di Croce L, Okret S, Kersten S, Gustafsson JA, Parker M, Wahli W, Beato M (1999) Steroid and nuclear receptors. EMBO J 18:6201–6210
Domènech J, Mir G, Huguet G, Capdevila M, Molinas M, Atrian S (2006) Plant metallothionein domains: functional insight into physiological metal binding and protein folding. Biochimie 88:583–593
Dorian C, Gattone VH II, Klaassen CD (1992) Accumulation and degradation of the protein moiety of cadmium-metallothionein (CdMT) in the mouse kidney. Toxicol Appl Pharmacol 117:242–248
Droge W (2002) Free radicals in the physiological control of cell function. Physiol Rev 82:47–95
Edwards SE, Maxson P, Miranda ML, Fry RC (2015) Cadmium levels in a North Carolina Cohort: identifying risk factors for elevated levels during pregnancy. J Expo Sci Environ Epidemiol 25:427–432
El-Refaiy AI, Eissa F (2012) Protective effects of ascorbic acid and zinc against cadmium-induced histopathological, histochemical and cytogenetic changes in rats. Comun Sci 3:162–180
Falnoga I, Zelenik Pevec A, Slejkovec Z, Znidaric MT, Zajc I, Mlakar SJ et al (2012) Arsenic trioxide (ATO) influences the gene expression of metallothioneins in human glioblastoma cells. Biol Trace Elem Res 149:331–339
Formigari A, Irato P, Santon A (2007) Zinc, antioxidant systems and metallothionein in metal mediated-apoptosis: biochemical and cytochemical aspects. Comp Biochem Physiol C Toxicol Pharmacol 146:443–459
Garla R, Ganger R, Mohanty BP, Verma S, Bansal MP, Garg ML (2016) Metallothionein does not sequester arsenic(III) ions in condition of acute arsenic toxicity. Toxicology 366–367:68–73. doi:10.1016/j.tox.2016.08.008
Garrett SH, Sens MA, Todd JH, Somji S, Sens DA (1999) Expression of MT-3 protein in the human kidney. Toxicol Lett 105:207–214
Ghaffari S (2008) Oxidative stress in the regulation of normal and neoplastic hematopoiesis. Antioxid Redox Signal 10:1923–1940
Gracy RW, Talent JM, Kong Y, Conrad CC (1999) Reactive oxygen species: the unavoidable environmental insult? Mutat Res 428:17–22
Grider A, Bailey LB, Cousins RJ (1990) Erythrocyte metallothionein as an index of zinc status in humans. Proc Natl Acad Sci U S A 87:1259–1262
Griese M, Kusenbach G, Lusebring K, Koster W, Roth B, Reinhardt D (1988) Glucocorticoid receptors in mononuclear blood cells and their correlation to endogenous and exogenous corticoids in healthy and asthmatic children. Eur J Pediatr 147:490–495
Groten JP, Luten JB, Bruggeman IM, Temmink JH, van Bladeren PJ (1992) Comparative toxicity and accumulation of cadmium chloride and cadmium-metallothionein in primary cells and cell lines of rat intestine, liver and kidney. Toxicol In Vitro 6:509–517
Groten JP, Koeman JH, van Nesselrooij JH, Luten JB, van Vlissingen JMF, Stenhuis WS et al (1994) Comparison of renal toxicity after long-term oral administration of cadmium chloride and cadmium-metallothionein in rats. Fundam Appl Toxicol 23:544–552
Hamer DH (1986) Metallothionein. Annu Rev Biochem 55:913–951
Haq F, Mahoney M, Koropatnick J (2003) Signaling events for metallothionein induction. Mutat Res 533:211–226
Hattangadi SM, Lodish HF (2007) Regulation of erythrocyte lifespan: do reactive oxygen species set the clock? J Clin Invest 117:2075–2077
Hennigar SR, Kelley AM, McClung JP (2016) Metallothionein and zinc transporter expression in circulating human blood cells as biomarkers of zinc status: a systematic review. Adv Nutr 7 (4):735–746. doi:10.3945/an.116.012518
Hidalgo J, Giralt M, Garvey JS, Armario A (1988) Physiological role of glucocorticoids on rat serum and liver metallothionein in basal and stress conditions. Am J Physiol 254:E71–E78

Holmstrom KM, Finkel T (2014) Cellular mechanisms and physiological consequences of redox-dependent signalling. Nat Rev Mol Cell Biol 15:411–421

Huber KL, Cousins RJ (1993a) Zinc metabolism and metallothionein expression in bone marrow during erythropoiesis. Am J Phys Endocrinol Metab 264:E770–E775

Huber KL, Cousins RJ (1993b) Metallothionein expression in rat bone marrow is dependent on dietary zinc but not dependent on interleukin-1 or interleukin-6. J Nutr 123:642–648

Ignatowicz E, Wozniak A, Kulza M, Senczuk-Przybylowska M, Cimino F, Piekoszewski W et al (2013) Exposure to alcohol and tobacco smoke causes oxidative stress in rats. Pharmacol Rep 65:906–913

Irato P, Santovito G, Piccinni E, Albergoni V (2001) Oxidative burst and metallothionein as a scavenger in macrophages. Immunol Cell Biol 79:251–254

Irvine GW, Summers KL, Stillman MJ (2013) Cysteine accessibility during As^{3+} metalation of the α- and β-domains of recombinant human MT1A. Biochem Biophys Res Commun 433:477–483

Järup L, Åkesson A (2009) Current status of cadmium as an environmental health problem. Toxicol Appl Pharmacol 238:201–208

Jiang L-J, Maret W, Vallee BL (1998) The glutathione redox couple modulates zinc transfer from metallothionein to zinc-depleted sorbitol dehydrogenase. Proc Natl Acad Sci U S A 95:3483–3488

Jonai H, Yamada H, Suzuki K, Otsuka F, Koizumi S (1992) Estimation of metallothionein synthesis in cadmium-exposed human lymphocytes by gel electrophoresis and silver staining. Ind Health 30:129–137

Kagi JH (1991) Overview of metallothionein. Methods Enzymol 205:613–626

Kang YJ (1999) The antioxidant function of metallothionein in the heart. Proc Soc Exp Biol Med 222:263–273

Karin M, Herschman HR (1980) Glucocorticoid hormone receptor mediated induction of metallothionein synthesis in HeLa cells. J Cell Physiol 103:35–40

Karin M, Imbra RJ, Heguy A, Wong G (1985) Interleukin 1 regulates human metallothionein gene expression. Mol Cell Biol 5:2866–2869

Kavushansky A, Ben-Shachar D, Richter-Levin G, Klein E (2009) Physical stress differs from psychosocial stress in the pattern and time-course of behavioral responses, serum corticosterone and expression of plasticity-related genes in the rat. Stress 12:412–425

Kelly EJ, Sandgren EP, Brinster RL, Palmiter RD (1997) A pair of adjacent glucocorticoid response elements regulate expression of two mouse metallothionein genes. Proc Natl Acad Sci U S A 94:10045–10050

Klaassen CD, Liu J, Diwan BA (2009) Metallothionein protection of cadmium toxicity. Toxicol Appl Pharmacol 238:215–220

Koh M, Kim H-J (2001) The effects of metallothionein on the activity of enzymes involved in removal of reactive oxygen species. Bull Kor Chem Soc 22:362–366

Krężel A, Hao Q, Maret W (2007) The zinc/thiolate redox biochemistry of metallothionein and the control of zinc ion fluctuations in cell signaling. Arch Biochem Biophys 463:188–200

Leszczyszyn OI, Imam HT, Blindauer CA (2013) Diversity and distribution of plant metallothioneins: a review of structure, properties and functions. Metallomics 5:1146–1169

Liu J, Cheng M-L, Yang Q, Shan K-R, Shen J, Zhou Y et al (2007) Blood metallothionein transcript as a biomarker for metal sensitivity: low blood metallothionein transcripts in arsenicosis patients from Guizhou, China. Environ Health Perspect 115:1101–1106

Lu J, Jin T, Nordberg G, Nordberg M (2001) Metallothionein gene expression in peripheral lymphocytes from cadmium-exposed workers. Cell Stress Chaperones 6:97–104

Lu J, Jin T, Nordberg G, Nordberg M (2005) Metallothionein gene expression in peripheral lymphocytes and renal dysfunction in a population environmentally exposed to cadmium. Toxicol Appl Pharmacol 206:150–156

Lynes MA, Garvey JS, Lawrence DA (1990) Extracellular metallothionein effects on lymphocyte activities. Mol Immunol 27:211–219

Lynes MA, Borghesi LA, Youn J, Olson EA (1993) Immunomodulatory activities of extracellular metallothionein. I. Metallothionein effects on antibody production. Toxicology 85:161–177
Mahbub ES, Haque N, Salma U, Ahmed A (2011) Immune modulation in response to stress and relaxation. Pak J Biol Sci 14:363–374
Mao J, Yu H, Wang C, Sun L, Jiang W, Zhang P et al (2012) Metallothionein MT1M is a tumor suppressor of human hepatocellular carcinomas. Carcinogenesis 33:2568–2577
Maret W (1994) Oxidative metal release from metallothionein via zinc-thiol/disulfide interchange. Proc Natl Acad Sci U S A 91:237–241
Margoshes M, Vallee BL (1957) A cadmium protein from equine kidney cortex. J Am Chem Soc 79:4813–4814
Michel V, Peinnequin A, Alonso A, Buguet A, Cespuglio R, Canini F (2007) Effect of glucocorticoid depletion on heat-induced Hsp70, IL-1β and TNF-α gene expression. Brain Res 1164:63–71
Min KS, Ohyanagi N, Ohta M, Onosaka S, Tanaka K (1995) Effect of erythropoiesis on splenic cadmium-metallothionein level following an injection of $CdCl_2$ in mice. Toxicol Appl Pharmacol 134:235–240
Moffatt P, Denizeau F (1997) Metallothionein in physiological and physiopathological processes. Drug Metab Rev 29:261–307
Moffatt P, Seguin C (1998) Expression of the gene encoding metallothionein-3 in organs of the reproductive system. DNA Cell Biol 17:501–510
Mohmand J, Eqani SA, Fasola M, Alamdar A, Mustafa I, Ali N, Liu L, Peng S, Shen H (2015) Human exposure to toxic metals via contaminated dust: bio-accumulation trends and their potential risk estimation. Chemosphere 132:142–151. doi:10.1016/j.chemosphere.2015.03.004
Moleirinho A, Carneiro J, Matthiesen R, Silva RM, Amorim A, Azevedo L (2011) Gains, losses and changes of function after gene duplication: study of the metallothionein family. PLoS One 6:e18487
Nagamine T, Nakajima K (2013) Development of a high sensitivity ELISA for the assay of metallothionein. Curr Pharm Biotechnol 14(4):427–431
Nakajima K, Kodaira T, Kato M, Nakazato K, Tokita Y, Kikuchi H, Sekine H, Suzuki K, Nagamine T (2010) Development of an enzyme-linked immunosorbent assay for metallothionein-I and -II in plasma of humans and experimental animals. Clin Chim Acta 2411(9–10):758–761. doi:10.1016/j.cca.2010.02.058
Nakazato K, Tomioka S, Nakajima K, Saito H, Kato M, Kodaira T, Yatsuzuka S, Shimomura Y, Hiroki T, Motoyama K, Kodama H, Nagamine T (2014) Determination of the serum metallothionein (MT)1/2 concentration in patients with Wilson's disease and Menkes disease. J Trace Elem Med Biol 28(4):441–447. doi:10.1016/j.jtemb.2014.07.013
Neal JW, Singhrao SK, Jasani B, Newman GR (1996) Immunocytochemically detectable metallothionein is expressed by astrocytes in the ischaemic human brain. Neuropathol Appl Neurobiol 22:243–247
Nordberg GF, Kjellström T (1979) Metabolic model for cadmium in man. Environ Health Perspect 28:211–217
Nordberg M, Nordberg GF (2000) Toxicological aspects of metallothionein. Cell Mol Biol 46:451–463
Nourani MR, Ebrahimi M, Roudkenar MH, Vahedi E, Ghanei M, Imani Fooladi AA (2011) Sulfur mustard induces expression of metallothionein-1a in human airway epithelial cells. Int J Gen Med 4:413–419
Oda N, Sogawa CA, Sogawa N, Onodera K, Furuta H, Yamamoto T (2001) Metallothionein expression and localization in rat bone tissue after cadmium injection. Toxicol Lett 123:143–150
Oh KB, Watanabe T, Matsuoka H (1999) A novel copper-binding protein with characteristics of a metallothionein from a clinical isolate of Candida albicans. Microbiology 145 (Pt 9):2423–2429

Pauwels M, van Weyenbergh J, Soumillion A, Proost P, De Ley M (1994) Induction by zinc of specific metallothionein isoforms in human monocytes. Eur J Biochem 220:105–110
Phillippi JA, Klyachko EA, Kenny JP IV, Eskay MA, Gorman RC, Gleason TG (2009) Basal and oxidative stress-induced expression of metallothionein is decreased in ascending aortic aneurysms of bicuspid aortic valve patients. Circulation 119:2498–2506
Prozialeck WC, Wellington DR, Lamar PC (1993) Comparison of the cytotoxic effects of cadmium chloride and cadmium-metallothionein in LLC-PK1 cells. Life Sci 53:Pl337–Pl342
Qu W, Waalkes MP (2015) Metallothionein blocks oxidative DNA damage induced by acute inorganic arsenic exposure. Toxicol Appl Pharmacol 282:267–274
Qu C-S, Ma Z-W, Yang J, Liu Y, Bi J, Huang L (2012) Human exposure pathways of heavy metals in a lead-zinc mining area, Jiangsu Province, China. PLoS One 7(11):e46793. doi:10.1371/journal.pone.0046793
Quaife C, Hammer RE, Mottet NK, Palmiter RD (1986) Glucocorticoid regulation of metallothionein during murine development. Dev Biol 118:549–555
Quaife CJ, Findley SD, Erickson JC, Froelick GJ, Kelly EJ, Zambrowicz BP et al (1994) Induction of a new metallothionein isoform (MT-IV) occurs during differentiation of stratified squamous epithelia. Biochemistry 33:7250–7259
Rahman MT, De Ley M (2001) Metallothionein isogene transcription in red blood cell precursors from human cord blood. Eur J Biochem 268:849–856
Rahman MT, De Ley M (2008) Metallothionein in human thrombocyte precursors, CD61+ megakaryocytes. Cell Biol Toxicol 24:19–25
Rahman MT, De Ley M (2016) Arsenic induction of metallothionein and metallothionein induction against arsenic cytotoxicity. Rev Environ Contam Toxicol 240:151–168. doi:10.1007/398_2016_2
Rahman MT, Vandingenen A, De Ley M (2000) Metallothionein biosynthesis in human RBC precursors. Cell Physiol Biochem 10:237–242
Rigby DKE, Stillman MJ (2006) Metal-dependent protein folding: metallation of metallothionein. J Inorg Biochem 100:2101–2107
Robinson JM (2008a) Reactive oxygen species in phagocytic leukocytes. Histochem Cell Biol 130:281–297
Robinson NJ (2008b) A bacterial copper metallothionein. Nat Chem Biol 4:582–583
Robinson NJ, Whitehall SK, Cavet JS (2001) Microbial metallothioneins. Adv Microb Physiol 44:183–213
Ronchetti SA, Bianchi MS, Duvilanski BH, Cabilla JP (2016) In vivo and in vitro arsenic exposition induces oxidative stress in anterior pituitary gland. Int J Toxicol 35(4):463–475. doi:10.1177/1091581816645797
Ruttkay-Nedecky B, Nejdl L, Gumulec J, Zitka O, Masarik M, Eckschlager T et al (2013) The role of metallothionein in oxidative stress. Int J Mol Sci 14:6044–6066
Sabolic I, Breljak D, Skarica M, Herak-Kramberger CM (2010) Role of metallothionein in cadmium traffic and toxicity in kidneys and other mammalian organs. Biometals 23:897–926
Sánchez-Rodríguez JE, Bartolomé M, Cañas AI, Huetos O, Navarro C, Rodríguez AC et al (2015) Anti-smoking legislation and its effects on urinary cotinine and cadmium levels. Environ Res 136:227–233
Santos SS, Brunialti MK, Rigato O, Machado FR, Silva E, Salomao R (2012) Generation of nitric oxide and reactive oxygen species by neutrophils and monocytes from septic patients and association with outcomes. Shock 38:18–23
Satarug S, Garrett SH, Sens MA, Sens DA (2010) Cadmium, environmental exposure, and health outcomes. Environ Health Perspect 118:182–190
Sato M, Bremner I (1993) Oxygen free radicals and metallothionein. Free Radic Biol Med 14:325–337
Sato M, Mehra RK, Bremner I (1984) Measurement of plasma metallothionein-I in the assessment of the zinc status of zinc-deficient and stressed rats. J Nutr 114:1683–1689

Schwarz MA, Lazo JS, Yalowich JC, Reynolds I, Kagan VE, Tyurin V et al (1994) Cytoplasmic metallothionein overexpression protects NIH 3T3 cells from tert-butyl hydroperoxide toxicity. J Biol Chem 269:15238–15243

Selvaraj A, Balamurugan K, Yepiskoposyan H, Zhou H, Egli D, Georgiev O et al (2005) Metal-responsive transcription factor (MTF-1) handles both extremes, copper load and copper starvation, by activating different genes. Genes Dev 19:891–896

Shi M, Wang X, Yamanaka T, Ogita F, Nakatani K, Takeuchi T (2007) Effects of anaerobic exercise and aerobic exercise on biomarkers of oxidative stress. Environ Health Prev Med 12:202–208

Slusser A, Zheng Y, Zhou XD, Somji S, Sens DA, Sens MA et al (2015) Metallothionein isoform 3 expression in human skin, related cancers and human skin derived cell cultures. Toxicol Lett 232:141–148

Smirnova IV, Bittel DC, Ravindra R, Jiang HM, Andrews GK (2000) Zinc and cadmium can promote rapid nuclear translocation of metal response element-binding transcription factor-1. J Biol Chem 275:9377–9384

Souza V, Escobar Mdel C, Bucio L, Hernández E, Gutiérrez-Ruiz MC (2004) Zinc pretreatment prevents hepatic stellate cells from cadmium-produced oxidative damage. Cell Biol Toxicol 20:241–251

Spiering R, van der Zee R, Wagenaar J, Kapetis D, Zolezzi F, van Eden W et al (2012) Tolerogenic dendritic cells that inhibit autoimmune arthritis can be induced by a combination of carvacrol and thermal stress. PLoS One 7:e46336

Spiering R, Wagenaar-Hilbers J, Huijgen V, van der Zee R, van Kooten PJ, van Eden W et al (2014) Membrane-bound metallothionein 1 of murine dendritic cells promotes the expansion of regulatory T cells in vitro. Toxicol Sci 138:69–75

Sullivan VK, Cousins RJ (1997) Competitive reverse transcriptase–polymerase chain reaction shows that dietary zinc supplementation in humans increases monocyte metallothionein mRNA levels. J Nutr 127:694–698

Sullivan VK, Burnett FR, Cousins RJ (1998) Metallothionein expression is increased in monocytes and erythrocytes of young men during zinc supplementation. J Nutr 128:707–713

Suzuki K, Nakajima K, Otaki N, Kimura M (1994) Metallothionein in developing human brain. Biol Signals 3:188–192

Tanaka K, Nomura H, Onosaka S, Min K (1981) Release of hepatic cadmium by carbon tetrachloride treatment. Toxicol Appl Pharmacol 59:535–539

Tanaka K, Min KS, Onosaka S, Fukuhara C, Ueda M (1985) The origin of metallothionein in red blood cells. Toxicol Appl Pharmacol 78:63–68

Tanaka K, Min KS, Ohyanagi N, Onosaka S, Fukuhara C (1986) Fate of erythrocyte Cd-metallothionein in mice. Toxicol Appl Pharmacol 83:197–202

Tanaka K, Min K, Onosaka S, Fukuhara C (1987) Synthesis and degradation of erythrocyte metallothionein in cadmium-administered mice. Experientia Suppl 52:525–532

Thornalley PJ, Vašák M (1985) Possible role for metallothionein in protection against radiation-induced oxidative stress. Kinetics and mechanism of its reaction with superoxide and hydroxyl radicals. Biochim Biophys Acta 827:36–44

Tsantes AE, Bonovas S, Travlou A, Sitaras NM (2006) Redox imbalance, macrocytosis, and RBC homeostasis. Antioxid Redox Signal 8:1205–1216

Uchida Y, Takio K, Titani K, Ihara Y, Tomonaga M (1991) The growth inhibitory factor that is deficient in Alzheimer's disease is a 68 amino acid metallothionein-like protein. Neuron 7 (2):337–347

Vandeghinste N, Proost P, De Ley M (2000) Metallothionein isoform gene expression in zinc-treated human peripheral blood lymphocytes. Cell Mol Biol 46:419–433

Vasak M (2005) Advances in metallothionein structure and functions. J Trace Elem Med Biol 19:13–17

Vašák M, Meloni G (2011) Chemistry and biology of mammalian metallothioneins. J Biol Inorg Chem 16:1067

Waldron KJ, Rutherford JC, Ford D, Robinson NJ (2009) Metalloproteins and metal sensing. Nature 460:823–830

Werynska B, Pula B, Muszczynska-Bernhard B, Gomulkiewicz A, Jethon A, Podhorska-Okolow M et al (2013) Expression of metallothionein-III in patients with non-small cell lung cancer. Anticancer Res 33:965–974

Wittman R, Hu H (2002) Cadmium exposure and nephropathy in a 28-year-old female metals worker. Environ Health Perspect 110:1261–1266

Yamada H, Koizumi S (1991) Metallothionein induction in human peripheral blood lymphocytes by heavy metals. Chem Biol Interact 78:347–354

Yamada H, Koizumi S (2001) Lymphocyte metallothionein-mRNA as a sensitive biomarker of cadmium exposure. Ind Health 39:29–32

Yang M, Chitambar CR (2008) Role of oxidative stress in the induction of metallothionein-2A and heme oxygenase-1 gene expression by the antineoplastic agent gallium nitrate in human lymphoma cells. Free Radic Biol Med 45:763–772

Youn J, Lynes MA (1999) Metallothionein-induced suppression of cytotoxic T lymphocyte function: an important immunoregulatory control. Toxicol Sci 52:199–208

Youn J, Borghesi LA, Olson EA, Lynes MA (1995) Immunomodulatory activities of extracellular metallothionein. II. Effects on macrophage functions. J Toxicol Environ Health 45:397–413

Zalups RK, Ahmad S (2003) Molecular handling of cadmium in transporting epithelia. Toxicol Appl Pharmacol 186:163–188

Zhang B, Georgiev O, Hagmann M, Gunes C, Cramer M, Faller P et al (2003) Activity of metal-responsive transcription factor 1 by toxic heavy metals and H_2O_2 in vitro is modulated by metallothionein. Mol Cell Biol 23:8471–8485

Zhou P, Kalakonda N, Comenzo RL (2005) Changes in gene expression profiles of multiple myeloma cells induced by arsenic trioxide (ATO): possible mechanisms to explain ATO resistance in vivo. Br J Haematol 128:636–644

Zhou XD, Sens DA, Sens MA, Namburi VBRK, Singh RK, Garrett SH et al (2006) Metallothionein-1 and -2 expression in cadmium- or arsenic-derived human malignant urothelial cells and tumor heterotransplants and as a prognostic indicator in human bladder cancer. Toxicol Sci 91:467–475

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Cannabinoids as Modulators of Cell Death: Clinical Applications and Future Directions

B.M. Fonseca, N.A. Teixeira, and G. Correia-da-Silva

Abstract Endocannabinoids are bioactive lipids that modulate various physiological processes through G-protein-coupled receptors (CB1 and CB2) and other putative targets. By sharing the activation of the same receptors, some phytocannabinoids and a multitude of synthetic cannabinoids mimic the effects of endocannabinoids. In recent years, a growing interest has been dedicated to the study of cannabinoids properties for their analgesic, antioxidant, anti-inflammatory and neuroprotective effects. In addition to these well-recognized effects, various studies suggest that cannabinoids may affect cell survival, cell proliferation or cell death. These observations indicate that cannabinoids may play an important role in the regulation of cellular homeostasis and, thus, may contribute to tissue remodelling and cancer treatment. For a long time, the study of cannabinoid receptor signalling has been focused on the classical adenylyl cyclase/cyclic AMP/protein kinase A (PKA) pathway. However, this pathway does not totally explain the wide array of biological responses to cannabinoids. In addition, the diversity of receptors and signalling pathways that endocannabinoids modulate offers an interesting opportunity for the development of specific molecules to disturb selectively the endogenous system. Moreover, emerging evidences suggest that cannabinoids ability to limit cell proliferation and to induce tumour-selective cell death may offer a novel strategy in cancer treatment. This review describes the main properties of cannabinoids in cell death and attempts to clarify the different pathways triggered by these compounds that may help to understand the complexity of respective molecular mechanisms and explore the potential clinical benefit of cannabinoids use in cancer therapies.

Keywords Apoptosis • Autophagy • Cancer • Cannabinoids

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Contents

1 Introduction

Cannabis sativa plant has a long social and medicinal history dating back thousands of years. However, it was only in 1964 that Gaoni and Mechoulam discovered the main psychoactive compound, the Δ9-tetrahydrocannabinol (Δ9-THC) (Gaoni and Mechoulam 1964). Although it was initially suggested that Δ9-THC would elicit its effects non-specifically, the synthesis and biological studies of Δ9-THC and its synthetic analogues revealed that its principal pharmacological actions were enantioselective and, in 1990s, cannabinoid receptors were discovered and cloned (Devane et al. 1988; Matsuda et al. 1990; Munro et al. 1993). Two cannabinoid receptors were described so far, CB1 and CB2, which are mainly distinguished by their physiological actions and locations within the body. Although CB1 is widely distributed throughout the brain, it is expressed in peripheral tissues, such as vascular endothelium, intestine, liver and reproductive tissues (Fonseca et al. 2009a). Originally thought to be absent in the central nervous system, the CB2 is primarily expressed in immune cells and the CB2 mRNA was also detected in cerebellar granule cells (Skaper et al. 1996), in the brainstem (Van Sickle et al. 2005) and in other peripheral tissues (Fonseca et al. 2009a).

The term cannabinoid began to be used to characterize the terpenophenolic constituents of *Cannabis sativa* plant, but currently, it has a more widely definition including the molecules that interact with the cannabinoid receptors or present structural similarities with Δ9-THC. Thus, among the cannabinoid "family", there are the endocannabinoids (eCBs), the phytocannabinoids and the synthetic cannabinoids (Fig. 1).

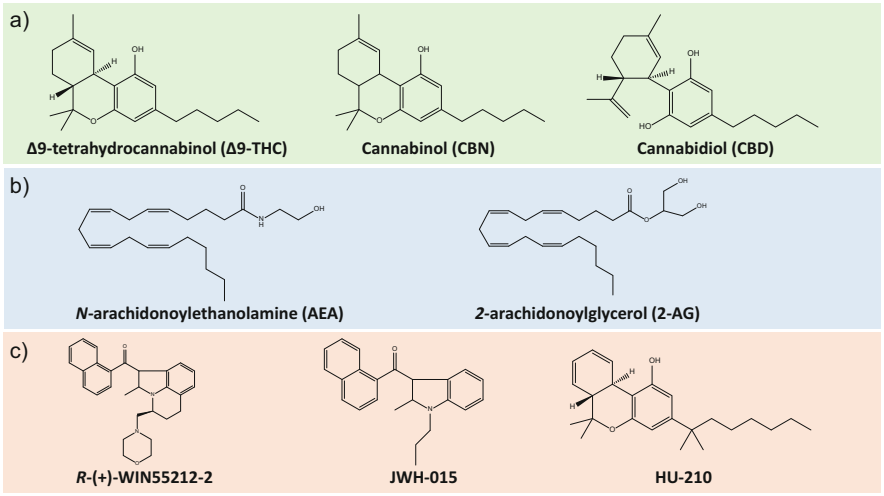

Fig. 1 Chemical structure of the main endogenous, synthetic and phytocannabinoids. (**a**) The most relevant phytocannabinoids of cannabis plant (*Cannabis sativa L.*) consist of Δ9-tetrahydrocannabinol (Δ9-THC), cannabidiol (CBD) and cannabinol (CBN). (**b**) The best-known and well-studied endocannabinoids are anandamide (AEA) and 2-arachidonoylglycerol (2-AG). (**c**) Synthetic cannabinoids are functionally similar to Δ9-THC, though many of these compounds are not structurally related to the so-called classical cannabinoids

1.1 Endocannabinoid System

The identification of cannabinoid receptors intensified the search for endogenous ligand(s) that might mimic Δ9-THC actions. The first endocannabinoid to be isolated was *N*-arachidonoylethanolamine (anandamide (AEA)) in 1992 (Devane et al. 1992), followed by 2-arachidonoylglycerol (2-AG), which together constitute the best characterized members of eCBs family (Mechoulam et al. 1995). Other minor compounds that belong to the "endocannabinoid family" are 2-arachidonoylglycerylether (noladin ether, 2-AGE), *O*-arachidonoylethanolamine (virodhamine), *N*-arachidonoyldopamine (NADA) and *N*-arachidonoylglycine (NAGly).

Besides cannabinoid receptors and eCBs, the enzymes responsible for synthesis, transport and degradation of eCBs comprise the endocannabinoid system. Recently, the endocannabinoid biosynthesis and degradation was nicely reviewed by Ligresti et al. (2016). The major biosynthetic pathway for AEA is through the transfer of arachidonic acid (AA) from phosphatidylcholine to the head group of phosphatidylethanolamine by a Ca^{2+}-dependent *N*-acyltransferase (NAT) to generate *N*-arachidonoyl-phosphatidylethanolamine (NAPE), which is subsequently converted into AEA via alternative enzymatic pathways. The well-studied and most important path is the direct conversion, catalysed by an NAPE-selective phospholipase D (NAPE-PLD) (Liu et al. 2006). Additionally, phospholipase C (PLC) and secreted phospholipase A2 (sPLA2)-catalyse Ca^{2+}-independent pathways. The hydrolysis of NAPE by PLC yields a phosphorylated precursor (pAEA), which generates AEA

through the action of phosphatases, including the putative tyrosine phosphatase non-receptor 22 (PTPN22) and phosphatidylinositol-3, 4,5-trisphosphate 5-phosphatase 1 (INPP5D) (Liu et al. 2006, 2008). Alternatively, through sequential deacylations by sPLA2 and αβ-hydrolase (ABHD)-4, NAPE generates lyso-NAPE and glycerophospho (GP)-AEA, which yields AEA by a metal-dependent phosphodiesterase action (Simon and Cravatt 2006).

The biosynthesis of 2-AG occurs from phosphatidylinositol (PI) through a two-step process with formation of 1,2-diacylglycerol catalysed by a phospholipase such as PLC, which is then hydrolysed to 2-AG by either of two diacylglycerol lipases (DAGLs), DAGLα or DAGLβ (Bisogno et al. 2003). An alternative pathway involves the formation of 2-arachidonoyl-lyso PI by phospholipase A1 (PLA1) and subsequent hydrolysis by a lyso-PLC (Higgs and Glomset 1994).

Despite the complexity and controversy on eCBs trafficking, it is still accepted that eCBs are not stored in vesicles but are instead synthesized on demand. Then, eCBs are released to extracellular space becoming available to activate cannabinoid receptors (Gabrielli et al. 2015). In central nervous system, eCBs are released from depolarized postsynaptic neurons, binding and activating CB1 receptors at pre-synaptic terminals, and acting as retrograde messengers (Kano 2014). They are then rapidly removed from the extracellular space by a selective cellular reuptake mechanism followed by intracellular enzymatic hydrolysis.

Anandamide is predominantly degraded by the intracellular membrane enzyme, fatty acid amide hydrolase (FAAH), whereas 2-AG is hydrolysed mainly by monoacylglycerol lipase (MAGL) and to a lesser extent, also by FAAH, ABHD-6 and ABHD-12 (Deutsch and Chin 1993; Dinh et al. 2002). Endocannabinoids can also be oxygenated by cyclooxygenase-2 (COX-2), lipoxygenases (LOXs) and cytochromes P450 (CYP450s) (Snider et al. 2008; Muccioli 2010).

The endocannabinoid system is involved in the regulation of various biological processes, including energy homeostasis, immune response, neurotransmission, reproduction and cell choice between survival and death (Mechoulam et al. 2014). Thus, cannabinoids attracted significant attention in recent years. In particular, some cannabinoids may offer potential applications as antitumour drugs, based on the ability to inhibit cell proliferation/induce cell death and a variety of pathways are likely to contribute to these effects.

1.2 Cannabinoid Signalling

Cannabinoid receptors belong to the Class A of G-protein coupled receptors (GPCRs) superfamily, which comprise seven transmembrane helices connected by three intracellular and three extracellular loops with a N-terminal extracellular domain and a C-terminal intracellular domain (Svizenska et al. 2008).

The activation of cannabinoid receptors primarily leads to the inhibition of adenylyl cyclase via $G\alpha_{i/o}$, resulting in the reduction of the cyclic AMP-stimulated PKA activity, which impacts important cellular signalling events

including voltage-dependent current flow at A-type K^+ channels (Wade et al. 2004), tyrosine phosphorylation of focal adhesion kinase (FAK) (Derkinderen et al. 1996) and RhoA/Rho-associated protein kinase (ROCK) signal pathway (Laezza et al. 2008). Also, via pertussis toxin (PTX)-sensitive $G\alpha_{i/o}$ proteins, the cannabinoid receptors inhibit *N*- and *P*/*Q*-type voltage-gated Ca^{2+} channels (Mackie et al. 1995; Pan et al. 1996) and regulate the phosphorylation and activation of different members of mitogen-activated protein kinases (MAPKs) pathway (Bouaboula et al. 1995). It includes extracellular signal-regulated kinase (ERK), p38 MAPK and c-Jun N-terminal kinase (JNK; also known as stress-activated protein kinase or SAPK) (Pertwee 2000). It was also described that CB1 can induce the activation of protein kinase B (PKB, also known as Akt) with the involvement of phosphoinositide 3-kinase (PI3K) pathway and subsequent induction of Raf-1 translocation to the membrane and phosphorylation of ERK kinase (del Pulgar et al. 2000; Sanchez et al. 2003).

Uncommonly, CB1 receptor has also been found to cause an increase in intracellular Ca^{2+} levels by coupling phospholipase C (PLC) involving the βγ subunits from $G_{i/o}$ (Lograno and Romano 2004) or by a PTX-insensitive manner requiring Gq/11 and PLC (Lauckner et al. 2005). It was also observed that cannabinoids may modulate cell function, through heparin-bound epidermal growth factor receptor (EGFR) (Preet et al. 2011).

On the other hand, there are also evidences that CB1 activation may stimulate adenylyl cyclase via G_s proteins, but with low efficacy compared to $G\alpha_{i/o}$ (Eldeeb et al. 2016), emphasizing the complexity of CB1 transduction pathways. By increasing concentrations of cannabinoids, the successive activation of G_s and of $G_{i/o}$ proteins may explain a biphasic concentration-response profile associated to cannabinoids, allowing, for both, the fine-tuning and adaptation of diverse functional responses elicited by CB1 activation. On the contrary, CB2 receptor activation does not couple G_s protein and conflicting data have been reported about the CB2-modulation of calcium channels or inward rectification of potassium channels (Felder et al. 1995; Zoratti et al. 2003).

Cannabinoids can also modulate the intracellular levels of ceramide, which is a ubiquitous sphingolipid second messenger with an important role in the control of cell fate. Activation of CB1 can produce two peaks of ceramide. Short-term ceramide generation involves sphingomyelin hydrolysis via sphingomyelinase (SMase) activation through the adaptor protein FAN, acting on metabolic regulation (Sanchez et al. 2001b). Long-term ceramide generation may occur via serine palmitoyltransferase (SPT) induction and enhanced de novo ceramide synthesis (Velasco et al. 2005; Herrera et al. 2006).

Moreover, besides these signalling pathways mediated by cannabinoid receptors and directly involved in cell survival, proliferation and apoptosis, there are also evidences that some cannabinoids may stimulate transient receptor potential vanilloid subtype 1 (TRPV1) (Zygmunt et al. 1999) and peroxisome proliferator-activated receptors (PPARs) (O'Sullivan 2007). In addition, inhibition of COX-2 may mediate the proapoptotic and antiproliferative effects of AEA and of some synthetic cannabinoids.

2 Cannabinoids and Cell Death

Numerous studies demonstrate that cannabinoids may affect cell proliferation and death by a multitude of signalling pathways. The first works that were focused on the classical cannabinoid receptor signalling involved adenylyl cyclase/cyclic AMP/protein kinase A (PKA) pathway. However, this pathway does not sufficiently explain the wide array of biological responses to cannabinoids. These include growth – as well as death-promoting effects that are in part shared with other receptors, such as TRPV1and PPAR receptors (Fig. 2).

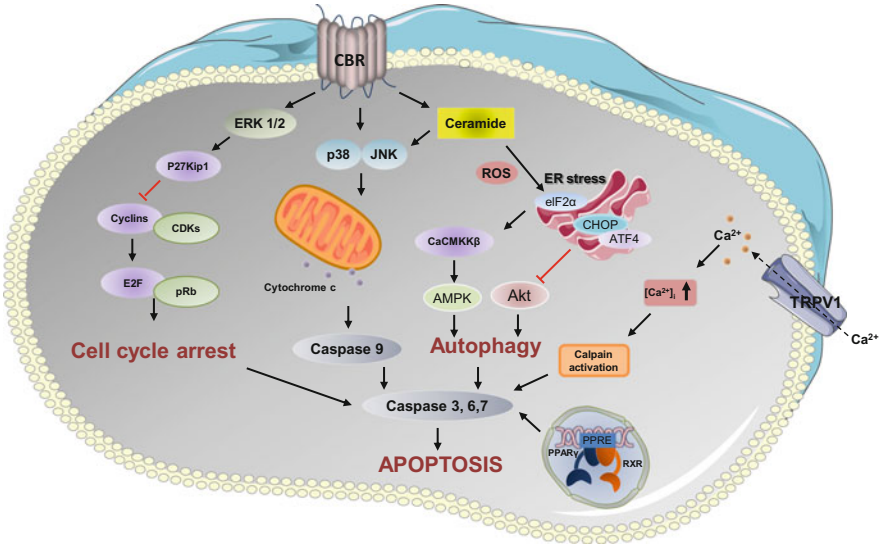

Fig. 2 Main signalling pathways involved in cannabinoid-induced cell death. Activation of cannabinoid receptors results in the activation of JNK and p38 MAPK, which may induce the release of cytochrome *c* and activation of caspase-9 and -3/-6/-7. Cannabinoids may also signal via ceramide generation engaging JNK/p38 MAPK pathway, resulting in apoptosis with mitochondria involvement or leading to ROS generation and endoplasmic reticulum (ER) stress. On the other hand, ER stress can also stimulate autophagy, which may be upstream of apoptosis in cannabinoid-induced cell death. It may occur via upregulation of TRIB3 and subsequent inhibition of the serine–threonine kinase Akt/mammalian target of rapamycin C (Akt/mTORC1) axis, or via adenosine monophosphate-activated kinase (AMPK) via CaMKKβ. Activation of ERK1/2 leads to the induction of cyclin kinase inhibitor p27/KIP1with modulation of cell cycle regulatory molecules, resulting in cell cycle arrest and apoptosis. Inside the cell, cannabinoids can bind to PPARγ or TRPV1 receptor. The latter was shown to be the responsible for AEA-induced cell death, coupled to oxidative stress, intracellular Ca^{2+} increase and calpain activation

2.1 Cannabinoid Receptors and Cell Death

There are various evidences about the cannabinoid capacity to modulate cell death through activation of cannabinoid receptors. Although the majority of observations indicate that cannabinoids induce apoptosis through CB1, there are also studies showing similar effects via CB2 or non-CB1/CB2 activation.

A common pathway resulting from cannabinoids-induced apoptosis through cannabinoid receptors involves phosphorylation of p38 MAPK followed by mitochondrial membrane depolarization and caspase activation as observed with synthetic cannabinoids in lymphoma B-cells (Gustafsson et al. 2006), epidermal cells (Casanova et al. 2003) and endothelial cells (Rajesh et al. 2010). Moreover, in cerebellar granule cells, the synthetic cannabinoid WIN55,212-2 (WIN) has been shown to induce apoptosis via CB1 and downregulation of the anti-apoptotic Bcl-xL (Pozzoli et al. 2006).

Contrary to synthetic cannabinoids, Δ9-THC did not present effects on JNK or p38 MAPK pathways. Instead, it may inhibit cell cycle progression or induce apoptosis through down-regulation of ERK and PI3K/Akt survival pathways (Greenhough et al. 2007; Jia et al. 2006; Do et al. 2004). Also in human breast cancer cells, this phytocannabinoid reduces cell proliferation by blocking the progression of the cell cycle and by inducing apoptosis, through activation of CB2 receptor (Caffarel et al. 2006, 2008).

In line with the effects of synthetic and phytocannabinoids, eCBs have also been shown to be involved in cell death modulation. In human breast carcinoma cells, AEA inhibited cAMP formation and induced cell cycle arrest, through CB1 receptor (De Petrocellis et al. 1998; Melck et al. 1999, 2000), whereas in prostate cancer and decidual cells, eCBs activated the apoptotic pathway without modifying cell cycle stage (Orellana-Serradell et al. 2015; Fonseca et al. 2010). Thus, by modulating the balance among ERK, JNK and p38 MAPK activities, the CB1 receptor activation might regulate cell proliferation, differentiation and death in response to environmental stimuli.

The activation of CB1 by different cannabinoid members also leads to sphingomyelin breakdown through the adaptor protein FAN, independently of $G_{i/o}$ proteins (Sanchez et al. 2001b). As shown in C6 glioma cells (Galve-Roperh et al. 2000; Sanchez et al. 1998) and primary astrocytes (Blazquez et al. 2000), the sustained increase of ceramide levels involves JNK/p38 MAPK phosphorylation resulting in apoptotic cell death. Ceramide levels were also found to be increased in different cancer (Velasco et al. 2005; Cianchi et al. 2008) and non-cancer cells (Fonseca et al. 2009b, 2013) in response to CB1 activation. The CB2 receptor was also involved on sustained ceramide biosynthesis stimulation by Δ9-THC in human leukaemia Jurkat (Herrera et al. 2006) and pancreatic tumour cells (Carracedo et al. 2006), resulting in upregulation of p38 MAPK, ER stress-related genes and apoptosis. The involvement of CB2 in cell death was also reported in macrophage J774-1 cells (Yamaori et al. 2013) and human cytotrophoblasts (Costa et al. 2014a, 2015a, b).

Interestingly, CB1 receptor activation also presented protective roles in diverse cell types. In fact, the modulation of PI3K/Akt pathway by WIN emerged as a survival signalling pathway on glial cells (Gomez Del Pulgar et al. 2002). Nevertheless, a common event in cannabinoid-induced apoptosis is either the inhibition of survival pathways or the activation of MAPKs interrelated signal transduction pathways with consequent depolarization of mitochondria via cytochrome *c* release (Jia et al. 2006; Greenhough et al. 2007).

The CB1 can adopt multiple conformations and these apparent differences triggered by cannabinoids are probably a result of conformational heterogeneity of cannabinoid receptors that allows the binding of different ligands and interaction with different intracellular effectors. Besides the recent work of Daniel Rosenbaum et al. to determine the structure of bound-human CB1 receptor, additional structural studies of cannabinoid receptors in different conformational states bound to ligands will be necessary to clarify such peculiar receptors (Shao et al. 2016).

2.2 Cannabinoids-Induced Cell Death Through Non-CB1/CB2 Receptors

Independently of cannabinoid receptors, TRPV1 receptor was shown to be responsible for AEA-induced apoptosis in rat and human neurons (Kim et al. 2005; Maccarrone et al. 2000), lymphoma cells (Maccarrone et al. 2000) and cytotrophoblasts (Costa et al. 2014b). Similarly, AEA induced apoptosis of human epidermal melanocyte through a TRPV1-mediated pathway (Pucci et al. 2012), though it also stimulated the activity of human melanocyte via CB1 receptor, suggesting a potential protective role for eCBs via CB1 (Pucci et al. 2012). Again, the stimulation of cannabinoid receptors was protective against the TRPV1-mediated antiproliferative effects of AEA in neuroblastoma (Maccarrone et al. 2000) and uterine cervix cancer cells (Contassot et al. 2004). Cannabidiol (CBD), which shares with AEA the ability to activate the TRPV1, also induced apoptosis of MDA-MB-231 human breast cancer cells via TRPV1, increasing intracellular Ca^{2+} levels and reactive oxygen species (Ligresti et al. 2006).

Cannabinoids may also induce apoptosis through PPARs independently of cannabinoid receptor activation as observed in human hepatocellular carcinoma cells by up-regulation of PPARγ-dependent pathways (Hong et al. 2013; Vara et al. 2013). Moreover, in lung cancer cells, CBD increased COX-2–dependent prostaglandins (PGs), which caused a translocation of PPAR-γ to the nucleus and induction of apoptosis (Ramer et al. 2013). Previous observations identified that 2-AG was able to suppress IL-2 expression via a CB1/2 receptor-independent activation of PPAR-γ, suggesting that 2-AG may be able to directly activate PPAR-γ (Rockwell et al. 2006). However, these effects are still in debate as in T cells, AEA or 2-AG suppresses IL-2 in a COX-2-dependent way (Rockwell et al. 2008). Moreover, it was also observed that AEA induces COX-2-dependent cell

death in colon and keratinocytes cancer cells without cannabinoid receptor engagement (Patsos et al. 2010; Van Dross 2009). Moreover, prostamides, resulting from AEA-oxidative metabolism by COX-2, were shown to induce apoptosis of decidual cells (Almada et al. 2015).

2.3 *Cannabinoids Mediated Receptor-Independent Cell Death*

In PC12 and chondrocyte cells, AEA inhibited cell proliferation and activated MAPK pathway independent of cannabinoid or vanilloid receptors (Sarker and Maruyama 2003; Sarker et al. 2000; Gomez et al. 2014), similarly to Δ9-THC and other phytocannabinoids in prostate cancer cells (Ruiz et al. 1999; De Petrocellis et al. 2013). Also in osteosarcoma cells, AEA induced apoptosis via intracellular Ca^{2+} increase (Hsu et al. 2007) and, in rat neurons, it caused neurotoxicity involving calpain activation in a receptor independent manner (Movsesyan et al. 2004), suggesting that more sophisticated mechanisms should be involved in the regulation of cell death by cannabinoids.

Potentially, AEA may exert its effects by direct inhibition of T-type Ca^{2+} channels (Chemin et al. 2001) and through membrane lipid rafts (Sarker and Maruyama 2003). The phytocannabinoid CBD induces apoptosis of murine primary microglial cells with marked activation of both caspase-8 and -9 and a significant increase in hypodiploid cells and DNA strand breaks. The specific antagonists for vanilloid and cannabinoid receptors did not counteract the apoptosis induced by CBD, whereas methyl-β-cyclodextrin (MCD), a lipid raft disruptor, potently attenuated CBD-induced microglial apoptosis and caspase activation (Wu et al. 2012).

Some studies enhanced also COX-2 contribution to cannabinoid-induced apoptosis in resistant colon cancer cells and tumourigenic keratinocytes (Patsos et al. 2010; Van Dross 2009), reinforcing the importance of eCBs-oxidative pathways. For example, the treatment of human glioma cells with the stable analogue of AEA (methanandamide) resulted in the induction of apoptosis via lipid raft-mediated events with an increase in ceramide levels, induction of COX-2 and subsequent prostaglandin E2 synthesis, involving p38 MAPK and ERK activation (Hinz et al. 2004a, b).

Moreover, it has been described that AEA may recruit the death receptor/ligand Fas/FasL to lipid rafts implicating the death-inducing signalling complex (DISC) formation and caspase-8 activation, culminating in the intrinsic apoptotic pathway activation by a cannabinoid-receptor independent mechanism (DeMorrow et al. 2007).

More alternative mechanisms have also been identified. In activated hepatic stellate cells CBD also induced apoptosis eliciting an endoplasmic reticulum (ER) stress response characterized by changes in ER morphology and initiation of RNA-dependent protein kinase-like ER kinase-, activating transcription factor-6-,

and inositol-requiring ER-to-nucleus signal kinase-1 (IRE1)-mediated signalling cascades. Furthermore, CBD caused downstream activation of the pro-apoptotic IRE1/ASK1/JNK pathway, leading to hepatic cell death (Lim et al. 2011). Also in freshly isolated monocytes, CBD markedly enhanced apoptosis, while cultured monocytes were insensitive, which was associated with the antioxidant capacity of these cells (Wu et al. 2010). Interestingly, AEA and the synthetic cannabinoid agonist HU 210 caused a decrease in cell viability of P19 embryonal carcinoma cells, effect blocked by the antioxidants α-tocopherol and *N*-acetylcysteine (Gustafsson et al. 2013), suggesting that the antioxidant capacity of the cells may be relevant for cell protection independent from specific receptor activation.

2.4 Cannabinoids and Autophagy-Associated Cell Death

The effects of cannabinoids are not only associated with the canonical apoptotic pathway and numerous studies reported a cannabinoid-induced autophagic mechanism in various cell types. Macroautophagy (hereafter referred to as autophagy) is a conserved catabolic process through which cytoplasmic constituents are nonselectively degraded in lysosomes. Although 2016 was recorded in science as the "year of autophagy" due to the 2016 Nobel Prize in Physiology and Medicine awarded to Yoshinori Ohsumi, the molecular regulation of autophagy remains an important area of research.

On a molecular level, autophagy is mechanistically regulated by various protein factors, recently reviewed by Kaur and Debnath (2015). Briefly, a family of more than 30 autophagy-related proteins (Atgs) is involved (Nakatogawa et al. 2009). These factors guide the selection and targeting of the cargo and control the growth of the autophagosome. Then, this double membrane vesicle fuses with lysosomes for degradation of its contents, releasing the recycled metabolites back into the cytoplasm. The PI3K/Akt/mTOR axis and the adenosine monophosphate-activated (AMP) kinase are the two major signaling pathways that lead to induction of autophagy. While mTOR is a serine/threonine protein kinase whose activity is inhibited by nutrient starvation, AMP kinase is activated by an increase in the intracellular AMP/ATP ratio as a consequence of energy deficit (Cuervo and Wong 2014).

The cross-talk between apoptosis and autophagy is complex, and sometimes contradictory, but critical to the overall cell fate. The role of cannabinoids in autophagy is extensively studied and it was recently reviewed (Costa et al. 2016), thus, this section will provide a brief overview of the main evidences of cannabinoids-induced autophagy associated with cell death mechanisms.

The synthetic cannabinoid WIN55,212-2 induced G2/M cell cycle arrest and programmed cell death in human osteosarcoma MG63 and Saos-2 cells (Notaro et al. 2014). Similar effects were observed in colon cancer cell lines (Pellerito et al. 2014). Both these effects were accompanied by ER stress and conversion of the cytosolic form of the autophagosome marker LC3-I into LC3-II. Furthermore, the

Δ9-THC and the CB2 agonist JWH-015 also stimulated autophagy on hepatocellular carcinoma cell lines through two different pathways (Vara et al. 2013). The induction of ER stress requires the upregulation of the pseudo-kinase tribbles homolog 3 (TRIB3) and subsequent inhibition of Akt/mammalian target of rapamycin C 1 (Akt/mTORC1) axis, or the activation of AMPk via calmodulin-activated kinase kinase (CaMKKβ) (Vara et al. 2013). In glioma cells, Δ9-THC, through CB1 receptor, stimulates ceramide de novo synthesis activating an early ER stress response that leads to eIF2α phosphorylation and, then, induction of ER stress promoting autophagy via TRIB3-dependent inhibition of Akt/mTORC1 axis (Salazar et al. 2009). Also the combination of Δ9-THC with classical chemotherapeutic drugs enhanced autophagy in glioma xenografts supporting the use of combined therapies (Lonardi et al. 2005; Torres et al. 2011).

The phytocannabinoid CBD, the synthetic cannabinoid arachidonyl-2′-chloroethylamide (ACEA) and AEA induced autophagy in a dose-dependent manner in fully differentiated Caco-2 cells, a model of mature intestinal epithelium (Koay et al. 2014). Whereas ACEA and AEA promoted canonical autophagy, which was a CB1-mediated effect, CBD was able to bypass the CB1 receptor and the canonical pathway to induce autophagy, albeit to a lesser extent. The CBD induced-apoptosis of MDA-MB-231 breast cancer cells was also associated to ER stress, with subsequent inhibition of Akt and mTOR signalling, as demonstrated by decreased levels of cyclin D1 and phosphorylated mTOR and 4EBP1 (Shrivastava et al. 2011). The authors also showed a cross-talk between autophagy and apoptosis, though these effects were independent of cannabinoid or TRPV1 receptors (Shrivastava et al. 2011).

3 Cannabinoids as Anticancer Agents

In addition to the well-known palliative effects on some cancer-associated symptoms, the ability of cannabinoids to limit cell proliferation and to induce tumour-selective cell death may offer a promising strategy in cancer treatment. The antitumour effects of cannabinoids were firstly shown by Munson et al. (1975), though the ability of cannabinoids to regulate the cellular signalling pathways critical for cell growth and survival only advanced after the characterization of cannabinoid receptors.

In addition to the above-described cell death promoting effects, cannabinoids as anticancer agents may also involve restriction of cell growth and regulation of extracellular proteases activity and respective inhibitors, preventing, in this way, the invasiveness of different types of cancer cells (Blazquez et al. 2008; Ramer and Hinz 2008). Moreover, cannabinoids have also been shown to have antiangiogenic effects by down-regulating the active forms of VEGF receptors (VEGFR1 and VEGFR2), induce apoptosis of vascular endothelial cells, and constrain migration and proliferation of these cells (Casanova et al. 2003). As most of the information of cannabinoids effects in cancer is derived from pre-clinical studies, next section will

only discuss recent findings from clinical studies, which are mainly based in cannabinoid-licensed pharmaceuticals as well as from studies with cannabis.

3.1 Rationale and Possible Clinical Applications

Cannabinoid-derived medicines have been on the market for more than 30 years. Dronabinol or synthetic THC is sold as an oral medication under the brand name Marinol®. Dronabinol is well tolerated and effective for treating chemotherapy-induced nausea and vomiting and their clinical efficacy was recently reviewed (May and Glode 2016). Also nabilone (Cesamet®), a synthetic cannabinoid, is approved in the United States and other countries for the prevention of chemotherapy-induced nausea and vomiting in cancer patients. Sativex®, an oromucosal spray containing Δ9-THC and CBD as well as minor cannabinoids, was later approved for multiple sclerosis spasticity in 28 countries. Clinical trials showed Sativex® to be efficacious for pain relief in patients with advanced cancer (Johnson et al. 2010). Currently, in Canada, it is additionally approved as an adjuvant analgesic for cancer pain.

Interestingly, Δ9-THC or cannabis extract was unsuccessful for treating patients with cancer-related anorexia-cachexia syndrome in a clinical trial phase III (Strasser et al. 2006). However, in Australia, a new clinical trial (ACTRN12616001036404) is enrolling participants to study the ability of oral THC/CBD cannabis extract to control emesis and nausea in patients receiving chemotherapy.

Thus, despite the emergence of preclinical data, the use of cannabis-based medicines in the clinical practice is restricted to palliative use and the clinical studies evaluating cannabinoid efficacy for treating cancer are limited (Table 1). In 2006, a pilot clinical study indicated that Δ9-THC administered intratumourally to patients with glioblastoma that had previously failed standard therapy was effective and safe (Guzman et al. 2006). Notably, the Δ9-THC anticancer action in vivo also implicated the molecular mechanisms observed in vitro, namely stimulation of autophagy and apoptosis, inhibition of cell proliferation, decreased VEGF signalling and extracellular proteases activity (Guzman et al. 2006). Clinical trials are also evaluating the use of CBD alone (NCT02255292) and dexanabinol (NCT01489826; NCT01654497), a synthetic cannabinoid, in patients with advanced tumours, though no data has been released yet. Also the effects of smoked cannabis (CBD:Δ9-THC) in pain and inflammation are being evaluated in lung cancer patients (NCT02675842).

Combined therapies also demonstrated to inhibit tumour growth with auspicious results. The combined treatment of Δ9-THC with classical chemotherapeutic drugs, such as the alkylating agent temozolomide (TMZ; Temodar®), produces a strong anticancer action in xenografts generated with glioma cells (Lonardi et al. 2005), even in TMZ-resistant tumours (Torres et al. 2011). More recently, it was observed that cannabinoid agonists acted synergistically with gemcitabine in pancreatic cancer cells (Donadelli et al. 2011) and increased the antineoplastic activity of paclitaxel (Miyato et al. 2009) and of 5-fluorouracil (Gustafsson et al. 2009) to reduce cell viability in gastric and colorectal carcinoma cells, respectively. This

Table 1 Clinical studies of cannabinoids in cancer treatment

Identifier	Phase	Condition	Intervention	Study design	Results
Pilot study	I	Glioblastoma	Δ9-THC	Nine patients with glioblastoma multiforme were enrolled after standard therapy failure, including surgery and radiotherapy	Δ9-THC administration was safe though the effects on patient survival were unclear (Guzman et al. 2006)
NCT02432612	I	Advanced cancer	Sativex®	An open-label trial to assess the pharmacokinetic properties and tolerability of Sativex®	This study has been withdrawn prior to enrollment
NCT01812603	I, II	Glioblastoma	Sativex®	An open-label trial with a single group assignment to assess tolerability, safety and pharmacodynamics of Sativex® in combination with dose-intense temozolomide in glioblastoma patients	This study has been completed but no results were published yet
NCT01812616	I, II	Glioblastoma	Sativex®	Double-blind, placebo-controlled study to investigate tolerability, safety and pharmacodynamics of Sativex® in combination with dose-intense temozolomide in glioblastoma patients	This study has been completed but no results were published yet
NCT01489826	I	Solid tumours	Dexanabinol	An open-label trial to assess safety and pharmacokinetics of dexanabinol	This study has been completed but no results were published yet
NCT01654497	I	Brain cancer	Dexanabinol	An open-label trial to assess safety and CNS pharmacokinetics of dexanabinol	In progress
NCT02675842	I	Lung cancer	Smoked cannabis CBD:Δ9-THC	Double-blind, placebo-controlled study to investigate the efficacy of cannabis in participants undergoing radiation therapy for lung cancer	In progress
NCT02423239	I	Hepatocellular and pancreatic cancer	Dexanabinol	An open-label trial to assess safety and efficacy of dexanabinol in combination with standard chemotherapies	In progress

(continued)

Table 1 (continued)

Identifier	Phase	Condition	Intervention	Study design	Results
ACTRN12616001036404	II, III	Any cancer	Δ9-THC: CBD	Double-blind, placebo-controlled study to investigate the efficacy of cannabis extract in chemotherapy induced nausea and vomiting	In progress

Guzman M, Duarte MJ, Blazquez C, Ravina J, Rosa MC, Galve-Roperh I, Sanchez C, Velasco G, Gonzalez-Feria L (2006) A pilot clinical study of Delta9-tetrahydrocannabinol in patients with recurrent glioblastoma multiforme. British journal of cancer 95 (2):197–203. doi:10.1038/sj.bjc.6603236

further supports the use of combined therapies of cannabinoids with the classical chemotherapeutic agents. The safety and efficacy of Sativex® (NCT01812603; NCT01812616) and of dexanabinol (NCT02423239) in combination with standard chemotherapies in patients with recurrent glioblastoma and hepatocellular or pancreatic cancer is being evaluated and may provide important information about adverse effects, disease progression and overall survival in cannabinoid-combined therapies.

Considering all the available evidences, cannabinoids-related drugs present reasonable safety profile, especially with respect to toxic adverse effects associated to current chemotherapeutics, though the use of cannabinoids for medicinal purposes is largely limited due to their psychotropic effects. However, non-psychotropic compounds and combined therapy of cannabinoids with classical cytotoxic drugs may enhance the efficacy of current cancer treatments with lower side effects.

The main evidences about the potential therapeutics of cannabinoids still result from preclinical data, thus, new clinical studies may help to determine the benefits from cannabinoids use alone or in combination in cancer, for other than their palliative effects.

3.2 Perspectives, Obstacles and Future Directions

The current data points to cannabinoids as important molecules modulating various physiological processes, including the restriction of cancer cells growth, supporting the therapeutic actions of cannabinoids and specifically their potential as anticancer agents. Although many drugs used today can cause addiction and are misused and abused, the social concerns of cannabinoid medicines persist, particularly after the CB1 antagonist rimonabant, used for weight loss, induced serious neuropsychiatric effects (Di Marzo and Despres 2009). Nevertheless, clinical evidences of cannabinoids benefits are accumulating and cannabinoid derived drugs are available, in some countries for specific conditions.

Despite such promising pre-clinical studies, an emerging strategy to block tumour growth in vitro is the increase in eCBs levels through manipulation of the endocannabinoid system. This strategy was efficiently used in colorectal cancer cells and in thyroid carcinoma cells by preventing eCBs cellular reuptake with VDM-11 or by inhibiting enzymatic degradation with arachidonoyl-serotonin (AA-5-HT) (Ligresti et al. 2003; Bifulco et al. 2004; Izzo et al. 2008). Also the inhibition of FAAH, by the entourage compound palmitoylethanolamide (PEA), enhanced the anti-proliferative effects of AEA on human breast cancer cells (Di Marzo et al. 2001). More recently, it was observed that pharmacological inhibition of 2-AG degradation with URB602, a MAGL inhibitor, also attenuates the experimental colon carcinogenesis (Pagano et al. 2017). Although promising, the effective manipulation of endocannabinoid system to enhance eCBs tone requires development of more selective molecules and further in vitro and in vivo

assays. Additionally, and besides the difficulties targeting such inhibitors in vivo, it is also important for the evaluation of short- and long-term toxicities resulting from the inhibition of eCBs degradation. The unanticipated severe adverse events of an experimental FAAH inhibitor, BIA 10-2474, used in a phase 1 trial show the importance of more studies in this class of compounds (Kerbrat et al. 2016). Although the underlying mechanism of this toxic cerebral syndrome remains unknown, it is now clear that alternative eCBs-metabolic pathways are exacerbated when primarily routes are inhibited, giving rise to several compounds, that may have a harmful effect (Almada et al. 2015). Thus, the oxidative metabolism of eCBs potential/complications is demanding further investigation. Likewise, the eCBs precursors are also intermediates for synthesis of other mediators and, thus, their levels may be affected as a result of manipulating the main eCBs metabolic pathway.

Also the influence of medicinal cannabis on the pharmacokinetics of common anticancer agents must be evaluated. Although a clinical study observed that coadministration of medicinal cannabis, as herbal tea, in cancer patients treated with irinotecan or docetaxel does not significantly influence the plasma pharmacokinetics of these drugs (Engels et al. 2007), there is still a major lack of long-term pharmacokinetic data and information about the interactions of cannabis-derived drugs with anticancer agents.

In cannabinoid-resistant glioma cells, midkine (MDK), which encodes a secreted heparin-binding growth factor, played a direct role in the resistance to Δ9-THC action through stimulation of anaplastic lymphoma kinase (ALK) (Lorente et al. 2011). Similarly, increased amphiregulin expression was associated with increased ERK activation, which mediated the resistance to Δ9-THC by blunting the expression of p8 and trb3-two genes involved in cannabinoid-induced apoptosis of glioma cells (Lorente et al. 2009). Pharmacologic inhibition of ALK or silencing of either MDK or amphiregulin rendered the resistant tumour xenografts sensitive to cannabinoid antitumoural action (Lorente et al. 2009, 2011). Nevertheless, TMZ-resistant tumours benefit from the combined treatment of Δ9-THC with the chemotherapeutic TMZ (Torres et al. 2011). Recently, Velasco et al. (2016) reviewed the molecular mechanisms of cannabinoids as antitumour agents, particularly the resistance mechanisms and opportunities for their use in combination therapy. These strategies that enhanced cannabinoid action in resistant tumours provide a rationale for combination therapies capable of increasing cannabinoid antineoplastic activity.

Likewise, other potentially interesting strategies to enhance cannabinoid anticancer action could be the combination of cannabinoids with ER stress or autophagy inducers (or both) or with inhibitors of the mTORC1 axis. This strategy has not been attempted with cannabinoid but previous studies observed that in some circumstances, classic anticancer drugs may benefit from combination with ER stress inducers and autophagy inhibitors (Xu et al. 2014; Sui et al. 2013).

4 Concluding Remarks

The last years were rich in the expansion of scientific knowledge regarding endocannabinoid system, particularly its biochemical, physiological and pharmacological effects. Recent investigations have shown that besides its well-known anti-inflammatory, analgesic and antiemetic properties, cannabinoids may also induce cell death and anti-proliferative effects by receptor independent or dependent pathways. Thus, our knowledge about cannabinoid properties encompasses the identification of endogenous ligands and their entire metabolism as well as the signalling pathways through which cannabinoids exert their action.

Besides the emergence of medicinal cannabis use as anticancer agents in several countries, the manipulation of endocannabinoid system may constitute an appellative target, contributing to the development of new drugs. Although contradictory results have been shown, CB1 and CB2 are upregulated in various cancer cells. In human biopsies from astrocytoma and glioblastoma, upregulation of CB2 was directly related to tumour malignancy (Sanchez et al. 2001a; Ellert-Miklaszewska et al. 2007). Furthermore, as observed in colorectal (Ligresti et al. 2003) and endometrial cancer (Guida et al. 2010), eCBs levels are often more abundant than in non-tumoural tissues, which may indicate a compensatory mechanism to counteract tumour cell proliferation.

Current evidences indicate that the modulation of several components of signal transduction pathways, including ceramide biosynthesis, MAPKs and Akt/mTORC1 axis by cannabinoids might support important effects on cell homeostasis control. Moreover, the recent insights into the molecular mechanisms of cannabinoids action and resistance increased our understanding about their potential as anticancer drugs and highlighted combined therapy approaches.

Various clinical assays are underway and, besides the clinical outcome, they may provide important information about cannabinoids tolerability, safety and adverse effects in cancer treatment. Moreover, with a well-defined endocannabinoid system manipulation and development of selective drugs acting directly on the components of this system, more clinical studies to clarify cannabinoids efficacy will be expected.

Conflict of Interest Statement

The authors declare no conflicts of interest in preparing this article.

References

Almada M, Piscitelli F, Fonseca BM, Di Marzo V, Correia-da-Silva G, Teixeira N (2015) Anandamide and decidual remodelling: COX-2 oxidative metabolism as a key regulator. Biochim Biophys Acta 1851(11):1473–1481. doi:10.1016/j.bbalip.2015.08.011

Bifulco M, Laezza C, Valenti M, Ligresti A, Portella G, Dim V (2004) A new strategy to block tumor growth by inhibiting endocannabinoid inactivation. FASEB J 18(13):1606–1608. doi:10.1096/fj.04-1754fje

Bisogno T, Howell F, Williams G, Minassi A, Cascio MG, Ligresti A, Matias I, Schiano-Moriello-A, Paul P, Williams EJ, Gangadharan U, Hobbs C, Di Marzo V, Doherty P (2003) Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. J Cell Biol 163(3):463–468. doi:10.1083/jcb.200305129

Blazquez C, Galve-Roperh I, Guzman M (2000) De novo-synthesized ceramide signals apoptosis in astrocytes via extracellular signal-regulated kinase. FASEB J 14(14):2315–2322. doi:10.1096/fj.00-0122com

Blazquez C, Salazar M, Carracedo A, Lorente M, Egia A, Gonzalez-Feria L, Haro A, Velasco G, Guzman M (2008) Cannabinoids inhibit glioma cell invasion by down-regulating matrix metalloproteinase-2 expression. Cancer Res 68(6):1945–1952. doi:10.1158/0008-5472.CAN-07-5176

Bouaboula M, Poinot-Chazel C, Bourrie B, Canat X, Calandra B, Rinaldi-Carmona M, Le Fur G, Casellas P (1995) Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB1. Biochem J 312(Pt 2):637–641

Caffarel MM, Sarrio D, Palacios J, Guzman M, Sanchez C (2006) Delta9-tetrahydrocannabinol inhibits cell cycle progression in human breast cancer cells through Cdc2 regulation. Cancer Res 66(13):6615–6621. doi:10.1158/0008-5472.CAN-05-4566

Caffarel MM, Moreno-Bueno G, Cerutti C, Palacios J, Guzman M, Mechta-Grigoriou F, Sanchez C (2008) JunD is involved in the antiproliferative effect of Delta9-tetrahydrocannabinol on human breast cancer cells. Oncogene 27(37):5033–5044. doi:10.1038/onc.2008.145

Carracedo A, Gironella M, Lorente M, Garcia S, Guzman M, Velasco G, Iovanna JL (2006) Cannabinoids induce apoptosis of pancreatic tumor cells via endoplasmic reticulum stress-related genes. Cancer Res 66(13):6748–6755. doi:10.1158/0008-5472.CAN-06-0169

Casanova ML, Blazquez C, Martinez-Palacio J, Villanueva C, Fernandez-Acenero MJ, Huffman JW, Jorcano JL, Guzman M (2003) Inhibition of skin tumor growth and angiogenesis in vivo by activation of cannabinoid receptors. J Clin Invest 111(1):43–50. doi:10.1172/JCI16116

Chemin J, Monteil A, Perez-Reyes E, Nargeot J, Lory P (2001) Direct inhibition of T-type calcium channels by the endogenous cannabinoid anandamide. EMBO J 20(24):7033–7040. doi:10.1093/emboj/20.24.7033

Cianchi F, Papucci L, Schiavone N, Lulli M, Magnelli L, Vinci MC, Messerini L, Manera C, Ronconi E, Romagnani P, Donnini M, Perigli G, Trallori G, Tanganelli E, Capaccioli S, Masini E (2008) Cannabinoid receptor activation induces apoptosis through tumor necrosis factor alpha – mediated ceramide De novo synthesis in colon cancer cells. Clin Cancer Res 14(23):7691–7700. doi:10.1158/1078-0432.CCR-08-0799

Contassot E, Tenan M, Schnuriger V, Pelte MF, Dietrich PY (2004) Arachidonyl ethanolamide induces apoptosis of uterine cervix cancer cells via aberrantly expressed vanilloid receptor-1. Gynecol Oncol 93(1):182–188. doi:10.1016/j.ygyno.2003.12.040

Costa MA, Fonseca BM, Keating E, Teixeira NA, Correia-da-Silva G (2014a) 2-Arachidonoylglycerol effects in cytotrophoblasts: metabolic enzymes expression and apoptosis in BeWo cells. Reproduction 147(3):301–311. doi:10.1530/REP-13-0563

Costa MA, Fonseca BM, Keating E, Teixeira NA, Correia-Da-Silva G (2014b) Transient receptor potential vanilloid 1 is expressed in human cytotrophoblasts: induction of cell apoptosis and impairment of syncytialization. Int J Biochem Cell Biol 57:177–185. doi:10.1016/j.biocel.2014.10.008

Costa MA, Fonseca BM, Teixeira NA, Correia-da-Silva G (2015a) The endocannabinoid anandamide induces apoptosis in cytotrophoblast cells: involvement of both mitochondrial and death receptor pathways. Placenta 36(1):69–76. doi:10.1016/j.placenta.2014.10.011

Costa MA, Keating E, Fonseca BM, Teixeira NA, Correia-da-Silva G (2015b) 2-Arachidonoylglycerol impairs human cytotrophoblast cells syncytialization: influence of endocannabinoid signalling in placental development. Mol Cell Endocrinol 399:386–394. doi:10.1016/j.mce.2014.09.005

Costa L, Amaral C, Teixeira N, Correia-da-Silva G, Fonseca BM (2016) Cannabinoid-induced autophagy: protective or death role? Prostaglandins Other Lipid Mediators 122:54–63. doi:10.1016/j.prostaglandins.2015.12.006

Cuervo AM, Wong E (2014) Chaperone-mediated autophagy: roles in disease and aging. Cell Res 24(1):92–104. doi:10.1038/cr.2013.153

De Petrocellis L, Melck D, Palmisano A, Bisogno T, Laezza C, Bifulco M, Di Marzo V (1998) The endogenous cannabinoid anandamide inhibits human breast cancer cell proliferation. Proc Natl Acad Sci U S A 95(14):8375–8380

De Petrocellis L, Ligresti A, Schiano Moriello A, Iappelli M, Verde R, Stott CG, Cristino L, Orlando P, Di Marzo V (2013) Non-THC cannabinoids inhibit prostate carcinoma growth in vitro and in vivo: pro-apoptotic effects and underlying mechanisms. Br J Pharmacol 168 (1):79–102. doi:10.1111/j.1476-5381.2012.02027.x

del Pulgar T, Velasco G, Guzman M (2000) The CB1 cannabinoid receptor is coupled to the activation of protein kinase B/Akt. Biochem J 347:369–373

DeMorrow S, Glaser S, Francis H, Venter J, Vaculin B, Vaculin S, Alpini G (2007) Opposing actions of endocannabinoids on cholangiocarcinoma growth: recruitment of Fas and Fas ligand to lipid rafts. J Biol Chem 282(17):13098–13113. doi:10.1074/jbc.M608238200

Derkinderen P, Toutant M, Burgaya F, Le Bert M, Siciliano JC, de Franciscis V, Gelman M, Girault JA (1996) Regulation of a neuronal form of focal adhesion kinase by anandamide. Science 273(5282):1719–1722

Deutsch DG, Chin SA (1993) Enzymatic synthesis and degradation of anandamide, a cannabinoid receptor agonist. Biochem Pharmacol 46(5):791–796

Devane WA, Dysarz FA III, Johnson MR, Melvin LS, Howlett AC (1988) Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol 34(5):605–613

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258(5090):1946–1949. doi:10.1126/science.1470919

Di Marzo V, Despres JP (2009) CB1 antagonists for obesity – what lessons have we learned from rimonabant? Nat Rev Endocrinol 5(11):633–638. doi:10.1038/nrendo.2009.197

Di Marzo V, Melck D, Orlando P, Bisogno T, Zagoory O, Bifulco M, Vogel Z, De Petrocellis L (2001) Palmitoylethanolamide inhibits the expression of fatty acid amide hydrolase and enhances the anti-proliferative effect of anandamide in human breast cancer cells. Biochem J 358(Pt 1):249–255

Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, Kathuria S, Piomelli D (2002) Brain monoglyceride lipase participating in endocannabinoid inactivation. Proc Natl Acad Sci U S A 99(16):10819–10824. doi:10.1073/pnas.152334899

Do Y, McKallip RJ, Nagarkatti M, Nagarkatti PS (2004) Activation through cannabinoid receptors 1 and 2 on dendritic cells triggers NF-kappaB-dependent apoptosis: novel role for endogenous and exogenous cannabinoids in immunoregulation. J Immunol 173(4):2373–2382

Donadelli M, Dando I, Zaniboni T, Costanzo C, Dalla Pozza E, Scupoli MT, Scarpa A, Zappavigna S, Marra M, Abbruzzese A, Bifulco M, Caraglia M, Palmieri M (2011) Gemcitabine/cannabinoid combination triggers autophagy in pancreatic cancer cells through a ROS-mediated mechanism. Cell Death Dis 2:e152. doi:10.1038/cddis.2011.36

Eldeeb K, Leone-Kabler S, Howlett AC (2016) CB1 cannabinoid receptor-mediated increases in cyclic AMP accumulation are correlated with reduced Gi/o function. J Basic Clin Physiol Pharmacol 27(3):311–322. doi:10.1515/jbcpp-2015-0096

Ellert-Miklaszewska A, Grajkowska W, Gabrusiewicz K, Kaminska B, Konarska L (2007) Distinctive pattern of cannabinoid receptor type II (CB2) expression in adult and pediatric brain tumors. Brain Res 1137(1):161–169. doi:10.1016/j.brainres.2006.12.060

Engels FK, de Jong FA, Sparreboom A, Mathot RAA, Loos WJ, Kitzen JJEM, de Bruijn P, Verweij J, Mathijssen RHJ (2007) Medicinal cannabis does not influence the clinical pharmacokinetics of irinotecan and docetaxel. Oncologist 12(3):291–300. doi:10.1634/theoncologist.12-3-291

Felder CC, Joyce KE, Briley EM, Mansouri J, Mackie K, Blond O, Lai Y, Ma AL, Mitchell RL (1995) Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. Mol Pharmacol 48(3):443–450

Fonseca BM, Correia-da-Silva G, Taylor AH, Konje JC, Bell SC, Teixeira NA (2009a) Spatio-temporal expression patterns of anandamide-binding receptors in rat implantation sites: evidence for a role of the endocannabinoid system during the period of placental development. Reprod Biol Endocrinol 7:121. doi:10.1186/1477-7827-7-121

Fonseca BM, Correia-da-Silva G, Teixeira NA (2009b) Anandamide-induced cell death: dual effects in primary rat decidual cell cultures. Placenta 30(8):686–692. doi:10.1016/j.placenta.2009.05.012

Fonseca BM, Correia-da-Silva G, Taylor AH, Lam PM, Marczylo TH, Bell SC, Konje JC, Teixeira NA (2010) The endocannabinoid 2-arachidonoylglycerol (2-AG) and metabolizing enzymes during rat fetoplacental development: a role in uterine remodelling. Int J Biochem Cell Biol 42(11):1884–1892. doi:10.1016/j.biocel.2010.08.006

Fonseca BM, Correia-da-Silva G, Teixeira NA (2013) The endocannabinoid anandamide induces apoptosis of rat decidual cells through a mechanism involving ceramide synthesis and p38 MAPK activation. Apoptosis 18(12):1526–1535. doi:10.1007/s10495-013-0892-9

Gabrielli M, Battista N, Riganti L, Prada I, Antonucci F, Cantone L, Matteoli M, Maccarrone M, Verderio C (2015) Active endocannabinoids are secreted on extracellular membrane vesicles. EMBO Rep 16(2):213–220. doi:10.15252/embr.201439668

Galve-Roperh I, Sanchez C, Cortes ML, Gomez del Pulgar T, Izquierdo M, Guzman M (2000) Anti-tumoral action of cannabinoids: involvement of sustained ceramide accumulation and extracellular signal-regulated kinase activation. Nat Med 6(3):313–319. doi:10.1038/73171

Gaoni Y, Mechoulam R (1964) Isolation, structure, and partial synthesis of an active constituent of hashish. J Am Chem Soc 86(8):1646–1647. doi:10.1021/Ja01062a046

Gomez Del Pulgar T, De Ceballos ML, Guzman M, Velasco G (2002) Cannabinoids protect astrocytes from ceramide-induced apoptosis through the phosphatidylinositol 3-kinase/protein kinase B pathway. J Biol Chem 277(39):36527–36533. doi:10.1074/jbc.M205797200

Gomez R, Conde J, Scotece M, Lopez V, Lago F, Gomez Reino JJ, Gualillo O (2014) Endogenous cannabinoid anandamide impairs cell growth and induces apoptosis in chondrocytes. J Orthop Res 32(9):1137–1146. doi:10.1002/jor.22660

Greenhough A, Patsos HA, Williams AC, Paraskeva C (2007) The cannabinoid delta(9)-tetrahydrocannabinol inhibits RAS-MAPK and PI3K-AKT survival signalling and induces BAD-mediated apoptosis in colorectal cancer cells. Int J Cancer 121(10):2172–2180. doi:10.1002/ijc.22917

Guida M, Ligresti A, De Filippis D, D'Amico A, Petrosino S, Cipriano M, Bifulco G, Simonetti S, Orlando P, Insabato L, Nappi C, Di Spiezio SA, Di Marzo V, Iuvone T (2010) The levels of the endocannabinoid receptor CB2 and its ligand 2-arachidonoylglycerol are elevated in endometrial carcinoma. Endocrinology 151(3):921–928. doi:10.1210/en.2009-0883

Gustafsson K, Christensson B, Sander B, Flygare J (2006) Cannabinoid receptor-mediated apoptosis induced by R(+)-methanandamide and Win55,212-2 is associated with ceramide accumulation and p38 activation in mantle cell lymphoma. Mol Pharmacol 70(5):1612–1620. doi:10.1124/mol.106.025981

Gustafsson SB, Lindgren T, Jonsson M, Jacobsson SO (2009) Cannabinoid receptor-independent cytotoxic effects of cannabinoids in human colorectal carcinoma cells: synergism with 5-fluorouracil. Cancer Chemother Pharmacol 63(4):691–701. doi:10.1007/s00280-008-0788-5

Gustafsson SB, Wallenius A, Zackrisson H, Popova D, Forshell LP, Jacobsson SOP (2013) Effects of cannabinoids and related fatty acids upon the viability of P19 embryonal carcinoma cells. Arch Toxicol 87(11):1939–1951. doi:10.1007/s00204-013-1051-3

Guzman M, Duarte MJ, Blazquez C, Ravina J, Rosa MC, Galve-Roperh I, Sanchez C, Velasco G, Gonzalez-Feria L (2006) A pilot clinical study of Delta9-tetrahydrocannabinol in patients with recurrent glioblastoma multiforme. Br J Cancer 95(2):197–203. doi:10.1038/sj.bjc.6603236

Herrera B, Carracedo A, Diez-Zaera M, Gomez del Pulgar T, Guzman M, Velasco G (2006) The CB2 cannabinoid receptor signals apoptosis via ceramide-dependent activation of the mitochondrial intrinsic pathway. Exp Cell Res 312(11):2121–2131. doi:10.1016/j.yexcr.2006.03.009

Higgs HN, Glomset JA (1994) Identification of a phosphatidic acid-preferring phospholipase A1 from bovine brain and testis. Proc Natl Acad Sci U S A 91(20):9574–9578

Hinz B, Ramer R, Eichele K, Weinzierl U, Brune K (2004a) R(+)-methanandamide-induced cyclooxygenase-2 expression in H4 human neuroglioma cells: possible involvement of membrane lipid rafts. Biochem Biophys Res Commun 324(2):621–626. doi:10.1016/j.bbrc.2004.09.095

Hinz B, Ramer R, Eichele K, Weinzierl U, Brune K (2004b) Up-regulation of cyclooxygenase-2 expression is involved in R(+)-methanandamide-induced apoptotic death of human neuroglioma cells. Mol Pharmacol 66(6):1643–1651. doi:10.1124/mol.104.002618

Hong Y, Zhou Y, Wang Y, Xiao S, Liao DJ, Zhao Q (2013) PPARgamma mediates the effects of WIN55,212-2, an synthetic cannabinoid, on the proliferation and apoptosis of the BEL-7402 hepatocarcinoma cells. Mol Biol Rep 40(11):6287–6293. doi:10.1007/s11033-013-2741-x

Hsu SS, Huang CJ, Cheng HH, Chou CT, Lee HY, Wang JL, Chen IS, Liu SI, Lu YC, Chang HT, Huang JK, Chen JS, Jan CR (2007) Anandamide-induced Ca2+ elevation leading to p38 MAPK phosphorylation and subsequent cell death via apoptosis in human osteosarcoma cells. Toxicology 231(1):21–29. doi:10.1016/j.tox.2006.11.005

Izzo AA, Aviello G, Petrosino S, Orlando P, Marsicano G, Lutz B, Borrelli F, Capasso R, Nigam S, Capasso F, Di Marzo V, Endocannabinoid Research G (2008) Increased endocannabinoid levels reduce the development of precancerous lesions in the mouse colon. J Mol Med 86 (1):89–98. doi:10.1007/s00109-007-0248-4

Jia W, Hegde VL, Singh NP, Sisco D, Grant S, Nagarkatti M, Nagarkatti PS (2006) Delta9-tetrahydrocannabinol-induced apoptosis in Jurkat leukemia T cells is regulated by translocation of bad to mitochondria. Mol Cancer Res 4(8):549–562. doi:10.1158/1541-7786.MCR-05-0193

Johnson JR, Burnell-Nugent M, Lossignol D, Ganae-Motan ED, Potts R, Fallon MT (2010) Multicenter, double-blind, randomized, placebo-controlled, parallel-group study of the efficacy, safety, and tolerability of THC:CBD extract and THC extract in patients with intractable cancer-related pain. J Pain Symptom Manag 39(2):167–179. doi:10.1016/j.jpainsymman.2009.06.008

Kano M (2014) Control of synaptic function by endocannabinoid-mediated retrograde signaling. Proc Jpn Acad Ser B Phys Biol Sci 90(7):235–250

Kaur J, Debnath J (2015) Autophagy at the crossroads of catabolism and anabolism. Nat Rev Mol Cell Biol 16(8):461–472. doi:10.1038/nrm4024

Kerbrat A, Ferre JC, Fillatre P, Ronziere T, Vannier S, Carsin-Nicol B, Lavoue S, Verin M, Gauvrit JY, Le Tulzo Y, Edan G (2016) Acute neurologic disorder from an inhibitor of fatty acid amide hydrolase. N Engl J Med 375(18):1717–1725. doi:10.1056/NEJMoa1604221

Kim SR, Lee DY, Chung ES, Oh UT, Kim SU, Jin BK (2005) Transient receptor potential vanilloid subtype 1 mediates cell death of mesencephalic dopaminergic neurons in vivo and in vitro. J Neurosci 25(3):662–671. doi:10.1523/JNEUROSCI.4166-04.2005

Koay LC, Rigby RJ, Wright KL (2014) Cannabinoid-induced autophagy regulates suppressor of cytokine signaling-3 in intestinal epithelium. Am J Physiol Gastrointest Liver Physiol 307(2): G140–G148. doi:10.1152/ajpgi.00317.2013

Laezza C, Pisanti S, Malfitano AM, Bifulco M (2008) The anandamide analog, Met-F-AEA, controls human breast cancer cell migration via the RHOA/RHO kinase signaling pathway. Endocr Relat Cancer 15(4):965–974. doi:10.1677/ERC-08-0030

Lauckner JE, Hille B, Mackie K (2005) The cannabinoid agonist WIN55,212-2 increases intracellular calcium via CB1 receptor coupling to Gq/11 G proteins. Proc Natl Acad Sci U S A 102 (52):19144–19149. doi:10.1073/pnas.0509588102

Ligresti A, Bisogno T, Matias I, De Petrocellis L, Cascio MG, Cosenza V, D'Argenio G, Scaglione G, Bifulco M, Sorrentini I, Di Marzo V (2003) Possible endocannabinoid control of colorectal cancer growth. Gastroenterology 125(3):677–687

Ligresti A, Moriello AS, Starowicz K, Matias I, Pisanti S, De Petrocellis L, Laezza C, Portella G, Bifulco M, Di Marzo V (2006) Antitumor activity of plant cannabinoids with emphasis on the effect of cannabidiol on human breast carcinoma. J Pharmacol Exp Ther 318(3):1375–1387. doi:10.1124/jpet.106.105247

Ligresti A, De Petrocellis L, Di Marzo V (2016) From phytocannabinoids to cannabinoid receptors and endocannabinoids: pleiotropic physiological and pathological roles through complex pharmacology. Physiol Rev 96(4):1593–1659. doi:10.1152/physrev.00002.2016

Lim MP, Devi LA, Rozenfeld R (2011) Cannabidiol causes activated hepatic stellate cell death through a mechanism of endoplasmic reticulum stress-induced apoptosis. Cell Death Dis 2: e170. doi:10.1038/cddis.2011.52

Liu J, Wang L, Harvey-White J, Osei-Hyiaman D, Razdan R, Gong Q, Chan AC, Zhou Z, Huang BX, Kim HY, Kunos G (2006) A biosynthetic pathway for anandamide. Proc Natl Acad Sci U S A 103(36):13345–13350. doi:10.1073/pnas.0601832103

Liu J, Wang L, Harvey-White J, Huang BX, Kim HY, Luquet S, Palmiter RD, Krystal G, Rai R, Mahadevan A, Razdan RK, Kunos G (2008) Multiple pathways involved in the biosynthesis of anandamide. Neuropharmacology 54(1):1–7. doi:10.1016/j.neuropharm.2007.05.020

Lograno MD, Romano MR (2004) Cannabinoid agonists induce contractile responses through G (i/o)-dependent activation of phospholipase C in the bovine ciliary muscle. Eur J Pharmacol 494(1):55–62. doi:10.1016/j.ejphar.2004.04.039

Lonardi S, Tosoni A, Brandes AA (2005) Adjuvant chemotherapy in the treatment of high grade gliomas. Cancer Treat Rev 31(2):79–89. doi:10.1016/j.ctrv.2004.12.005

Lorente M, Carracedo A, Torres S, Natali F, Egia A, Hernandez-Tiedra S, Salazar M, Blazquez C, Guzman M, Velasco G (2009) Amphiregulin is a factor for resistance of glioma cells to cannabinoid-induced apoptosis. Glia 57(13):1374–1385. doi:10.1002/glia.20856

Lorente M, Torres S, Salazar M, Carracedo A, Hernandez-Tiedra S, Rodriguez-Fornes F, Garcia-Taboada E, Melendez B, Mollejo M, Campos-Martin Y, Lakatosh SA, Barcia J, Guzman M, Velasco G (2011) Stimulation of the midkine/ALK axis renders glioma cells resistant to cannabinoid antitumoral action. Cell Death Differ 18(6):959–973. doi:10.1038/cdd.2010.170

Maccarrone M, Lorenzon T, Bari M, Melino G, Finazzi-Agro A (2000) Anandamide induces apoptosis in human cells via vanilloid receptors – evidence for a protective role of cannabinoid receptors. J Biol Chem 275(41):31938–31945. doi:10.1074/jbc.M005722200

Mackie K, Lai Y, Westenbroek R, Mitchell R (1995) Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in AtT20 cells transfected with rat brain cannabinoid receptor. J Neurosci 15(10):6552–6561

Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346(6284):561–564. doi:10.1038/346561a0

May MB, Glode AE (2016) Dronabinol for chemotherapy-induced nausea and vomiting unresponsive to antiemetics. Cancer Manag Res 8:49–55. doi:10.2147/CMAR.S81425

Mechoulam R, Benshabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR, Pertwee RG, Griffin G, Bayewitch M, Barg J, Vogel Z (1995) Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol 50(1):83–90. doi:10.1016/0006-2952(95)00109-D

Mechoulam R, Hanus LO, Pertwee R, Howlett AC (2014) Early phytocannabinoid chemistry to endocannabinoids and beyond. Nat Rev Neurosci 15(11):757–764. doi:10.1038/nrn3811

Melck D, Rueda D, Galve-Roperh I, De Petrocellis L, Guzman M, Di Marzo V (1999) Involvement of the cAMP/protein kinase A pathway and of mitogen-activated protein kinase in the

anti-proliferative effects of anandamide in human breast cancer cells. FEBS Lett 463 (3):235–240

Melck D, De Petrocellis L, Orlando P, Bisogno T, Laezza C, Bifulco M, Di Marzo V (2000) Suppression of nerve growth factor Trk receptors and prolactin receptors by endocannabinoids leads to inhibition of human breast and prostate cancer cell proliferation. Endocrinology 141 (1):118–126. doi:10.1210/endo.141.1.7239

Miyato H, Kitayama J, Yamashita H, Souma D, Asakage M, Yamada J, Nagawa H (2009) Pharmacological synergism between cannabinoids and paclitaxel in gastric cancer cell lines. J Surg Res 155(1):40–47. doi:10.1016/j.jss.2008.06.045

Movsesyan VA, Stoica BA, Yakovlev AG, Knoblach SM, Lea PM, Cernak I, Vink R, Faden AI (2004) Anandamide-induced cell death in primary neuronal cultures: role of calpain and caspase pathways. Cell Death Differ 11(10):1121–1132. doi:10.1038/sj.cdd.4401442

Muccioli GG (2010) Endocannabinoid biosynthesis and inactivation, from simple to complex. Drug Discov Today 15(11–12):474–483. doi:10.1016/j.drudis.2010.03.007

Munro S, Thomas KL, Abu-Shaar M (1993) Molecular characterization of a peripheral receptor for cannabinoids. Nature 365(6441):61–65. doi:10.1038/365061a0

Munson AE, Harris LS, Friedman MA, Dewey WL, Carchman RA (1975) Antineoplastic activity of cannabinoids. J Natl Cancer Inst 55(3):597–602

Nakatogawa H, Suzuki K, Kamada Y, Ohsumi Y (2009) Dynamics and diversity in autophagy mechanisms: lessons from yeast. Nat Rev Mol Cell Biol 10(7):458–467. doi:10.1038/nrm2708

Notaro A, Sabella S, Pellerito O, Di Fiore R, De Blasio A, Vento R, Calvaruso G, Giuliano M (2014) Involvement of PAR-4 in cannabinoid-dependent sensitization of osteosarcoma cells to TRAIL-induced apoptosis. Int J Biol Sci 10(5):466–478. doi:10.7150/ijbs.8337

O'Sullivan SE (2007) Cannabinoids go nuclear: evidence for activation of peroxisome proliferator-activated receptors. Br J Pharmacol 152(5):576–582. doi:10.1038/sj.bjp.0707423

Orellana-Serradell O, Poblete CE, Sanchez C, Castellon EA, Gallegos I, Huidobro C, Llanos MN, Contreras HR (2015) Proapoptotic effect of endocannabinoids in prostate cancer cells. Oncol Rep 33(4):1599–1608. doi:10.3892/or.2015.3746

Pagano E, Borrelli F, Orlando P, Romano B, Monti M, Morbidelli L, Aviello G, Imperatore R, Capasso R, Piscitelli F, Buono L, Di Marzo V, Izzo AA (2017) Pharmacological inhibition of MAGL attenuates experimental colon carcinogenesis. Pharmacol Res 119:227–236. doi:10.1016/j.phrs.2017.02.002

Pan X, Ikeda SR, Lewis DL (1996) Rat brain cannabinoid receptor modulates N-type Ca2+ channels in a neuronal expression system. Mol Pharmacol 49(4):707–714

Patsos HA, Greenhough A, Hicks DJ, Al Kharusi M, Collard TJ, Lane JD, Paraskeva C, Williams AC (2010) The endogenous cannabinoid, anandamide, induces COX-2-dependent cell death in apoptosis-resistant colon cancer cells. Int J Oncol 37(1):187–193. doi:10.3892/ijo_00000666

Pellerito O, Notaro A, Sabella S, De Blasio A, Vento R, Calvaruso G, Giuliano M (2014) WIN induces apoptotic cell death in human colon cancer cells through a block of autophagic flux dependent on PPAR gamma down-regulation. Apoptosis 19(6):1029–1042. doi:10.1007/s10495-014-0985-0

Pertwee RG (2000) Cannabinoid receptor ligands: clinical and neuropharmacological considerations, relevant to future drug discovery and development. Expert Opin Investig Drugs 9 (7):1553–1571. doi:10.1517/13543784.9.7.1553

Pozzoli G, Tringali G, Vairano M, D'Amico M, Navarra P, Martire M (2006) Cannabinoid agonist WIN55,212-2 induces apoptosis in cerebellar granule cells via activation of the CB1 receptor and downregulation of bcl-xL gene expression. J Neurosci Res 83(6):1058–1065. doi:10.1002/jnr.20794

Preet A, Qamri Z, Nasser MW, Prasad A, Shilo K, Zou X, Groopman JE, Ganju RK (2011) Cannabinoid receptors, CB1 and CB2, as novel targets for inhibition of non-small cell lung cancer growth and metastasis. Cancer Prev Res 4(1):65–75. doi:10.1158/1940-6207.CAPR-10-0181

Pucci M, Pasquariello N, Battista N, Di Tommaso M, Rapino C, Fezza F, Zuccolo M, Jourdain R, Agro AF, Breton L, Maccarrone M (2012) Endocannabinoids stimulate human melanogenesis

via type-1 cannabinoid receptor. J Biol Chem 287(19):15466–15478. doi:10.1074/jbc.M111.314880

Rajesh M, Mukhopadhyay P, Hasko G, Liaudet L, Mackie K, Pacher P (2010) Cannabinoid-1 receptor activation induces reactive oxygen species-dependent and -independent mitogen-activated protein kinase activation and cell death in human coronary artery endothelial cells. Br J Pharmacol 160(3):688–700. doi:10.1111/j.1476-5381.2010.00712.x

Ramer R, Hinz B (2008) Inhibition of cancer cell invasion by cannabinoids via increased expression of tissue inhibitor of matrix metalloproteinases-1. J Natl Cancer Inst 100 (1):59–69. doi:10.1093/jnci/djm268

Ramer R, Heinemann K, Merkord J, Rohde H, Salamon A, Linnebacher M, Hinz B (2013) COX-2 and PPAR-gamma confer cannabidiol-induced apoptosis of human lung cancer cells. Mol Cancer Ther 12(1):69–82. doi:10.1158/1535-7163.MCT-12-0335

Rockwell CE, Snider NT, Thompson JT, Heuvel JPV, Kaminski NE (2006) Interleukin-2 suppression by 2-arachidonyl glycerol is mediated through peroxisome proliferator-activated receptor gamma independently of cannabinoid receptors 1 and 2. Mol Pharmacol 70 (1):101–111. doi:10.1124/mol.105.019117

Rockwell CE, Raman P, Kaplan BLF, Kaminski NE (2008) A COX-2 metabolite of the endogenous cannabinoid, 2-arachidonyl glycerol, mediates suppression of IL-2 secretion in activated Jurkat T cells. Biochem Pharmacol 76(3):353–361. doi:10.1016/j.bcp.2008.05.005

Ruiz L, Miguel A, Diaz-Laviada I (1999) Delta9-tetrahydrocannabinol induces apoptosis in human prostate PC-3 cells via a receptor-independent mechanism. FEBS Lett 458(3):400–404

Salazar M, Carracedo A, Salanueva IJ, Hernandez-Tiedra S, Lorente M, Egia A, Vazquez P, Blazquez C, Torres S, Garcia S, Nowak J, Fimia GM, Piacentini M, Cecconi F, Pandolfi PP, Gonzalez-Feria L, Iovanna JL, Guzman M, Boya P, Velasco G (2009) Cannabinoid action induces autophagy-mediated cell death through stimulation of ER stress in human glioma cells. J Clin Invest 119(5):1359–1372

Sanchez C, Galve-Roperh I, Canova C, Brachet P, Guzman M (1998) Delta9-tetrahydrocannabinol induces apoptosis in C6 glioma cells. FEBS Lett 436(1):6–10

Sanchez C, de Ceballos ML, Gomez del Pulgar T, Rueda D, Corbacho C, Velasco G, Galve-Roperh I, Huffman JW, Ramon y Cajal S, Guzman M (2001a) Inhibition of glioma growth in vivo by selective activation of the CB(2) cannabinoid receptor. Cancer Res 61(15):5784–5789

Sanchez C, Rueda D, Segui B, Galve-Roperh I, Levade T, Guzman M (2001b) The CB1 cannabinoid receptor of astrocytes is coupled to sphingomyelin hydrolysis through the adaptor protein fan. Mol Pharmacol 59(5):955–959

Sanchez MG, Ruiz-Llorente L, Sanchez AM, Diaz-Laviada I (2003) Activation of phosphoinositide 3-kinase/PKB pathway by CB1 and CB2 cannabinoid receptors expressed in prostate PC-3 cells. Involvement in Raf-1 stimulation and NGF induction. Cell Signal 15(9):851–859. doi:10.1016/S0898-6568(03)00036-6

Sarker KP, Maruyama I (2003) Anandamide induces cell death independently of cannabinoid receptors or vanilloid receptor 1: possible involvement of lipid rafts. Cell Mol Life Sci 60 (6):1200–1208. doi:10.1007/s00018-003-3055-2

Sarker KP, Obara S, Nakata M, Kitajima I, Maruyama I (2000) Anandamide induces apoptosis of PC-12 cells: involvement of superoxide and caspase-3. FEBS Lett 472(1):39–44

Shao Z, Yin J, Chapman K, Grzemska M, Clark L, Wang J, Rosenbaum DM (2016) High-resolution crystal structure of the human CB1 cannabinoid receptor. Nature 540:602–606. doi:10.1038/nature20613

Shrivastava A, Kuzontkoski PM, Groopman JE, Prasad A (2011) Cannabidiol induces programmed cell death in breast cancer cells by coordinating the cross-talk between apoptosis and autophagy. Mol Cancer Ther 10(7):1161–1172. doi:10.1158/1535-7163.MCT-10-1100

Simon GM, Cravatt BF (2006) Endocannabinoid biosynthesis proceeding through glycerophospho-N-acyl ethanolamine and a role for alpha/beta-hydrolase 4 in this pathway. J Biol Chem 281 (36):26465–26472. doi:10.1074/jbc.M604660200

Skaper SD, Buriani A, Dal Toso R, Petrelli L, Romanello S, Facci L, Leon A (1996) The ALIAmide palmitoylethanolamide and cannabinoids, but not anandamide, are protective in a delayed postglutamate paradigm of excitotoxic death in cerebellar granule neurons. Proc Natl Acad Sci U S A 93(9):3984–3989

Snider NT, Sikora MJ, Sridar C, Feuerstein TJ, Rae JM, Hollenberg PF (2008) The endocannabinoid anandamide is a substrate for the human polymorphic cytochrome P450 2D6. J Pharmacol Exp Ther 327(2):538–545. doi:10.1124/jpet.108.141796

Strasser F, Luftner D, Possinger K, Ernst G, Ruhstaller T, Meissner W, Ko YD, Schnelle M, Reif M, Cerny T (2006) Comparison of orally administered cannabis extract and delta-9-tetrahydrocannabinol in treating patients with cancer-related anorexia-cachexia syndrome: a multicenter, phase III, randomized, double-blind, placebo-controlled clinical trial from the Cannabis-In-Cachexia-Study-Group. J Clin Oncol Off J Am Soc Clin Oncol 24 (21):3394–3400. doi:10.1200/JCO.2005.05.1847

Sui X, Chen R, Wang Z, Huang Z, Kong N, Zhang M, Han W, Lou F, Yang J, Zhang Q, Wang X, He C, Pan H (2013) Autophagy and chemotherapy resistance: a promising therapeutic target for cancer treatment. Cell Death Dis 4:e838. doi:10.1038/cddis.2013.350

Svizenska I, Dubovy P, Sulcova A (2008) Cannabinoid receptors 1 and 2 (CB1 and CB2), their distribution, ligands and functional involvement in nervous system structures – a short review. Pharmacol Biochem Behav 90(4):501–511. doi:10.1016/j.pbb.2008.05.010

Torres S, Lorente M, Rodriguez-Fornes F, Hernandez-Tiedra S, Salazar M, Garcia-Taboada E, Barcia J, Guzman M, Velasco G (2011) A combined preclinical therapy of cannabinoids and temozolomide against glioma. Mol Cancer Ther 10(1):90–103. doi:10.1158/1535-7163.MCT-10-0688

Van Dross RT (2009) Metabolism of anandamide by COX-2 is necessary for endocannabinoid-induced cell death in tumorigenic keratinocytes. Mol Carcinog 48(8):724–732. doi:10.1002/mc.20515

Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K, Stella N, Makriyannis A, Piomelli D, Davison JS, Marnett LJ, Di Marzo V, Pittman QJ, Patel KD, Sharkey KA (2005) Identification and functional characterization of brainstem cannabinoid CB2 receptors. Science 310(5746):329–332. doi:10.1126/science.1115740

Vara D, Morell C, Rodriguez-Henche N, Diaz-Laviada I (2013) Involvement of PPAR gamma in the antitumoral action of cannabinoids on hepatocellular carcinoma. Cell Death Dis 4:e618. doi:10.1038/cddis.2013.141

Velasco G, Galve-Roperh I, Sanchez C, Blazquez C, Haro A, Guzman M (2005) Cannabinoids and ceramide: two lipids acting hand-by-hand. Life Sci 77(14):1723–1731. doi:10.1016/j.lfs.2005.05.015

Velasco G, Sanchez C, Guzman M (2016) Anticancer mechanisms of cannabinoids. Curr Oncol 23 (2):S23–S32. doi:10.3747/co.23.3080

Wade MR, Tzavara ET, Nomikos GG (2004) Cannabinoids reduce cAMP levels in the striatum of freely moving rats: an in vivo microdialysis study. Brain Res 1005(1–2):117–123. doi:10.1016/j.brainres.2004.01.039

Wu HY, Chang AC, Wang CC, Kuo FH, Lee CY, Liu DZ, Jan TR (2010) Cannabidiol induced a contrasting pro-apoptotic effect between freshly isolated and precultured human monocytes. Toxicol Appl Pharmacol 246(3):141–147. doi:10.1016/j.taap.2010.05.003

Wu HY, Goble K, Mecha M, Wang CC, Huang CH, Guaza C, Jan TR (2012) Cannabidiol-induced apoptosis in murine microglial cells through lipid raft. Glia 60(7):1182–1190. doi:10.1002/glia.22345

Xu Y, Wang C, Li Z (2014) A new strategy of promoting cisplatin chemotherapeutic efficiency by targeting endoplasmic reticulum stress. Mol Clin Oncol 2(1):3–7. doi:10.3892/mco.2013.202

Yamaori S, Ishii H, Chiba K, Yamamoto I, Watanabe K (2013) Delta-tetrahydrocannabinol induces cytotoxicity in macrophage J774-1 cells: involvement of cannabinoid receptor 2 and p38 MAPK. Toxicology 314(2–3):254–261. doi:10.1016/j.tox.2013.10.007

Zoratti C, Kipmen-Korgun D, Osibow K, Malli R, Graier WF (2003) Anandamide initiates Ca(2+) signaling via CB2 receptor linked to phospholipase C in calf pulmonary endothelial cells. Br J Pharmacol 140(8):1351–1362. doi:10.1038/sj.bjp.0705529

Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sorgard M, Di Marzo V, Julius D, Hogestatt ED (1999) Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature 400(6743):452–457. doi:10.1038/22761