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Cytochrome P450 epoxygenases and cancer: A genetic and a molecular perspective

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ABSTRACT

Cytochrome CYP450 epoxygenases catalyze the epoxidation of polyunsaturated fatty acids including arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid. The arachidonic acid-derived products are potent pro-angiogenic lipids and promote tumor development and growth. On the other hand, eicosapentaenoic acid- and docosahexaenoic acid-derived products inhibit angiogenesis and play a protective role in certain pathological conditions including cancer. Increased expression of CYP2C epoxygenases, together with increased levels of their arachidonic acid-derived products, is often observed in tumors and tumor-associated vasculature, making these enzymes an ideal target for anti-cancer therapies. Yet, given the pro- and anti-angiogenic action of these enzymes, a better understanding of the specific roles of their products in the regulation of endothelial cell function and cancer development is required. In this review, we provide an overview of the role of CYP450 epoxygenase-derived lipids, with emphasis on arachidonic acid-derived products, in the regulation of endothelial cell function both in physiological and pathological conditions. Moreover, we discuss the impact of genetic polymorphisms in CYP450 epoxygenases on cancer risk, and we discuss advantages and limitation of approaches to target these enzymes and their products in pathological angiogenesis and cancer.

Contents

Abbreviations: AA, arachidonic acid; AKT, protein kinase B; CI, confidence interval; COX, cyclooxygenase; CRC, colorectal cancer; CYP450, cytochrome P450; EC, endothelial cell; EDP, epoxydocosapentaenoic acid; EEQ, epoxyeicosatetraenoic acid; EET, epoxyeicosatrienoic acid; ERK, extracellular signal–regulated kinase; FGF, fibroblast growth factor; GPR, G protein-coupled receptor; LOX, lipoxygenase; NSAIDs, nonsteroidal anti-inflammatory drugs; NSCLC, non-small cell lung cancer; PPAR, peroxisome proliferator-activated receptor; PTUPB, (4-(5-phenyl-3-{3-[3-(4-trifluoromethyl-phenyl)-ureido]-propyl}-pyrazol-1-yl)-benzenesulfonamide; PUFA, polyunsaturated fatty acid; sEH, soluble epoxide hydrolase; SNP, single nucleotide polymorphism; VEGF, vascular endothelial growth factor; WT, wild-type.

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1. Introduction

1.1. Overview of arachidonic acid metabolism pathway

Eicosanoids represent a class of lipid signaling molecules with prominent roles in inflammation, cancer, and angiogenesis (Capdevila & Falck, 2001; Schneider & Pozzi, 2011). Various eicosanoids can be generated from arachidonic acid (AA) through the cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 monooxygenase (CYP450) pathways. Each pathway uses unique enzymes to synthesize specific eicosanoids, and each eicosanoid elicits distinct biological effects. In cancer, these biological effects may be exerted on tumor or microenvironment cells (Hanahan & Weinberg, 2011; Schneider & Pozzi, 2011). The effects of COX- and LOX-derived eicosanoids have been relatively well studied in cancer, especially those of tumor-promoting prostaglandin E_2. While most eicosanoids synthesized by the COX and LOX pathways promote tumorigenesis, exceptions exist, such as prostaglandin I_2 (Schneider & Pozzi, 2011). In addition to the COX- and LOX-derived eicosanoids, metabolites derived from CYP450 monooxygenases also regulate cancer development and growth and they can exert both pro- and anti-tumorigenic effects.

The AA CYP450 monooxygenases consist of epoxygenases (CYP2 isoforms) and ω-hydroxylases (CYP4 isoforms) (Schneider & Pozzi, 2011) (Fig. 1). CYP450 ω-hydroxylases oxidize AA to 19- or 20-hydroxyeicosatetraenoic acids. Epoxidation of AA by the CYP450 epoxygenases produces four epoxyeicosatrienoic acid (EET) regioisomers: 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET (Zeldin, 2001) (Fig. 1). Subsequent metabolism by the soluble epoxide hydrolase (sEH) converts EETs into corresponding dihydroxyeicosatrienoic acids, lipids with less potent biological effects (Campbell et al., 2002; Campbell & Falck, 2007; Chacos et al., 1983; Yu et al., 2000; Zeldin et al., 1993) (Fig. 1).

In addition to AA, CYP450 epoxygenases catalyze the epoxidation of other lipids and xenobiotic substrates (Fer et al., 2008; Oliw, 1994; Spector & Kim, 2015; Van Booven et al., 2010). Metabolism of the ω-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) by CYP450 epoxygenases generates epoxyeicosatetraenoic acid (EEQ) and epoxydocosapentaenoic acid (EDP) regioisomers, respectively (Fer et al., 2008; Westphal, Konkel, & Schunck, 2011) (Fig. 2). EEQs and EDPs can be subsequently hydrolyzed by sEH to dihydroxyeicosatetraenoic acids and dihydroxydocosapentaenoic acids, respectively (Arnold et al., 2010) (Fig. 2). Finally, as phase I detoxification enzymes, the CYP450 epoxygenases oxidize a number of xenobiotics including amiodarone, cyclosporine, non-steroidal anti-inflammatory drugs (NSAIDs), paclitaxel, tamoxifen, and warfarin (Kerb et al., 2009; C. A. Lee et al., 2010; C. R. Lee, Goldstein, & Pieper, 2002; Rahman, Korzekwa, Grogan, Gonzalez, & Harris, 1994). In this review, we focus on the putative role of EETs as pro-angiogenic and pro-tumorigenic lipids, their mechanism of action, and approaches to target these lipids in pathological conditions.

2. CYP450 epoxygenase-derived EETs

Synthesis of EETs represents one mechanism by which CYP450 epoxygenases influence a diverse number of biological processes. CYP2C8, CYP2C9, CYP2J2, and CYP3A4 are the major enzymes responsible for the conversion of AA to EETs in humans, while Cyp2c44, the functional homolog of CYP2C9, is one of the major CYP450 epoxygenase in mice (Daikh, Lasker, Raucy, & Koop, 1994; Pozzi et al., 2005, 2010; Rifkind, Lee, Chang, & Waxman, 1995; S. Wu, Moomaw, Tomer, Falck,

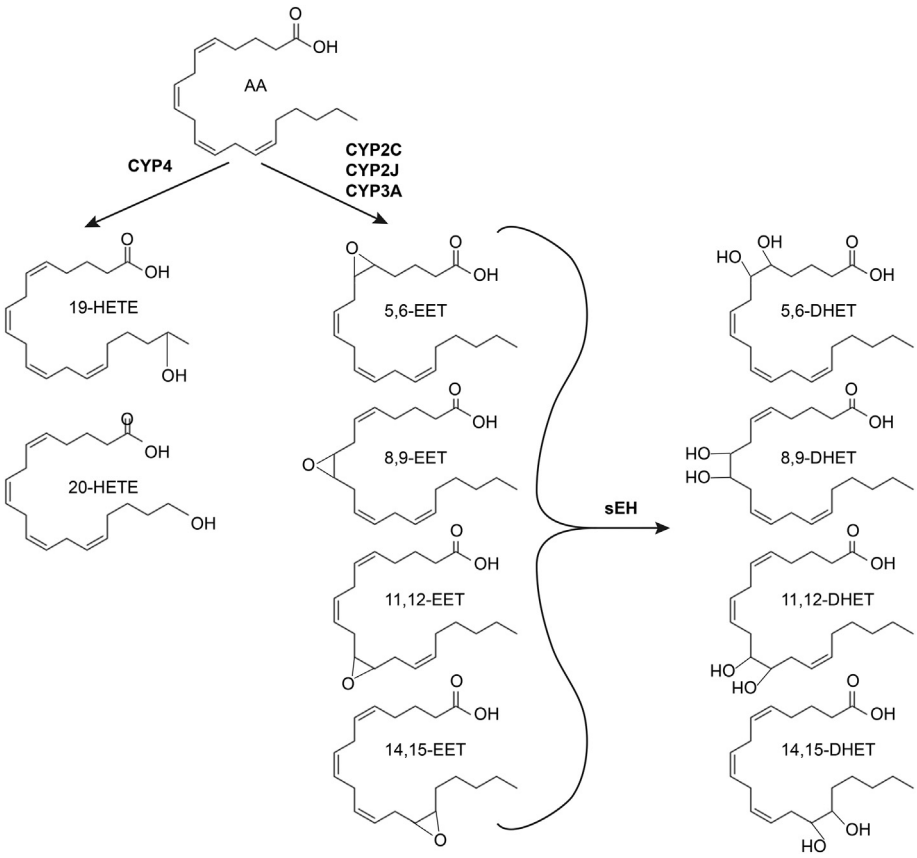

Fig. 1. Metabolism of arachidonic acid (AA) by the cytochrome P450 monooxygenases. AA is metabolized by P450 ω-hydroxylases (CYP4) to 19- or 20-hydroxyeicosatetraenoic acid (HETE). In addition, AA is metabolized by P450 epoxygenases (CYP2C, 2J, and 3A) to 5,6-, 8,9-, 11,12- or 14,15-epoxyeicosatranoic acid (EET). Subsequent metabolism by the soluble epoxide hydrolase (sEH) converts EETs into corresponding dihydroxyeicosatrienoic acids (DHET).

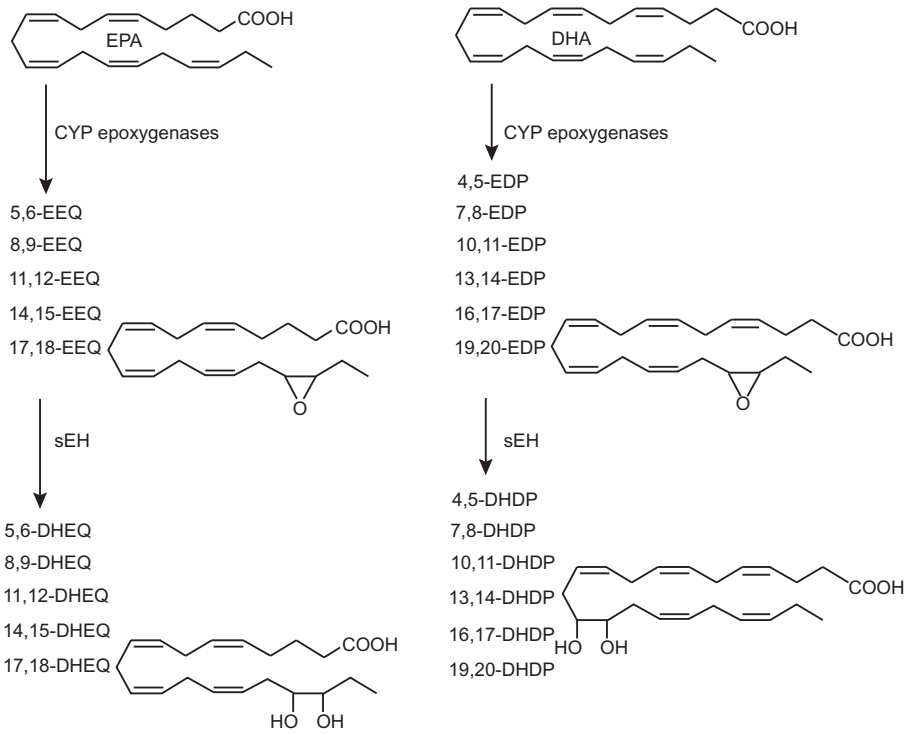

Fig. 2. Metabolism of ω-3 polyunsaturated fatty acids by CYP450 epoxygenases. Metabolism of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) by CYP450 epoxygenases generates 5 epoxyeicosatetraenoic acids (EEQ, 17,18-EEQ shown) and 6 epoxydocosapentaenoic acids (EDP, 19,20-EDP shown). EEQs and EDPs can be subsequently hydrolyzed by soluble hepoxihydrolase (sEH) to 5 dihydroxyeicosatetraenoic acids (DHEQ, 17,18-DHEQ shown) and 6 dihydroxydocosapentaenoic acids (DHDP, 13,14-DHDP shown).

& Zeldin, 1996; Zeldin, Dubois, Falck, & Capdevila, 1995). CYP450 epoxygenases exhibit regioselectivity in that a given epoxygenase is more efficient at producing a particular EET regioisomer(s) (Daikh, Lasker, Raucy, & Koop, 1994; Rifkind, Lee, Chang, & Waxman, 1995; S. Wu, Moomaw, Tomer, Falck, & Zeldin, 1996; Zeldin, Dubois, Falck, & Capdevila, 1995). Given their role in xenobiotic metabolism, it is unsurprising that CYP2C8, CYP2C9, and CYP2J2 are expressed in the liver. However, endothelial cell (EC) expression of these enzymes accounts for the majority of EETs synthesis (Delozier et al., 2007; S. Wu et al., 1996). Once produced and secreted by ECs, EETs act as autocrine and paracrine molecules affecting numerous processes of the vascular system. Among their effects, EETs inhibit vascular inflammation, promote vasodilation and natriuresis, and lower blood pressure making them an attractive target for cardiovascular disease interventions (Fleming, 2008; Spiecker & Liao, 2005). However, the ability of EETs to stimulate proliferation, migration and tubulogenesis of ECs, indicate that autocrine effects of EETs also have major implications in promoting both physiological and pathological angiogenesis. These effects temper the appeal of enhancing EET production for clinical benefit in cardiovascular disease.

2.1. Biological effects of EETs on murine and human ECs

Broadly, EETs produced either by ECs or by surrounding cells, stimulate EC proliferation, migration and tubulogenesis. Astrocytes secrete lipids that can promote cerebral capillary EC proliferation and tubulogenesis. Irreversible pharmacological inhibition of CYP450 epoxygenases with 17-octadecynoic (10 μM) eliminated these pro-angiogenic effects, indicating that astrocyte CYP450-derived metabolites likely mediate the effects on ECs (Munzenmaier & Harder, 2000). Subsequent studies by us and others confirmed that EETs synthesized by endothelial CYP450 epoxygenases promote EC function, including proliferation. While these studies collectively identified the four EET regioisomers as mitogenic, the magnitude of their effects varied by the specific EET regioisomer. All EET regioisomers used at a 0–5 μM range enhance pulmonary EC proliferation with maximum effect observed at 1 μM, but 5,6-EET induced the most proliferation (Pozzi et al., 2005). In line with this finding, EET regioisomers propagate their mitogenic effects through different signaling cascades, with protein kinase B (AKT) mediating the effects of 5,6- and 14,15-EETs and mitogen-activated protein kinase mediating the effects of 8,9- and 11,12-EETs in murine ECs (Pozzi et al., 2005). Furthermore, treatment of ECs with the sEH inhibitor adamantyl-cyclohexyl-urea (0–40 μM) enhances EC functions; whereas pharmacological inhibition of Cyp2c epoxygenase with *N*-methylsulfonyl-6-(2-proparglyloxyphenyl) hexanamide or ketoconazole (10–40 μM) or genetic deletion of *Cyp2c44* reduces EC functions (Pozzi et al., 2005, 2007, 2010). Similar to the selective effects of EETs on EC proliferation, not all EET regioisomers promote EC migration and tubulogenesis at the same efficacy. For example, we found that 5,6- and 8,9-EETs (1 μM each) enhanced EC functions by activating AKT and extracellular signal–regulated kinase (ERK), while the two other regioisomers lacked any effect (Pozzi et al., 2005). In addition to their *in vitro* effects on EC functions, EETs are also potent pro-angiogenic lipids *in vivo*. Injection of 5,6- or 8,9-EET (50 μM) over a period of 2 weeks in sponges subcutaneously implanted into mice, promoted neovascularization that was further enhanced by co-administrated sEH inhibitor adamantyl-cyclohexyl-urea (250 μM) to stabilize these two EET regioisomers (Pozzi et al., 2005). Consistent with this finding, a 7-day *in vivo* matrigel plug assay performed in rats or mice showed that matrigel premixed with 14,15-EET (150 μM or 50 μM, respectively) formed more hemoglobin-rich plugs than matrigel premixed with vehicle only (Medhora et al., 2003; B. Zhang, Cao, & Rao, 2006). Thus, EETs are key contributors of physiological angiogenesis and *de novo* vascularization.

Similar to the effects observed in murine ECs, EETs also promote human EC function. A study focusing on CYP2C9 showed that overexpression of this epoxygenase *via* adenovirus infection in human ECs derived from the lung or the umbilical vein increased their proliferation and tubulogenesis activity (Michaelis et al., 2003), while inhibition of CYP2C9 with sulfaphenazole (30 μM) abrogated these effects

(Michaelis et al., 2003). All four EET regioisomers stimulated human dermal microvascular EC tubulogenesis and migration when used at a 0-1 μM range with a maximum effect observed at 0.1 μM (B. Zhang, Cao, & Rao, 2006). 14,15-EET also promoted AKT phosphorylation in human dermal microvascular ECs within 30 min of treatment and this event was required for mediating 14,15-EET-dependent EC migration and tubulogenesis (B. Zhang et al., 2006). Thus, CYP450 epoxygenases promote EC function through EET synthesis and function in both human and murine ECs.

2.2. Role of EETs in tumorigenesis and pathological angiogenesis

Given the clear pro-angiogenic role of EETs, it is plausible that EETs support tumor growth *via* enhanced angiogenesis, but current knowledge of the direct role of these lipids in cancer is still limited. Mice expressing human *CYP2C8* or *CYP2J2* in the endothelium under the control of the Tie2 promoter or lacking sEH globally developed bigger tumors following injection of melanoma, fibrosarcoma, or lung carcinoma cells compared to wild-type (WT) mice (Panigrahy et al., 2012). Increased tumor growth was independent of the type of tumor cell injected and correlated with a 15-fold increase in plasma levels of 11,12- and 14,15-EET in sEH-null mice and a 33% increase in plasma levels of 14,15-EET in mice expressing endothelial CYP2C8. Thus, these results suggest that endothelial-derived EETs promote primary tumor growth (Panigrahy et al., 2012). Similar results were obtained by systemic administration of 14,15-EET *via* osmotic minipumps or treatment with the selective sEH inhibitors 1-(1-methanesulfonyl-piperidin-4-yl)-3-(4-trifluoromethoxy-phenyl)-urea and trans-4-[4-(3-adamantan-1-y1-ureido)-cyclohexyloxy]-benzoic acid *via* gavage. In contrast, mice expressing sEH in the endothelium under the control of the Tie-2 promoter or treated by gavage with the EET antagonist 14,15-epoxyeicosa-5(*Z*)-enoic acid showed reduced primary and metastatic tumor growth, and these effects were associated with decreased tumor-associated angiogenesis (Panigrahy et al., 2012). Finally, systemic administration of the 14,15-EET analogs 2-(13-(3-pentylureido) tridec-8(*Z*)-enamido)succinic acid and *N*-isopropyl-*N*-(5-((2-pivalamidobenzo[*d*]thiazol-4-yl)oxy)pentyl)heptanamide (both at 10 mg/kg in drinking water) restored tumor growth in mice lacking *Cyp2c44* (Skrypnyk et al., 2014), indicating that EETs support primary and metastatic tumor growth.

Studies in human cancers demonstrate a pro-tumorigenic role for EETs. Various primary tumors express CYP450 epoxygenases, whereas sEH expression is reduced in certain tumors (Enayetallah, French, & Grant, 2006; Jiang et al., 2005; Nithipatikom et al., 2010). Breast cancer tissues express high levels of multiple CYP450 epoxygenases and EETs (Luo et al., 2018; Wei et al., 2014). Overexpression of CYP2J2 *via* adenovirus infection or treatment with 8,9-EET, 11,12-EET, and 14,15-EET (0.1 μM) increased *in vitro* growth, migration and invasion of 4 different carcinoma call lines (Jiang et al., 2005, 2007). Moreover, overexpression of CYP2J2 in human breast cancer cells significantly enhanced primary growth and metastatic potential 14 weeks after tumor cell injection into mouse mammary glands (Jiang et al., 2005, 2007). The pro-tumorigenic action of CYP2J2 seems to reside in its ability to promote and downregulate the expression of pro-metastatic and anti-metastatic genes, respectively, including matrix metalloproteinases and CD82 (Jiang et al., 2005, 2007). In addition to these findings, treatment of human breast cancer cells with14,15-EET (0.1 μM) promotes invasion, epithelial-to-mesenchymal transition, and resistance to cisplatin, a commonly used chemotherapeutic agent (Luo et al., 2018). Mechanistically, 14,15-EET induces the expression of the matrix receptor integrin αvβ3 and promotes integrin-mediated pro-tumorigenic signaling including focal adhesion kinase and AKT activation. Downregulation of integrin αvβ3 prevents 14,15-EET-induced AKT activation, cell invasion and epithelial-to-mesenchymal transition, suggesting that integrins are key in mediating the pro-tumorigenic effects of EETs (Luo et al., 2018).

Finally, human prostate cancer cell lines express CYP450 epoxygenases such CYP2C8, CYP2C9, and CYP2J2 synthesize 11,12-EET as a major AA metabolite (Nithipatikom et al., 2010). Treatment of prostate cancer cells with 11,12-EET (0–10 μM) promoted cell invasion and migration with a maximum effect observed at 1 μM EET (Nithipatikom et al., 2010). Treatment with 11,12-EET (1 μM) also promoted the formation of myosin-containing stress fibers and cell stretching which were both prevented by treatment of cells with the EET antagonist 14,15-epoxyeicosa-5(*Z*)-enoic acid (5 μM) (Nithipatikom et al., 2010). Collectively, these studies indicate that EETs support tumorigenesis and tumor-associated angiogenesis *in vivo* as well as tumor cell proliferation and migration *in vitro*.

2.3. EET receptors

A key question is how EETs exert their biological effects. Ligand binding studies suggest the presence of receptors for EETs, some of which belong to the G protein-coupled receptor (GPR) family (Y. Chen, Falck, Tuniki, & Campbell, 2009; Wong, Lai, & Falck, 2000; Wong, Lai, Shen, Belosludtsev, & Falck, 1997; Wong et al., 1993). Downregulation of a G(s)-coupled receptor *via* siRNA in ECs abolished the ability of 11,12-EET (1 μM) to promote the rapid translocation of TRPC6 channel from the Golgi to the plasma membrane as well as tubulogenesis, strongly indicating that this receptor facilitates some EET functions (Ding et al., 2014). Recently, GPR40, known to be activated by medium and long chain fatty acids (Briscoe et al., 2003) has been described as an EET-binding receptor able to promote EET-mediated cell proliferation, activate growth factor receptors, and phosphorylate ERK in human embryonic kidney cells (Ma et al., 2015). These effects, however, were evident in cells overexpressing human GPR40 and required doses of EETs ranging from 5 to 20 μM, thus questioning the physiological role of EETs in activating this receptor. Interestingly, in human ECs, which express endogenous GPR40, this receptor seems to regulate 11,12-EET-induced ERK phosphorylation, since treatment with the GPR40 inhibitor GW1100 (10 μM) or siRNA-mediated GPR40 silencing decreased ERK activation induced by short term treatment with 11,12-EET (1 μM for 10 min) (Park et al., 2018).

Search for a 14,15-EET receptor has led to the identification of five low affinity prostaglandin receptor subtypes that increase intracellular levels of cyclic adenosine monophosphate after 14,15-EET treatment (Liu et al., 2017). All together, these studies provide evidence that EETs can exert their biological function by binding receptors that normally mediate prostanoid and/or fatty acid effects.

2.4. EET cross talk

EETs exert pro-angiogenic effects in multiple models through several signaling cascades (Cheranov et al., 2008; Medhora et al., 2003; Michaelis et al., 2003; Pozzi et al., 2005; Y. Wang et al., 2005; Yan, Chen, You, & Sun, 2008; B. Zhang et al., 2006). One mechanism whereby EETs exert their biological function is *via* cross talk with receptor tyrosine kinases (Fig. 3). EETs are a key component of the vascular endothelial growth factor (VEGF)-mediated signaling in ECs. Genetic or pharmacologic inhibition of murine Cyp2c44 diminished VEGF-stimulated EC proliferation, tubulogenesis as well as ERK and AKT activation (Pozzi et al., 2010; Yang, Wei, Pozzi, & Capdevila, 2009). Moreover, treatment of ECs with VEGF increases Cyp2c44 expression, indicating this CYP450 epoxygenase operates as a downstream effector of VEGF (Yang, Wei, Pozzi, & Capdevila, 2009). A study utilizing human ECs had similar findings. Specifically, VEGF induced *CYP2C8 RNA* expression and increased CYP2C8 protein and intracellular EET levels (Webler et al., 2008). *CYP2C8* knockdown impaired VEGF-stimulated EC sprouting and branching, while EET antagonists inhibited *in vivo* angiogenesis in response to VEGF (Webler et al., 2008). Interestingly, another study showed that 14,15-EET treatment enhanced EC *VEGF*

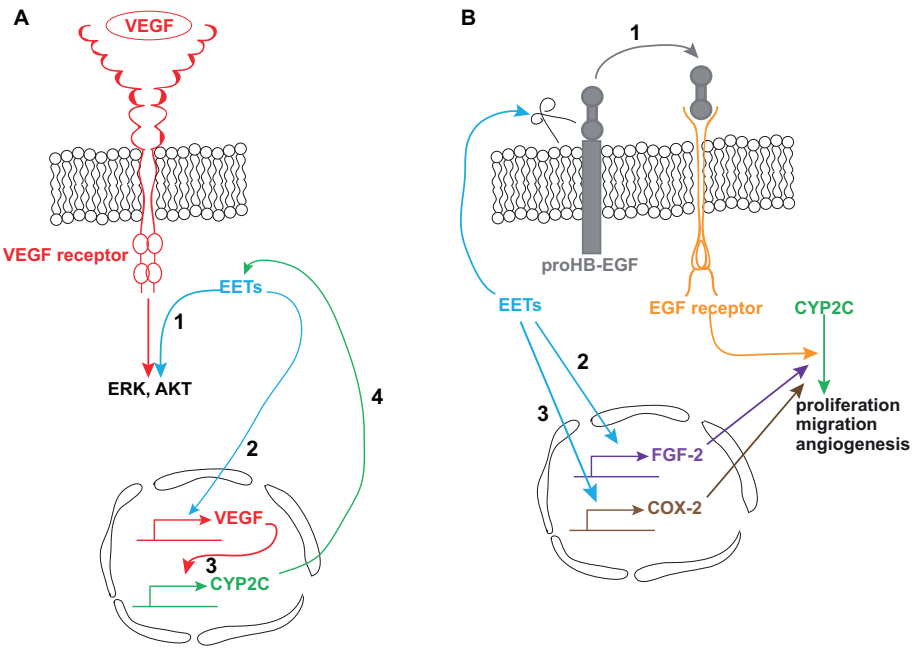

Fig. 3. Example of crosstalk between EETs and intracellular signaling. (A) EETs act downstream of the VEGF receptor potentiating VEGF-mediated activation of intracellular kinases (1). EETs can also promote the synthesis of VEGF (2) that, in turn, can promote the transcription of CYP2C (3) thus potentiating EET-mediated signaling (4). (B) EETs induce the release of HB-EGF and activation of EGF receptor (1) as well as synthesis of FGF-2 (2) and COX2 (3) thus potentiating CYP2C/EET-mediated proliferation migration and *in vivo* angiogenesis.

expression, indicating the presence of positive feedback loop between VEGF and EETs (Panigrahy et al., 2012).

Another membrane receptor that mediates the pro-angiogenic effects of EETs is the EGF receptor (Cheranov et al., 2008; Michaelis et al., 2003; B. Zhang et al., 2006) (Fig. 3). 14,15-EET treatment stimulates the cleavage and secretion of heparin binding-EGF and subsequent EGF receptor activation (J. K. Chen, Capdevila, & Harris, 2002) and inhibition of EGF receptor tyrosine kinase impairs CYP2C9-induced EC proliferation (Michaelis et al., 2003).

In addition to membrane receptors, EETs can exert their effects by acting on soluble ligands or intracellular enzymes (Michaelis, Falck, Schmidt, Busse, & Fleming, 2005; B. Zhang et al., 2006) (Fig. 3). Specifically, 14,15-EET increases the expression of the pro-angiogenic ligand fibroblast growth factor-2 (FGF-2) in a phosphoinositide 3-kinase-dependent manner. FGF-2 likely propagates the effects of 14,15-EET, as FGF-2 inhibition diminishes 14,15-EET-induced EC tubulogenesis and *in vivo* angiogenesis (B. Zhang et al., 2006). Another study found that both CYP2C9 overexpression and exogenous 11,12-EET upregulated COX2 expression in ECs and treatment with celecoxib, a COX2 inhibitor, blocked CYP2C9-mediated EC proliferation (Michaelis, Falck, Schmidt, Busse, & Fleming, 2005). Interestingly, besides promoting COX2 expression, EETs serve as substrates for COX enzymes (Carroll, Balazy, Margiotta, Falck, & McGiff, 1993; Moreland et al., 2007; Rand et al., 2017; J. Y. Zhang, Prakash, Yamashita, & Blair, 1992). COX acts on all the EET regioisomers, except 14,15-EET. However, among these regioisomers, COX enzymes preferentially metabolize 8,9-EET to form two major products: ct-8,9-epoxy-11-hydroxy-eicosatrienoic acid and ct-8,9-epoxy-15-hydroxy-eicosatrienoic acid (Fig. 4). sEH further metabolizes both products, but effects of these products are poorly understood in the context of EC biology and angiogenesis. One of the 8,9-EET products, *ct*-8,9-epoxy-11-hydroxy-eicosatrienoic acid, stimulated angiogenesis *in vivo*, whereas ct-8,9-epoxy-15-hydroxy-eicosatrienoic acid had no effect on angiogenesis (Fig. 4) (Rand et al., 2017). Thus, CYP2C and their products promote angiogenic effects by regulating the expression of growth factors and lipid metabolizing enzymes, as well as acting downstream growth factor receptor-mediated signaling.

3. Non AA-derived CYP450 epoxygenases products

3.1. Synthesis and activity of EDPs and EEQs

Metabolism of ω-3 PUFAs is another avenue by which the CYP450 epoxygenases affect biological processes. CYP2C8, CYP2C9, and CYP2J2 oxidize the double bonds in EPA and DHA (Fig. 2). Similar to their metabolism of AA, CYP450 epoxygenases exhibit regiospecificity and preferentially synthesize different regioisomers. CYP2C8 and CYP2J2 primarily generate 19,20-EDP and 17,18-EEQ from DHA and EPA, respectively, whereas CYP2C9 predominantly produces 10,11-EDP and 14,15-EEQ (Fer et al., 2008; Westphal, Konkel, & Schunck, 2011) (Fig. 2). sEH rapidly metabolizes most EDPs and EEQs regioisomers into dihydroxydocosapentaenoic acids and dihydroxyeicosatetraenoic acids, respectively (Fig. 2), but little is known about the function of the corresponding diols. However, sEH poorly metabolizes 19,20-EDP, making 19,20-EDP highly abundant in tissues (Morisseau et al., 2010).

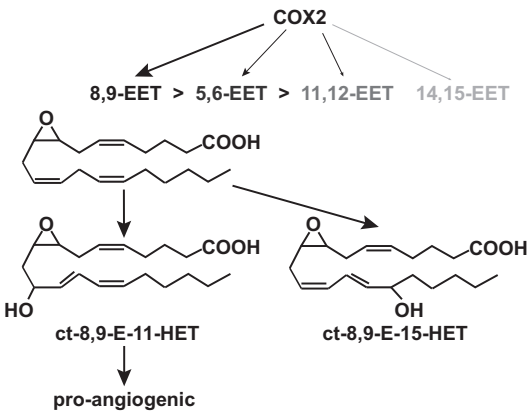

Fig. 4. Metabolism of EETs by COX2. EETs are substrates for COX2 with 8,9-EET and 14,15-EET servings as the most preferred and inactive substrate, respectively. 8.9-EET is converted into two products, of which only ct-8,9-E-11-HET exerts pro-angiogenic activity.

Similar to EETs, CYP450 epoxygenase-derived metabolites of ω-3 PUFAs exert cardio protective effects such as vasodilation, but their effects on angiogenesis are less well understood (Arnold et al., 2010; Fer et al., 2008; Morin, Sirois, Echave, Rizcallah, & Rousseau, 2009; Ye et al., 2002).

3.2. Effects of EDPs and EEQs on angiogenesis

Several studies have reported anti-angiogenic effects of diets rich in ω-3 PUFAs, but the specific active metabolite(s) mediating these effects is not well characterized. In addition to CYP2 epoxygenases, ω-3 PUFAs serve as substrates for COXs and LOXs, making it essential to understand the biological effects of each metabolite derived from these fatty acids. However, only a small number of studies have investigated the effects of specific metabolites (Groeger et al., 2010; Kim, Karanian, & Salem, 1990; Malkowski et al., 2001; Miller, Yamaguchi, & Ziboh, 1989; Sapieha et al., 2011). A 4 day *in vivo* matrigel plug study broadly characterized EDPs as anti-angiogenic lipids, with all EDP regioisomers (10 μg/plug) preventing VEGF-driven angiogenesis (100 ng/plug) and 19,20-EDP (10 μg/plug) inhibiting FGF-2-driven angiogenesis (0.5 μg/plug) (G. Zhang et al., 2013), implicating 19,20-EDP as a broad angiogenesis inhibitor. In addition, daily administration of 19,20-EDP (0.05 mg/kg) together with the sEH inhibitor trans-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (1 mg/kg) resulted in a 70% reduction of primary and metastatic triple-negative breast cancer tumor growth by reducing vascularization (G. Zhang et al., 2013). Consistent with the *in vivo* findings, 19,20-EDP inhibited EC tubulogenesis and migration and diminished VEGF-induced VEGF receptor 2 phosphorylation, indicating that this lipid acts upstream of growth factor signaling. In addition to 19,20-EDP, 17,18-EEQ has been shown to have anti-angiogenic activity. To this end, daily intraperitoneal injection of 17,18-EEQ (5 μg/kg) reduced angiogenesis and leukocyte adhesion in a mouse model of laser-induced choroidal neovascularization (Yanai et al., 2014). Furthermore, 17,18-EEQ reduced EC proliferation through mitogen-activated protein kinase-induced downregulation of cyclin D1 expression, although these effects were observed when this epoxy-EPA is used at high doses (10 μM) (Cui, Petrovic, & Murray, 2011).

Besides their direct effect on EC function, ω-3 PUFAs could also inhibit angiogenesis through decreased synthesis of angiogenic EETs. Increased dietary consumption of ω-3 PUFAs shifted CYP450 epoxygenase metabolism in favor of EDPs and EEQs synthesis over EET synthesis (Arnold et al., 2010; Fischer et al., 2014; Shearer, Harris, Pedersen, & Newman, 2010; Yanai et al., 2014). These alterations in metabolism correlated with reduced neovascularization in a mouse model of age-related macular degeneration. However, it is unclear whether EDPs or EEQs directly mediate the reduced neovascularization associated with ω-3 PUFA supplementation (Yanai et al., 2014).

Not all studies characterizing the effects of EDPs and EEQs found these molecules inhibit EC functions and angiogenesis. Both 8,9- and 11,12-EEQ (used at 10 μM) slightly enhanced EC proliferation *in vitro* (Cui, Petrovic, & Murray, 2011). Interestingly, mice expressing endothelial *CYP2C8* under the Tie-2 promoter showed more retinal neovascularization in a model of oxygen-induced retinopathy than WT mice following a ω-3 PUFA rich diet (Shao et al., 2014). ω-3 PUFA fed CYP2C8 expressing mice also showed significantly higher levels of plasma 19,20-EDP (~1.6-fold; $p = .029$) and 17,18-EEQ (~1.5-fold; $p = .030$) than WT mice. Moreover, 19,20-EDP and 17,18-EEQ (both used at 30 μM) enhanced endothelial cell sprouting in an *ex vivo* aortic ring explant assay (Shao et al., 2014). Finally, 19,20-EDP (used at 1 μM) enhanced the tubulogenic activity of human retinal microvascular endothelial cells at baseline and reversed the anti-tubulogogenic activity induced by fenofibric acid (20 μM) (Gong et al., 2016). These results suggest that the origin of the ECs may influence the effects of 19,20-EDP. Although these studies indicate ω-3 PUFA epoxides may inhibit angiogenesis in ECs outside of the retina, additional characterization of the biological effects of EDPs and EEQs is necessary in order to target these lipids in pathological conditions. A summary comparing and contrasting the actions of the various AA-, EPA-, DHA-derived CYP450 epoxygenase products on tumorigenesis and angiogenesis is provided in Table 1.

4. Targeting CYP450 epoxygenases and their products

4.1. Peroxisome proliferator-activated receptor alpha (PPARα)

Given the pro-angiogenic and pro-tumorigenic action of EETs, reduction of EET synthesis may provide clinical benefit for cancer patients. PPARα is a member of the peroxisome proliferator-activated receptors family of transcription factors, all of which regulate multiple biological processes including glucose and lipid metabolism. Ligand binding to the PPARα ligand binding domain activates transcriptional regulation activity of PPARα (Issemann & Green, 1990; Wahli, Braissant, & Desvergne, 1995). To date several studies have reported PPARα ligands possess anti-tumorigenic and anti-angiogenic effects (Panigrahy et al., 2008; Pozzi et al., 2007; Saidi, Holland, Charnock-Jones, & Smith, 2006; Varet et al., 2003). Further study of the effects of PPARα ligands provided mechanistic insight connecting the inhibitory effects to CYP450 epoxygenase expression. Treatment with PPARα ligands such as Wy-14,643 or fibrates downregulates *CYP2C9* and *Cyp2c44* expression in human and murine ECs, respectively, and reduces EET biosynthesis (Pozzi et al., 2007, 2010). In addition, PPARα ligands decreases *in vitro* EC proliferation, migration, and tubulogenesis (Goetze et al., 2002; Panigrahy et al., 2008; Pozzi et al., 2010; Varet et al., 2003). Mice treated with PPARα ligands also show reduced primary and metastatic tumor growth, tumor angiogenesis, endothelial *Cyp2c44* expression, and circulating EET levels (Skrypnyk et al., 2014). Taken together, these results indicate that activation of PPARα and consequent downregulation of *Cyp2c* expression may be a promising and safe anti-cancer approach.

4.2. EET antagonists and CYP2C inhibitors

Another approach to reduce EET production or effects is the use of CYP2C inhibitors or EET antagonists, respectively. *In vitro* studies performed on cancer cells show that inhibiting epoxygenase activity with a selective CYP epoxygenase inhibitor N-methanesulfonyl-6-(2-propargyloxyphenyl)hexanamide prevented tumor cell invasion (Nithipatikom et al., 2010). In addition, treatment of cells with synthetic EET antagonists, including 14,15-epoxyeicosa-5(*Z*)-enoic acid, prevented EET-induced tumor cell invasion and migration (Nithipatikom et al., 2010). Consistent with this finding, treatment of breast cancer cells with ketoconazole and azamulin, a selective CYP3A4 inhibitors, or 14,15-epoxyeicosa-5(Z)-enoic acid inhibited cell proliferation and conferred sensitivity to the selective estrogen receptor modulator 4-hydroxytamoxifen (Thuy Phuong et al., 2017). Finally, treatment with 14,15-epoxyeicosa-5(Z)-enoic acid significantly inhibited migration and proliferation of tumor cells and ECs overexpressing CYP2C9 (Sausville et al., 2018). Consistent with these *in vitro* findings, mice treated with EET antagonists showed reduced primary tumor growth and multi-organ metastatic potential, indicating that counteracting EET-mediated function can affect cancer growth and metastasis (Panigrahy et al., 2012).

4.3. Enhancing EDPs and EEQs synthesis

Given the inhibitory effects of EEQs and EDPs, increasing tissue levels of these ω-3-derived lipids may be effective in reducing tumor-associated vasculature without causing hypertension, a frequent side effect of angiogenic inhibitors (Morin, Sirois, Echave, Rizcallah, & Rousseau, 2009; Ye et al., 2002). Pharmacological inhibition of sEH is one avenue for consideration, if the main goal is to increase EDP concentration in tumor tissue. However, inhibiting sEH results in increased levels of pro-angiogenic EETs thereby promoting cancer growth (G. Zhang et al., 2013). The beneficial effects of sEH inhibitors appears

Table 1
Summary of the various effects of epoxygenases-derived products on cancer growth/dissemination and pathological angiogenesis.

Study	Target
	Tumorigenesis and tumor-associated angiogenesis
	Pro
Panigrahy et al. (2012)	Tie-2-CYP2C8 and Tie-2-CYP2J2 mice
Panigrahy et al. (2012)	Global sEH-null mice
Panigrahy et al. (2012); Skrypnyk et al. (2014)	Systemic administration of EETs and/or EETs analogs
Panigrahy et al. (2012)	Systemic administration of sEH inhibitors
Jiang et al. (2005, 2007)	Overexpression of CYP2J2 in cancer cells
	Anti
Pozzi et al. (2010); Skrypnyk et al. (2014)	Global Cyp2c44-null mice
Panigrahy et al. (2012)	Tie2-sEH mice
Panigrahy et al. (2012)	Systemic administration of EET antagonists
Zhang et al. (2013)	Administration of 19,20-EDP ± sEH inhibitors
	Matrigel plugs and/or sponge assays
	Pro
Pozzi et al. (2005); B. Zhang et al. (2006)	Administration of EETs
	Anti
G. Zhang et al. (2013)	EDPs
	In vitro cancer cell growth, invasion, epithelial-to-mesenchymal transition, resistance to chemotherapy
	Pro
Jiang et al. (2005, 2007)	Overexpression of CYP2J2 in cancer cells
Jiang et al. (2005, 2007); Nithipatikom et al. (2010); Luo et al. (2018)	Treatment with EETs
	Anti
Nithipatikom et al. (2010); Thuy Phuong et al. (2017)	CYP epoxygenase inhibitors
Nithipatikom et al. (2010); Sausville et al. (2018); Thuy Phuong et al. (2017)	Treatment with EET antagonists
	In vitro EC function (proliferation, migration tubulogenesis)
	Pro
Michaelis et al. (2003)	Overexpression of CYP2C9
Cui et al. (2011)	8,9- and 11,12-EEQ
Gong et al. (2016)	19,20-EDP (human retinal microvascular ECs)
Zhang et al. (2006); Pozzi et al. (2005, 2007, 2010),	Exogenous EETs ± sEH inhibitors
	Anti
Pozzi et al. (2010)	Cyp2c44-null ECs
Sausville et al. (2018)	Treatment with EET antagonists
Zhang et al. (2013)	Treatment with 19,20-EDP
Cui, Petrovic, & Murray (2011)	Treatment with 17,18-EEQ
Pozzi et al., 2005, 2007, 2010; Michaelis et al. (2003)	CYP2C/2 J inhibitors
	Ex vivo EC function (*e.g.*, aortic ring assays)
	Pro
Shao et al. (2014)	19,20-EDP and 17,18-EEQ
	Cancer independent pathological angiogenesis
	Pro
Shao et al. (2014)	Tie2-CYP2C8 (retinal neovascularization in a model of oxygen-induced retinopathy)
	Anti
Yanai et al. (2014)	17–18-EEQ (laser-induced angiogenesis)

to depend on the availability of ω-6 (AA) and ω-3 PUFAs. In tissues rich in ω-3 PUFAs, sEH inhibition attenuated angiogenesis, while promoting it in tissues rich in ω-6 PUFAs (Fromel et al., 2012). With the exception of a few organs, most tissues have higher ω-6 PUFA content (Brenna & Diau, 2007; Crawford, Casperd, & Sinclair, 1976; SanGiovanni & Chew, 2005) and high levels of ω-6 PUFA in the Western diet increases the tissue abundance of ω-6 PUFA, thereby increasing EET levels and potential angiogenesis after sEH inhibition (Simopoulos, 2008). Because targeting EDP is more desirable, changes in dietary ω-3 PUFA intake may represent an effective avenue to inhibit cancer growth and progression. Consistent with this logic, dietary supplementation of ω-3 PUFA increases plasma and tissue levels of EEQs and EDPs in rats (Arnold et al., 2010) and humans (Keenan et al., 2012; Shearer, Harris, Pedersen, & Newman, 2010). However, while numerous studies report beneficial effects of ω-3 PUFA supplements in both preventing cancer and sensitizing cancer cells to chemotherapy and radiation, it is unclear whether and how EEQs or EDPs mediate these effects. To determine which metabolites facilitate specific effects, it is necessary to individually examine the effects of ω-3 PUFA metabolites derived from the COX, LOX, and CYP450 epoxygenase enzymes and determine whether their effects are cancer type dependent. However, not all clinical trials find that ω-3 PUFA supplementation decreased cancer risk (Nabavi et al., 2015). Therefore, mores studies are needed to clearly determine whether and when targeting these metabolites is beneficial in cancer.

4.4. Heme binding biguanides

In addition to CYP2C8, CYP2C9 and CYP2J2, CYP3A4 converts AA to EETs in humans. CYP3A4 is highly expressed in breast cancer (Murray, Patimalla, Stewart, Miller, & Heys, 2010) and associated with breast cancer development and progression (Floriano-Sanchez, Rodriguez, Bandala, Coballase-Urrutia, & Lopez-Cruz, 2014). Furthermore, CYP3A4 AA epoxygenase activity is required for the growth of various breast cancer cell lines (Mitra et al., 2011), suggesting that blocking its activity might have beneficial anti-tumorigenic effects. Based on the finding that metformin, a heme binding biguanide used for the treatment of type 2 diabetes, blocks CYP3A4-induced drug metabolism (Choi & Lee, 2012) and reduces *in vitro* and *in vivo* breast cancer cell growth (Alimova et al., 2009; Orecchioni et al., 2015), Guo and colleagues investigated whether metformin binds CYP3A4 inhibiting its epoxygenase activity (Guo et al., 2017). Indeed, co-crystallization of metformin with soluble CYP3A4 revealed metaformin directly binds to the active site heme of CYP3A4 (Guo et al., 2017). Importantly, the biguanide compound (N1-hexyl-N5-benzyl-biguanide) blocks CYP3A4 AA epoxygenase activity with an IC50 of ~9 μM. The findings that this compound does not impair CYP2J2 AA epoxygenase activity and inhibits CYP2C8-mediated EET biosynthesis at high doses (~ 50 μM), suggests that selective CYP3A4 heme binding biguanide compounds can be devised for the treatment of breast cancer.

4.5. Concomitant inhibition of sEH and COX2

COX2 inhibition might have beneficial anti-angiogenic/anti-tumorigenic activity by reducing prostaglandin production and/or the formation of the pro-angiogenic *ct*-8,9-epoxy-11-hydroxy-eicosatrienoic acid from 8,9-EET (see above). In contrast, sEH inhibition should potentiate tumorigenesis and angiogenesis by decreasing EET hydroxylation. Interestingly, Zhang and colleagues found that dual inhibition of COX2 and sEH is beneficial in the setting of cancer. Unexpectedly, a combination of a low-dose COX-2 inhibitor celecoxib and a low-dose sEH inhibitor *4-[[trans-4-[[(tricyclo[3.3.1.13,7]dec-1-ylamino)carbonyl]amino] cyclohexyl]oxy]-benzoic acid* (20 mg/kg/day and 3 mg/kg/day, respectively) synergistically inhibited primary tumor growth and metastasis (G. Zhang et al., 2014). Furthermore, administration of a COX-2/sEH dual inhibitor, (4-(5-phenyl-3-{3-[3-(4-trifluoromethyl-phenyl)-ureido]-propyl}-pyrazol-1-yl)-benzenesulfonamide (PTUPB), inhibited primary growth and metastasis of Lewis lung carcinoma cells by suppressing tumor angiogenesis, without altering tumor cell function (G. Zhang et al., 2014). PTUPB prevented EC tube formation and aortic vessel spring *in vitro* and VEGF-induced angiogenesis in a matrigel plug assay. Mechanistically, PTUPB affects EC function by promoting cell cycle arrest at a G0/1 phase *via* inhibition of CKD3 and CDK6 (G. Zhang et al., 2014). Consistent with these data, systemic

administration of PTUPB (30 mg/kg/day) inhibited lung cancer cell tumor growth as well as associated angiogenesis. These effects were observed despite increased levels of pro-angiogenic EETs in the plasma of mice treated with the dual inhibitor (G. Zhang et al., 2014). A potential explanation for these anti-angiogenic and anti-tumorigenic effects are that the dual inhibitor reduces plasma levels of VEGF in tumor-bearing mice and, as mentioned above, this growth factor is required to promote the pro-angiogenic action of EETs.

Moreover, the same dual inhibitor has been shown to inhibit glioblastoma cell proliferation *via* cell cycle arrest *in vitro*, and to suppress glioblastoma growth and angiogenesis *in vivo* (Li et al., 2017). Expression and activation of the epidermal growth factor receptor and its downstream kinases, ERK and AKT, are reduced in glioblastoma cells treated with PTUPB, indicating that the epidermal growth factor-mediated signaling pathway is a potential target for this dual inhibitor (Li et al., 2017). Finally, PTUPB potentiates the action of chemotherapeutic agents such as cisplatin and gemcitabine (F. Wang et al., 2018). Mechanistically, PTUPB enhanced cisplatin-mediated apoptosis and decreased activation of pro-proliferative and pro-survival pathways including the MAPK and the mTOR pathways (F. Wang et al., 2018). All together, these data indicate that dual COX2/sEH inhibitors either alone or in combination with standard chemotherapy can play a protective role in reducing primary and metastatic tumor growth either by targeting ECs and/or tumor cells.

5. CYP450 epoxygenases in human tumors

5.1. CYP450 epoxygenase expression

Given the divergent effects on tumorigenesis of CYP450 epoxygenase-derived metabolites, it is critical to establish in what context these enzymes impede or promote cancer development and progression. One method to address the potential role of CYP450 epoxygenases in tumorigenesis is by examining the expression of these enzymes in tumors. All three major CYP450 epoxygenases are expressed by a variety of cancers (C. Chen et al., 2011; Cheng et al., 2010; Enayetallah, French, & Grant, 2006; Jiang et al., 2005, 2007; Schmelzle et al., 2011). Expression of CYP2C8 and CYP2C9 correlates with high Ki67 staining, a marker for proliferation, and CYP2J2 levels positively correlate with histological grade and tumor size (Schmelzle et al., 2011; Wei et al., 2014). Tumor microenvironment expression of CYP450 epoxygenases also occurs. In particular, human clear cell renal carcinoma, lung adenoma, and metastatic melanoma all express CYP2C9 in the tumor-associated vasculature indicating a role for endothelial CYP2C9 in tumor-associated angiogenesis and tumor growth (Pozzi et al., 2010).

5.2. Impact of genetic polymorphisms in CYP450 epoxygenases on cancer risk

In addition to expression levels, effects of genetic variation in CYP450 epoxygenases on tumor development and progression needs to be considered. Common genetic polymorphisms in *CYP2C8* and *CYP2C9* affect enzyme function (C. R. Lee, Goldstein, & Pieper, 2002; Zhou, Ingelman-Sundberg, & Lauschke, 2017). Genetic studies examining the association of CYP450 epoxygenase polymorphisms with cancer risk have focused predominantly on *CYP2C9*, specifically two variants that encode proteins with reduced enzymatic activity known as *CYP2C9*2* and *CYP2C9*3* (Rettie, Wienkers, Gonzalez, Trager, & Korzekwa, 1994; Steward et al., 1997). One study reported an association between *CYP2C9* genotype and colorectal cancer (CRC) risk where homozygotes for WT *CYP2C9*1* were more frequent in CRC patients compared to controls. In addition, the loss-of-function *CYP2C9*3* allele associated with lower risk of CRC compared to *CYP2C9*1* (odds ratio = 0.59, 95% confidence interval (CI) = 0.35–0.96) (Martinez et al., 2001); however, a large meta-analysis failed to replicate these associations (Liang, Hu, Cao, & Cai, 2012). Neither *CYP2C9*2* nor *CYP2C9*3* associated with lung cancer risk, but both hypomorphic alleles were overrepresented in head and neck cancer cases (Garcia-Martin et al., 2002; London, Daly, Leathart, Navidi, & Idle, 1996; Yadav et al., 2014). For all of the above studies, EET levels were not measured; thus, it is unknown whether *CYP2C9*2* or *CYP2C9*3* reduced EET synthesis in the relevant endothelium. Regardless, genetic variation in *CYP2C9* does not appear to strongly or consistently influence cancer risk based on previous studies, whereas the effects of *CYP2C8* and *CYP2J2* genetic variation on cancer risk have not been well studied and warrant further investigation in large cohorts.

5.3. Impact of CYP450 epoxygenases on cancer survival and response to therapy

Since CYP450 epoxygenase metabolites alter tumor angiogenesis and metastasis, polymorphisms in these enzymes may alter tumor progression, rather than risk of developing a primary tumor. Unfortunately, few studies address the role of genetics in cancer progression and survival. Our recent study indicated female patients with non-small cell lung cancer (NSCLC) with either the *CYP2C9*2* or *CYP2C9*3* allele survived longer than women homozygous for the *CYP2C9*1* allele (hazard ratio = 0.38, 95% CI = 0.16–0.94) (Sausville et al., 2018). Interestingly, compared to CYP2C9*1, both CYP2C9*2 and CYP2C9*3 variants synthesize fewer EETs, with CYP2C9*3 having the lowest enzymatic activity. These reductions in EET synthesis altered NSCLC tumorigenesis and angiogenesis. Specifically, human NSCLC cells expressing either CYP2C9*2 or CYP2C9*3 formed fewer, smaller and less vascularized tumors compared to tumor cells expressing CYP2C*1. This study indicates that CYP2C9*2 and CYP2C9*3 variants directly limit growth of NSCLC *via* reduced production of pro-tumorigenic and pro-angiogenic EETs (Sausville et al., 2018).

CYP450 epoxygenases metabolize a variety of xenobiotics used for chemoprevention or chemotherapy, potentially affecting response to cancer treatment. CYP2C9*1 oxidizes multiple NSAIDs that exert chemo preventive effects by blocking COX2 activity (Bort, Ponsoda, Carrasco, Gomez-Lechon, & Castell, 1996; Chesne et al., 1998; Hamman, Thompson, & Hall, 1997; Leemann, Transon, Bonnabry, & Dayer, 1993; Miners, Coulter, Tukey, Veronese, & Birkett, 1996; Tracy, Marra, Wrighton, Gonzalez, & Korzekwa, 1996; Zhao, Leemann, & Dayer, 1992). Similar to EET synthesis, variants in CYP2C9 affect NSAID metabolism. CYP2C9*3 reduced NSAID metabolism, and individuals with this variant have reduced NSAID clearance (Kirchheiner et al., 2003; Takanashi et al., 2000; Tang et al., 2001). However, few studies have found that *CYP2C9* variants modify the benefits of NSAID treatment in reducing CRC risk (McGreavey, et al., 2005; Siemes et al., 2009; Bigler et al., 2001). In contrast, Samowitz and colleagues reported differential effects of ibuprofen by *CYP2C9* genotype, with carriers of slower-metabolizing *CYP2C9* variants being more protected from CRC with ibuprofen treatment (p-value of interaction = 0.02) (Samowitz et al., 2006). Many other studies have examined the relationship between *CYP2C9* genotype and NSAID chemoprevention of colon adenoma or CRC, but there has been no consensus on effects.

Another example of gene-drug interactions is *CYP2C8* that serves as the major metabolizer of the chemotherapy drug paclitaxel converting it to the inactive form 6α-hydroxytaxol (Dai et al., 2001; Rahman, Korzekwa, Grogan, Gonzalez, & Harris, 1994). Genetic variation in *CYP2C8* may affect protein function and downstream paclitaxel detoxification and efficacy. Various studies found that CYP2C8*3 metabolizes paclitaxel less effectively than WT CYP2C8, but the impact of the *CYP2C8*3* allele on paclitaxel response and toxicity is controversial (Bergmann et al., 2011; Rowbotham, Boddy, Redfern, Veal, & Daly, 2010). One study showed that the *CYP2C8*3* allele associated with increased risk of paclitaxel-induced neuropathy compared to non-carriers (hazard ratio (per allele) = 1.93, 95% CI = 1.05–3.55); however, another study failed to replicate the association of *CYP2C8*3*

with neuropathy risk (Hertz et al., 2012, 2013). Based on the hypothesis that *CYP2C8* variants slow paclitaxel metabolism, these variants may associate with cancer survival due to genotype dependent treatment efficacy. However, *CYP2C8* variants did not associate with ovarian cancer time to recurrence or survival (Peethambaram et al., 2011; White et al., 2013). Neither study stratified by paclitaxel treatment, limiting the interpretation of the results given *CYP2C8* variants are predicted to alter paclitaxel clearance. Despite limitations, these studies suggest that CYP450 epoxygenases influence both chemoprevention and chemotherapy metabolism, but the effect on cancer risk, cancer-specific survival, and treatment efficacy is still not well understood.

5.4. Associations of genetic polymorphisms in genes that cross-talk with CYP450 epoxygenases and EETs in cancer risk

Given the cross talk of CYP450 epoxygenase-derived EETs with other pathways, genetic variation in mediators of these interactions could potentially impact cancer risk and progression. Two genes that could affect this cross talk are *EPHX2* and *PPARα*, encoding sEH and PPARα, that affect EET steady state and production, respectively. Thus far, few studies have characterized the association of variation in these two genes with cancer risk or survival. Several studies associated *PPARα* polymorphisms with breast cancer risk (Golembesky et al., 2008; Lianggeng, Baiwu, Maoshu, Jiming, & Youshan, 2017; C. T. Wu et al., 2012). The missense *PPARα* SNP, *PPARα L162V* (rs1800206), associated with increased risk of breast cancer. Specifically, carriers with at least one copy of *PPARα L162V*, had a 1.5-fold increased odds of developing breast cancer compared to non-carriers (95% CI = 1.20–1.93). Although functionally this association is not fully understood, one study indicated that the V162 variant induces the transactivation activity of PPARα less effectively, suggesting this variant is hypomorphic. Whether this variant affects the ability of PPARα to downregulate CYP450 epoxygenases has not been investigated, but given the predicted reduced function of the L162V polymorphism, increased CYP450 epoxygenase transcription may drive increased EET synthesis, tumorigenesis, and angiogenesis. If true, the minor allele might be expected to confer the observed higher risk of breast cancer in carriers (Flavell et al., 2000; Lianggeng, Baiwu, Maoshu, Jiming, & Youshan, 2017). Studies of the effects of genetic variation in *EPHX2* are lacking as well. To date, only one study reported a statistically significant association between rs2741354 in *EPHX2* and lung cancer risk (odds ratio = 0.91, 95% CI = 0.86–0.97) (Brenner et al., 2013). However, rs2741354 resides within an intron of *EPHX2*, so whether and how this SNP effects EPHX2 function remains unexplored. As with the CYP450 epoxygenases, it is not only poorly understood whether variation in *PPARα* or *EPHX2* impacts cancer risk, but also if genetic variation in these two genes affects EET synthesis. A summary of the genetic epidemiology studies referenced here is provided in Table 2.

6. Conclusion

The finding that some members of the CYP450 epoxygenase family are upregulated in diseases such as cancer makes them a potential target for anti-cancer therapy. However, whether the activity of these enzymes needs to be enhanced or inhibited depends on the type of cancer and the major products produced by these enzymes.

CYP450 epoxygenases play both pro- and anti-tumorigenic action since they can metabolize different substrates ranging from ω-3 and ω-6 PUFAs to chemotherapeutic agents. In this review, we have discussed the pro-tumorigenic action of these enzymes based to their ability to generate EETs from AA or to inactivate chemo preventive agents. Thus, decreasing CYP450 epoxygenase expression and/or activity might be viewed as a promising anti-cancer therapy. In support of this statement, mice that lack epoxygenases can be protected from tumor development and growth. In addition, downregulating CYP450 epoxygenase expression, inhibiting its enzymatic activity, or enhancing EET hydroxylation has proven anti-tumorigenic action. Furthermore, human genetic and mouse studies indicate that subjects with NSCLC carrying slow metabolizers P450 epoxygenase variants have improved survival and these effects are most likely due to reduced ability of these variants to generate pro-tumorigenic EETs.

The direct relationship of CYP450 epoxygenases to tumorigenesis is complicated by the fact that they can also generate anti-angiogenic factors or less efficiently inactivate pro-carcinogens. In this regard, tobacco intake results in a several fold increase in the risk to head and neck squamous cell carcinomas in individuals carrying slow metabolizers CYP2C variants as compared to individual carrying WT CYP2C (Yadav et al., 2010). Finally, there seems to be evidence that diets rich in ω-3 over ω-6 PUFAs might enhance the production of anti-angiogenic EDPs and EEQs. Thus, whether blocking CYP450 epoxygenases protects from the risk and prognosis of cancer depends on the cancer type, the treatment, the etiology of the cancer, and possibility the diet.

In conclusion, a better and deeper understanding of the biological effects of each CYP450 epoxygenase-derived metabolite is required to determine the types of cancer and the disease stage suitable for CYP450 epoxygenases targeted therapy.

Table 2
Studies characterizing the associations of genetic variation in CYP450 epoxygenases and related genes with cancer risk, survival, and treatment outcome.

Study	Gene Variation Studied	Cancer type	Phenotype	Sample Size	Study Location
Bergmann et al. (2011)	*CYP2C8*	Ovarian	Paclitaxel clearance	93	Denmark and Sweden
Hertz et al. (2012)	*CYP2C8*	Breast	Clinical complete response Treatment-induced toxicity	109	United States of America
Hertz et al. (2013)	*CYP2C8*	Breast	Treatment-induced toxicity	411	United States of America
Peethambaram et al. (2011)	*CYP2C8*	Ovarian	Recurrence Survival	445	United States of America
White et al. (2013)	*CYP2C8*	Ovarian r	Survival	10,084	Multiple
Garcia-Martin et al. (2002)	*CYP2C9*	Lung	Risk of disease	301	Spain
Liang, Hu, Cao, & Cai (2012)	*CYP2C9*	Colorectal	Risk of disease	20,879	Multiple
London, Daly, Leathart, Navidi, & Idle (1996);	*CYP2C9*	Lung	Risk of disease	1029	United States of America
Martinez et al. (2001)	*CYP2C9*	Colorectal	Risk of disease	279	Spain
McGreavey, et al. (2005)	*CYP2C9*	Colorectal	Protective effect of NSAIDs	982	United Kingdom
Samowitz et al. (2006)	*CYP2C9*	Colorectal	Protective effect of NSAIDs	3469	United States of America
Sausville et al. (2018)	*CYP2C9*	NSCLC	Survival	398	United States of America
Siemes et al. (2009)	*CYP2C9*	Colorectal	Protective effect of NSAIDs	6378	Netherlands
Yadav et al. (2014)	*CYP2C9*	Head and neck	Risk of disease	1500	India
Brenner et al. (2013)	*EPHX2*	Lung	Risk of disease	21,152	Multiple
Wu et al. (2012)	*PPARα*	Breast	Risk of disease	606	Taiwan
Golembesky et al. (2008)	*PPARα*	Breast	Risk of disease	3064	United States of America
Lianggeng et al. (2017)	*PPARα*	Breast	Risk of disease	862	China

For each study examining associations between *CYP2C8*, *CYP2C9*, *EPHX2*, or *PPARα*, the following are listed above: the relevant cancer type, the phenotype, sample size, and study location.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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References

Alimova, I. N., Liu, B., Fan, Z., Edgerton, S. M., Dillon, T., Lind, S. E., & Thor, A. D. (2009). Metformin inhibits breast cancer cell growth, colony formation and induces cell cycle arrest in vitro. *Cell Cycle 8*, 909–915.

Arnold, C., Markovic, M., Blossey, K., Wallukat, G., Fischer, R., Dechend, R., ... Schunck, W. H. (2010). Arachidonic acid-metabolizing cytochrome P450 enzymes are targets of {omega}-3 fatty acids. *The Journal of Biological Chemistry 285*, 32720–32733.

Bergmann, T. K., Brasch-Andersen, C., Green, H., Mirza, M., Pedersen, R. S., Nielsen, F., ... Brosen, K. (2011). Impact of CYP2C8*3 on paclitaxel clearance: A population pharmacokinetic and pharmacogenomic study in 93 patients with ovarian cancer. *The Pharmacogenomics Journal 11*, 113–120.

Bigler, J., Whitton, J., Lampe, J. W., Fosdick, L., Bostick, R. M., & Potter, J. D. (2001). CYP2C9 and UGT1A6 genotypes modulate the protective effect of aspirin on colon adenoma risk. *Cancer Research 61*, 3566–3569.

Bort, R., Ponsoda, X., Carrasco, E., Gomez-Lechon, M. J., & Castell, J. V. (1996). Metabolism of aceclofenac in humans. *Drug Metabolism and Disposition 24*, 834–841.

Brenna, J. T., & Diau, G. Y. (2007). The influence of dietary docosahexaenoic acid and arachidonic acid on central nervous system polyunsaturated fatty acid composition. *Prostaglandins, Leukotrienes, and Essential Fatty Acids 77*, 247–250.

Brenner, D. R., Brennan, P., Boffetta, P., Amos, C. I., Spitz, M. R., Chen, C., ... Hung, R. J. (2013). Hierarchical modeling identifies novel lung cancer susceptibility variants in inflammation pathways among 10,140 cases and 11,012 controls. *Human Genetics 132*, 579–589.

Briscoe, C. P., Tadayyon, M., Andrews, J. L., Benson, W. G., Chambers, J. K., Eilert, M. M., ... Muir, A. I. (2003). The orphan G protein-coupled receptor GPR40 is activated by medium and long chain fatty acids. *The Journal of Biological Chemistry 278*, 11303–11311.

Campbell, W. B., Deeter, C., Gauthier, K. M., Ingraham, R. H., Falck, J. R., & Li, P. L. (2002). 14,15-Dihydroxyeicosatrienoic acid relaxes bovine coronary arteries by activation of K(Ca) channels. *American Journal of Physiology. Heart and Circulatory Physiology 282*, H1656–H1664.

Campbell, W. B., & Falck, J. R. (2007). Arachidonic acid metabolites as endothelium-derived hyperpolarizing factors. *Hypertension 49*, 590–596.

Capdevila, J. H., & Falck, J. R. (2001). The CYP P450 arachidonic acid monooxygenases: From cell signaling to blood pressure regulation. *Biochemical and Biophysical Research Communications 285*, 571–576.

Carroll, M. A., Balazy, M., Margiotta, P., Falck, J. R., & McGiff, J. C. (1993). Renal vasodilator activity of 5,6-epoxyeicosatrienoic acid depends upon conversion by cyclooxygenase and release of prostaglandins. *The Journal of Biological Chemistry 268*, 12260–12266.

Chacos, N., Capdevila, J., Falck, J. R., Manna, S., Martin-Wixtrom, C., Gill, S. S., ... Estabrook, R. W. (1983). The reaction of arachidonic acid epoxides (epoxyeicosatrienoic acids) with a cytosolic epoxide hydrolase. *Archives of Biochemistry and Biophysics 223*, 639–648.

Chen, C., Wei, X., Rao, X., Wu, J., Yang, S., Chen, F., ... Wang, D. W. (2011). Cytochrome P450 2J2 is highly expressed in hematologic malignant diseases and promotes tumor cell growth. *The Journal of Pharmacology and Experimental Therapeutics 336*, 344–355.

Chen, J. K., Capdevila, J., & Harris, R. C. (2002). Heparin-binding EGF-like growth factor mediates the biological effects of P450 arachidonate epoxygenase metabolites in epithelial cells. *Proceedings of the National Academy of Sciences of the United States of America 99*, 6029–6034.

Chen, Y., Falck, J. R., Tuniki, V. R., & Campbell, W. B. (2009). 20-125Iodo-14,15-epoxyeicosa-5(Z)-enoic acid: A high-affinity radioligand used to characterize the epoxyeicosatrienoic acid antagonist binding site. *The Journal of Pharmacology and Experimental Therapeutics 331*, 1137–1145.

Cheng, L. M., Jiang, J. G., Sun, Z. Y., Chen, C., Dackor, R. T., Zeldin, D. C., & Wang, D. W. (2010). The epoxyeicosatrienoic acid-stimulated phosphorylation of EGF-R involves the activation of metalloproteinases and the release of HB-EGF in cancer cells. *Acta Pharmacologica Sinica 31*, 211–218.

Cheranov, S. Y., Karpurapu, M., Wang, D., Zhang, B., Venema, R. C., & Rao, G. N. (2008). An essential role for SRC-activated STAT-3 in 14,15-EET-induced VEGF expression and angiogenesis. *Blood 111*, 5581–5591.

Chesne, C., Guyomard, C., Guillouzo, A., Schmid, J., Ludwig, E., & Sauter, T. (1998). Metabolism of Meloxicam in human liver involves cytochromes P4502C9 and 3A4. *Xenobiotica 28*, 1–13.

Choi, Y. H., & Lee, M. G. (2012). Pharmacokinetic and pharmacodynamic interaction between nifedipine and metformin in rats: Competitive inhibition for metabolism of nifedipine and metformin by each other via CYP isozymes. *Xenobiotica 42*, 483–495.

Crawford, M. A., Casperd, N. M., & Sinclair, A. J. (1976). The long chain metabolites of linoleic avid linolenic acids in liver and brain in herbivores and carnivores. *Comparative Biochemistry and Physiology. B 54*, 395–401.

Cui, P. H., Petrovic, N., & Murray, M. (2011). The omega-3 epoxide of eicosapentaenoic acid inhibits endothelial cell proliferation by p38 MAP kinase activation and cyclin D1/CDK4 down-regulation. *British Journal of Pharmacology 162*, 1143–1155.

Dai, D., Zeldin, D. C., Blaisdell, J. A., Chanas, B., Coulter, S. J., Ghanayem, B. I., & Goldstein, J. A. (2001). Polymorphisms in human CYP2C8 decrease metabolism of the anticancer drug paclitaxel and arachidonic acid. *Pharmacogenetics 11*, 597–607.

Daikh, B. E., Lasker, J. M., Raucy, J. L., & Koop, D. R. (1994). Regio- and stereoselective epoxidation of arachidonic acid by human cytochromes P450 2C8 and 2C9. *The Journal of Pharmacology and Experimental Therapeutics 271*, 1427–1433.

Delozier, T. C., Kissling, G. E., Coulter, S. J., Dai, D., Foley, J. F., Bradbury, J. A., ... Goldstein, J. A. (2007). Detection of human CYP2C8, CYP2C9, and CYP2J2 in cardiovascular tissues. *Drug Metabolism and Disposition 35*, 682–688.

Ding, Y., Fromel, T., Popp, R., Falck, J. R., Schunck, W. H., & Fleming, I. (2014). The biological actions of 11,12-epoxyeicosatrienoic acid in endothelial cells are specific to the R/S-enantiomer and require the G(s) protein. *The Journal of Pharmacology and Experimental Therapeutics 350*, 14–21.

Enayetallah, A. E., French, R. A., & Grant, D. F. (2006). Distribution of soluble epoxide hydrolase, cytochrome P450 2C8, 2C9 and 2J2 in human malignant neoplasms. *Journal of Molecular Histology 37*, 133–141.

Fer, M., Dreano, Y., Lucas, D., Corcos, L., Salaun, J. P., Berthou, F., & Amet, Y. (2008). Metabolism of eicosapentaenoic and docosahexaenoic acids by recombinant human cytochromes P450. *Archives of Biochemistry and Biophysics 471*, 116–125.

Fischer, R., Konkel, A., Mehling, H., Blossey, K., Gapelyuk, A., Wessel, N., ... Schunck, W. H. (2014). Dietary omega-3 fatty acids modulate the eicosanoid profile in man primarily via the CYP-epoxygenase pathway. *Journal of Lipid Research 55*, 1150–1164.

Flavell, D. M., Pineda Torra, I., Jamshidi, Y., Evans, D., Diamond, J. R., Elkeles, R. S., ... Humphries, S. E. (2000). Variation in the PPARalpha gene is associated with altered function in vitro and plasma lipid concentrations in Type II diabetic subjects. *Diabetologia 43*, 673–680.

Fleming, I. (2008). Vascular cytochrome p450 enzymes: Physiology and pathophysiology. *Trends in Cardiovascular Medicine 18*, 20–25.

Floriano-Sanchez, E., Rodriguez, N. C., Bandala, C., Coballase-Urrutia, E., & Lopez-Cruz, J. (2014). CYP3A4 expression in breast cancer and its association with risk factors in Mexican women. *Asian Pacific Journal of Cancer Prevention 15*, 3805–3809.

Fromel, T., Jungblut, B., Hu, J., Trouvain, C., Barbosa-Sicard, E., Popp, R., ... Fleming, I. (2012). Soluble epoxide hydrolase regulates hematopoietic progenitor cell function via generation of fatty acid diols. *Proceedings of the National Academy of Sciences of the United States of America 109*, 9995–10000.

Garcia-Martin, E., Martinez, C., Ladero, J. M., Gamito, F. J., Rodriguez-Lescure, A., & Agundez, J. A. (2002). Influence of cytochrome P450 CYP2C9 genotypes in lung cancer risk. *Cancer Letters 180*, 41–46.

Goetze, S., Eilers, F., Bungenstock, A., Kintscher, U., Stawowy, P., Blaschke, F., ... Grafe, M. (2002). PPAR activators inhibit endothelial cell migration by targeting Akt. *Biochemical and Biophysical Research Communications 293*, 1431–1437.

Golembesky, A. K., Gammon, M. D., North, K. E., Bensen, J. T., Schroeder, J. C., Teitelbaum, S. L., ... Santella, R. M. (2008). Peroxisome proliferator-activated receptor-alpha (PPARA) genetic polymorphisms and breast cancer risk: A Long Island ancillary study. *Carcinogenesis 29*, 1944–1949.

Gong, Y., Shao, Z., Fu, Z., Edin, M. L., Sun, Y., Liegl, R. G., ... Smith, L. E. H. (2016). Fenofibrate inhibits cytochrome P450 epoxygenase 2C activity to suppress pathological ocular angiogenesis. *eBioMedicine 13*, 201–211.

Groeger, A. L., Cipollina, C., Cole, M. P., Woodcock, S. R., Bonacci, G., Rudolph, T. K., ... Schopfer, F. J. (2010). Cyclooxygenase-2 generates anti-inflammatory mediators from omega-3 fatty acids. *Nature Chemical Biology 6*, 433–441.

Guo, Z., Sevrioukova, I. F., Denisov, I. G., Zhang, X., Chiu, T. L., Thomas, D. G., ... Potter, D. A. (2017). Heme binding biguanides target cytochrome P450-dependent cancer cell mitochondria. *Cell Chemical Biology 24*, 1314.

Hamman, M. A., Thompson, G. A., & Hall, S. D. (1997). Regioselective and stereoselective metabolism of ibuprofen by human cytochrome P450 2C. *Biochemical Pharmacology 54*, 33–41.

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: The next generation. *Cell 144*, 646–674.

Hertz, D. L., Motsinger-Reif, A. A., Drobish, A., Winham, S. J., McLeod, H. L., Carey, L. A., & Dees, E. C. (2012). CYP2C8*3 predicts benefit/risk profile in breast cancer patients receiving neoadjuvant paclitaxel. *Breast Cancer Research and Treatment 134*, 401–410.

Hertz, D. L., Roy, S., Motsinger-Reif, A. A., Drobish, A., Clark, L. S., McLeod, H. L., ... Dees, E. C. (2013). CYP2C8*3 increases risk of neuropathy in breast cancer patients treated with paclitaxel. *Annals of Oncology 24*, 1472–1478.

Issemann, I., & Green, S. (1990). Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature 347*, 645–650.

Jiang, J. G., Chen, C. L., Card, J. W., Yang, S., Chen, J. X., Fu, X. N., ... Wang, D. W. (2005). Cytochrome P450 2J2 promotes the neoplastic phenotype of carcinoma cells and is up-regulated in human tumors. *Cancer Research 65*, 4707–4715.

Jiang, J. G., Ning, Y. G., Chen, C., Ma, D., Liu, Z. J., Yang, S., ... Wang, D. W. (2007). Cytochrome p450 epoxygenase promotes human cancer metastasis. *Cancer Research 67*, 6665–6674.

Keenan, A. H., Pedersen, T. L., Fillaus, K., Larson, M. K., Shearer, G. C., & Newman, J. W. (2012). Basal omega-3 fatty acid status affects fatty acid and oxylipin responses to high-dose n3-HUFA in healthy volunteers. *Journal of Lipid Research 53*, 1662–1669.

Kerb, R., Fux, R., Morike, K., Kremsner, P. G., Gil, J. P., Gleiter, C. H., & Schwab, M. (2009). Pharmacogenetics of antimalarial drugs: Effect on metabolism and transport. *The Lancet Infectious Diseases 9*, 760–774.

Kim, H. Y., Karanian, J. W., & Salem, N.,. J. (1990). Formation of 15-lipoxygenase product from docosahexaenoic acid (22:6w3) by human platelets. *Prostaglandins 40*, 539–549.

Kirchheiner, J., Stormer, E., Meisel, C., Steinbach, N., Roots, I., & Brockmoller, J. (2003). Influence of CYP2C9 genetic polymorphisms on pharmacokinetics of celecoxib and its metabolites. *Pharmacogenetics 13*, 473–480.

Lee, C. A., Neul, D., Clouser-Roche, A., Dalvie, D., Wester, M. R., Jiang, Y., ... Totah, R. A. (2010). Identification of novel substrates for human cytochrome P450 2J2. *Drug Metabolism and Disposition 38*, 347–356.

Lee, C. R., Goldstein, J. A., & Pieper, J. A. (2002). Cytochrome P450 2C9 polymorphisms: A comprehensive review of the in-vitro and human data. *Pharmacogenetics 12*, 251–263.

Leemann, T. D., Transon, C., Bonnabry, P., & Dayer, P. (1993). A major role for cytochrome P450TB (CYP2C subfamily) in the actions of non-steroidal antiinflammatory drugs. *Drugs under Experimental and Clinical Research 19*, 189–195.

Li, J., Zhou, Y., Wang, H., Gao, Y., Li, L., Hwang, S. H., ... Hammock, B. D. (2017). COX-2/sEH dual inhibitor PTUPB suppresses glioblastoma growth by targeting epidermal growth factor receptor and hyaluronan mediated motility receptor. *Oncotarget 8*, 87353–87363.

Liang, S., Hu, J., Cao, W., & Cai, S. (2012). Meta-analysis of cytochrome P-450 2C9 polymorphism and colorectal cancer risk. *PLoS One 7*, e49134.

Lianggeng, X., Baiwu, L., Maoshu, B., Jiming, L., & Youshan, L. (2017). Impact of interaction between PPAR alpha and PPAR gamma on breast cancer risk in the Chinese Han population. *Clinical Breast Cancer 17*, 336–340.

Liu, X., Qian, Z. Y., Xie, F., Fan, W., Nelson, J. W., Xiao, X., ... Alkayed, N. J. (2017). Functional screening for G protein-coupled receptor targets of 14,15-epoxyeicosatrienoic acid. *Prostaglandins & Other Lipid Mediators 132*, 31–40.

London, S. J., Daly, A. K., Leathart, J. B., Navidi, W. C., & Idle, J. R. (1996). Lung cancer risk in relation to the CYP2C9*1/CYP2C9*2 genetic polymorphism among African-Americans and Caucasians in Los Angeles County, California. *Pharmacogenetics 6*, 527–533.

Luo, J., Yao, J. F., Deng, X. F., Zheng, X. D., Jia, M., Wang, Y. Q., ... Zhu, J. H. (2018). 14, 15-EET induces breast cancer cell EMT and cisplatin resistance by up-regulating integrin alphavbeta3 and activating FAK/PI3K/AKT signaling. *Journal of Experimental & Clinical Cancer Research 37*, 23.

Ma, S. K., Wang, Y., Chen, J., Zhang, M. Z., Harris, R. C., & Chen, J. K. (2015). Overexpression of G-protein-coupled receptor 40 enhances the mitogenic response to epoxyeicosatrienoic acids. *PLoS One 10*, e0113130.

Malkowski, M. G., Thuresson, E. D., Lakkides, K. M., Rieke, C. J., Micielli, R., Smith, W. L., & Garavito, R. M. (2001). Structure of eicosapentaenoic and linoleic acids in the cyclooxygenase site of prostaglandin endoperoxide H synthase-1. *The Journal of Biological Chemistry 276*, 37547–37555.

Martinez, C., Garcia-Martin, E., Ladero, J. M., Sastre, J., Garcia-Gamito, F., Diaz-Rubio, M., & Agundez, J. A. (2001). Association of CYP2C9 genotypes leading to high enzyme activity and colorectal cancer risk. *Carcinogenesis 22*, 1323–1326.

McGreavey, L. E., Turner, F., Smith, G., Boylan, K., Timothy Bishop, D., Forman, D., ... Colorectal Cancer Study, G (2005). No evidence that polymorphisms in CYP2C8, CYP2C9, UGT1A6, PPARdelta and PPARgamma act as modifiers of the protective effect of regular NSAID use on the risk of colorectal carcinoma. *Pharmacogenetics and Genomics 15*, 713–721.

Medhora, M., Daniels, J., Mundey, K., Fisslthaler, B., Busse, R., Jacobs, E. R., & Harder, D. R. (2003). Epoxygenase-driven angiogenesis in human lung microvascular endothelial cells. *American Journal of Physiology. Heart and Circulatory Physiology 284*, H215–H224.

Michaelis, U. R., Falck, J. R., Schmidt, R., Busse, R., & Fleming, I. (2005). Cytochrome P4502C9-derived epoxyeicosatrienoic acids induce the expression of cyclooxygenase-2 in endothelial cells. *Arteriosclerosis, Thrombosis, and Vascular Biology 25*, 321–326.

Michaelis, U. R., Fisslthaler, B., Medhora, M., Harder, D., Fleming, I., & Busse, R. (2003). Cytochrome P450 2C9-derived epoxyeicosatrienoic acids induce angiogenesis via cross-talk with the epidermal growth factor receptor (EGFR). *The FASEB Journal 17*, 770–772.

Miller, C., Yamaguchi, R. Y., & Ziboh, V. A. (1989). Guinea pig epidermis generates putative anti-inflammatory metabolites from fish oil polyunsaturated fatty acids. *Lipids 24*, 998–1003.

Miners, J. O., Coulter, S., Tukey, R. H., Veronese, M. E., & Birkett, D. J. (1996). Cytochromes P450, 1A2, and 2C9 are responsible for the human hepatic O-demethylation of R- and S-naproxen. *Biochemical Pharmacology 51*, 1003–1008.

Mitra, R., Guo, Z., Milani, M., Mesaros, C., Rodriguez, M., Nguyen, J., ... Potter, D. A. (2011). CYP3A4 mediates growth of estrogen receptor-positive breast cancer cells in part by inducing nuclear translocation of phospho-Stat3 through biosynthesis of (+/−)-14,15-epoxyeicosatrienoic acid (EET). *The Journal of Biological Chemistry 286*, 17543–17559.

Moreland, K. T., Procknow, J. D., Sprague, R. S., Iverson, J. L., Lonigro, A. J., & Stephenson, A. H. (2007). Cyclooxygenase (COX)-1 and COX-2 participate in 5,6-epoxyeicosatrienoic acid-induced contraction of rabbit intralobar pulmonary arteries. *The Journal of Pharmacology and Experimental Therapeutics 321*, 446–454.

Morin, C., Sirois, M., Echave, V., Rizcallah, E., & Rousseau, E. (2009). Relaxing effects of 17 (18)-EpETE on arterial and airway smooth muscles in human lung. *American Journal of Physiology. Lung Cellular and Molecular Physiology 296*, L130–L139.

Morisseau, C., Inceoglu, B., Schmelzer, K., Tsai, H. J., Jinks, S. L., Hegedus, C. M., & Hammock, B. D. (2010). Naturally occurring monoepoxides of eicosapentaenoic acid and docosahexaenoic acid are bioactive antihyperalgesic lipids. *Journal of Lipid Research 51*, 3481–3490.

Munzenmaier, D. H., & Harder, D. R. (2000). Cerebral microvascular endothelial cell tube formation: Role of astrocytic epoxyeicosatrienoic acid release. *American Journal of Physiology. Heart and Circulatory Physiology 278*, H1163–H1167.

Murray, G. I., Patimalla, S., Stewart, K. N., Miller, I. D., & Heys, S. D. (2010). Profiling the expression of cytochrome P450 in breast cancer. *Histopathology 57*, 202–211.

Nabavi, S. F., Bilotto, S., Russo, G. L., Orhan, I. E., Habtemariam, S., Daglia, M., ... Nabavi, S. M. (2015). Omega-3 polyunsaturated fatty acids and cancer: Lessons learned from clinical trials. *Cancer Metastasis Reviews 34*, 359–380.

Nithipatikom, K., Brody, D. M., Tang, A. T., Manthati, V. L., Falck, J. R., Williams, C. L., & Campbell, W. B. (2010). Inhibition of carcinoma cell motility by epoxyeicosatrienoic acid (EET) antagonists. *Cancer Science 101*, 2629–2636.

Oliw, E. H. (1994). Oxygenation of polyunsaturated fatty acids by cytochrome P450 monooxygenases. *Progress in Lipid Research 33*, 329–354.

Orecchioni, S., Reggiani, F., Talarico, G., Mancuso, P., Calleri, A., Gregato, G., ... Bertolini, F. (2015). The biguanides metformin and phenformin inhibit angiogenesis, local and metastatic growth of breast cancer by targeting both neoplastic and microenvironment cells. *International Journal of Cancer 136*, E534–E544.

Panigrahy, D., Edin, M. L., Lee, C. R., Huang, S., Bielenberg, D. R., Butterfield, C. E., ... Zeldin, D. C. (2012). Epoxyeicosanoids stimulate multiorgan metastasis and tumor dormancy escape in mice. *The Journal of Clinical Investigation 122*, 178–191.

Panigrahy, D., Kaipainen, A., Huang, S., Butterfield, C. E., Barnes, C. M., Fannon, M., ... Kieran, M. W. (2008). PPARalpha agonist fenofibrate suppresses tumor growth through direct and indirect angiogenesis inhibition. *Proceedings of the National Academy of Sciences of the United States of America 105*, 985–990.

Park, S. K., Herrnreiter, A., Pfister, S. L., Gauthier, K. M., Falck, B. A., Falck, J. R., & Campbell, W. B. (2018). GPR40 is a low-affinity epoxyeicosatrienoic acid receptor in vascular cells. *The Journal of Biological Chemistry 293*, 10675–10691.

Peethambaram, P., Fridley, B. L., Vierkant, R. A., Larson, M. C., Kalli, K. R., Elliott, E. A., ... Goode, E. L. (2011). Polymorphisms in ABCB1 and ERCC2 associated with ovarian cancer outcome. *International Journal of Molecular Epidemiology and Genetics 2*, 185–195.

Pozzi, A., Ibanez, M. R., Gatica, A. E., Yang, S., Wei, S., Mei, S., ... Capdevila, J. H. (2007). Peroxisomal proliferator-activated receptor-alpha-dependent inhibition of endothelial cell proliferation and tumorigenesis. *The Journal of Biological Chemistry 282*, 17685–17695.

Pozzi, A., Macias-Perez, I., Abair, T., Wei, S., Su, Y., Zent, R., ... Capdevila, J. H. (2005). Characterization of 5,6- and 8,9-epoxyeicosatrienoic acids (5,6- and 8,9-EET) as potent in vivo angiogenic lipids. *The Journal of Biological Chemistry 280*, 27138–27146.

Pozzi, A., Popescu, V., Yang, S., Mei, S., Shi, M., Puolitaival, S. M., ... Capdevila, J. H. (2010). The anti-tumorigenic properties of peroxisomal proliferator-activated receptor alpha are arachidonic acid epoxygenase-mediated. *The Journal of Biological Chemistry 285*, 12840–12850.

Rahman, A., Korzekwa, K. R., Grogan, J., Gonzalez, F. J., & Harris, J. W. (1994). Selective biotransformation of taxol to 6 alpha-hydroxytaxol by human cytochrome P450 2C8. *Cancer Research 54*, 5543–5546.

Rand, A. A., Barnych, B., Morisseau, C., Cajka, T., Lee, K. S. S., Panigrahy, D., & Hammock, B. D. (2017). Cyclooxygenase-derived proangiogenic metabolites of epoxyeicosatrienoic acids. *Proceedings of the National Academy of Sciences of the United States of America 114*, 4370–4375.

Rettie, A. E., Wienkers, L. C., Gonzalez, F. J., Trager, W. F., & Korzekwa, K. R. (1994). Impaired (S)-warfarin metabolism catalysed by the R144C allelic variant of CYP2C9. *Pharmacogenetics 4*, 39–42.

Rifkind, A. B., Lee, C., Chang, T. K., & Waxman, D. J. (1995). Arachidonic acid metabolism by human cytochrome P450s 2C8, 2C9, 2E1, and 1A2: Regioselective oxygenation and evidence for a role for CYP2C enzymes in arachidonic acid epoxygenation in human liver microsomes. *Archives of Biochemistry and Biophysics 320*, 380–389.

Rowbotham, S. E., Boddy, A. V., Redfern, C. P., Veal, G. J., & Daly, A. K. (2010). Relevance of nonsynonymous CYP2C8 polymorphisms to 13-cis retinoic acid and paclitaxel hydroxylation. *Drug Metabolism and Disposition 38*, 1261–1266.

Saidi, S. A., Holland, C. M., Charnock-Jones, D. S., & Smith, S. K. (2006). In vitro and in vivo effects of the PPAR-alpha agonists fenofibrate and retinoic acid in endometrial cancer. *Molecular Cancer 5*, 13.

Samowitz, W. S., Wolff, R. K., Curtin, K., Sweeney, C., Ma, K. N., Andersen, K., ... Slattery, M. L. (2006). Interactions between CYP2C9 and UGT1A6 polymorphisms and nonsteroidal anti-inflammatory drugs in colorectal cancer prevention. *Clinical Gastroenterology and Hepatology 4*, 894–901.

SanGiovanni, J. P., & Chew, E. Y. (2005). The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina. *Progress in Retinal and Eye Research 24*, 87–138.

Sapieha, P., Stahl, A., Chen, J., Seaward, M. R., Willett, K. L., Krah, N. M., ... Smith, L. E. (2011). 5-Lipoxygenase metabolite 4-HDHA is a mediator of the antiangiogenic effect of omega-3 polyunsaturated fatty acids. *Science Translational Medicine 3*, 69ra12.

Sausville, L. N., Gangadhariah, M., Chiusa, M., Mei, S., Wei, S., Zent, R., ... Pozzi, A. (2018). The cytochrome P450 slow metabolizers CYP2C9*2 and CYP2C9*3 directly regulate tumorigenesis via reduced epoxyeicosatrienoic acid production. *Cancer Research 78*, 4865–4877.

Schmelzle, M., Dizdar, L., Matthaei, H., Baldus, S. E., Wolters, J., Lindenlauf, N., ... Stoecklein, N. H. (2011). Esophageal cancer proliferation is mediated by cytochrome P450 2C9 (CYP2C9). *Prostaglandins & Other Lipid Mediators 94*, 25–33.

Schneider, C., & Pozzi, A. (2011). Cyclooxygenases and lipoxygenases in cancer. *Cancer Metastasis Reviews 30*, 277–294.

Shao, Z., Fu, Z., Stahl, A., Joyal, J. S., Hatton, C., Juan, A., ... Smith, L. E. (2014). Cytochrome P450 2C8 omega3-long-chain polyunsaturated fatty acid metabolites increase mouse retinal pathologic neovascularization–brief report. *Arteriosclerosis, Thrombosis, and Vascular Biology 34*, 581–586.

Shearer, G. C., Harris, W. S., Pedersen, T. L., & Newman, J. W. (2010). Detection of omega-3 oxylipins in human plasma and response to treatment with omega-3 acid ethyl esters. *Journal of Lipid Research 51*, 2074–2081.

Siemes, C., Eijgelsheim, M., Dieleman, J. P., van Schaik, R. H., Uitterlinden, A. G., van Duijn, C. M., ... Visser, L. E. (2009). No modification of the beneficial effect of NSAIDs on colorectal cancer by CYP2C9 genotype. *The Netherlands Journal of Medicine 67*, 134–141.

Simopoulos, A. P. (2008). The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Experimental Biology and Medicine (Maywood, N.J.) 233*, 674–688.

Skrypnyk, N., Chen, X., Hu, W., Su, Y., Mont, S., Yang, S., ... Pozzi, A. (2014). PPARalpha activation can help prevent and treat non-small cell lung cancer. *Cancer Research 74*, 621–631.

Spector, A. A., & Kim, H. Y. (2015). Cytochrome P450 epoxygenase pathway of polyunsaturated fatty acid metabolism. *Biochimica et Biophysica Acta 1851*, 356–365.

Spiecker, M., & Liao, J. K. (2005). Vascular protective effects of cytochrome p450 epoxygenase-derived eicosanoids. *Archives of Biochemistry and Biophysics 433*, 413–420.

Steward, D. J., Haining, R. L., Henne, K. R., Davis, G., Rushmore, T. H., Trager, W. F., & Rettie, A. E. (1997). Genetic association between sensitivity to warfarin and expression of CYP2C9*3. *Pharmacogenetics 7*, 361–367.

Takanashi, K., Tainaka, H., Kobayashi, K., Yasumori, T., Hosakawa, M., & Chiba, K. (2000). CYP2C9 Ile359 and Leu359 variants: Enzyme kinetic study with seven substrates. *Pharmacogenetics 10*, 95–104.

Tang, C., Shou, M., Rushmore, T. H., Mei, Q., Sandhu, P., Woolf, E. J., ... Rodrigues, A. D. (2001). In-vitro metabolism of celecoxib, a cyclooxygenase-2 inhibitor, by allelic variant forms of human liver microsomal cytochrome P450 2C9: Correlation with CYP2C9 genotype and in-vivo pharmacokinetics. *Pharmacogenetics 11*, 223–235.

Thuy Phuong, N. T., Kim, J. W., Kim, J. A., Jeon, J. S., Lee, J. Y., Xu, W. J., ... Kang, K. W. (2017). Role of the CYP3A4-mediated 11,12-epoxyeicosatrienoic acid pathway in the development of tamoxifen-resistant breast cancer. *Oncotarget 8*, 71054–71069.

Tracy, T. S., Marra, C., Wrighton, S. A., Gonzalez, F. J., & Korzekwa, K. R. (1996). Studies of flurbiprofen 4′-hydroxylation. Additional evidence suggesting the sole involvement of cytochrome P450 2C9. *Biochemical Pharmacology 52*, 1305–1309.

Van Booven, D., Marsh, S., McLeod, H., Carrillo, M. W., Sangkuhl, K., Klein, T. E., & Altman, R. B. (2010). Cytochrome P450 2C9-CYP2C9. *Pharmacogenet Genomics 20*, 277–281.

Varet, J., Vincent, L., Mirshahi, P., Pille, J. V., Legrand, E., Opolon, P., ... Soria, C. (2003). Fenofibrate inhibits angiogenesis in vitro and in vivo. *Cellular and Molecular Life Sciences 60*, 810–819.

Wahli, W., Braissant, O., & Desvergne, B. (1995). Peroxisome proliferator activated receptors: Transcriptional regulators of adipogenesis, lipid metabolism and more. *Chemistry & Biology 2*, 261–266.

Wang, F., Zhang, H., Ma, A. H., Yu, W., Zimmermann, M., Yang, J., ... Pan, C. X. (2018). COX-2/sEH dual inhibitor PTUPB potentiates the antitumor efficacy of cisplatin. *Molecular Cancer Therapeutics 17*, 474–483.

Wang, Y., Wei, X., Xiao, X., Hui, R., Card, J. W., Carey, M. A., ... Zeldin, D. C. (2005). Arachidonic acid epoxygenase metabolites stimulate endothelial cell growth and angiogenesis via mitogen-activated protein kinase and phosphatidylinositol 3-kinase/Akt signaling pathways. *The Journal of Pharmacology and Experimental Therapeutics 314*, 522–532.

Webler, A. C., Michaelis, U. R., Popp, R., Barbosa-Sicard, E., Murugan, A., Falck, J. R., ... Fleming, I. (2008). Epoxyeicosatrienoic acids are part of the VEGF-activated signaling cascade leading to angiogenesis. *American Journal of Physiology. Cell Physiology 295*, C1292–C1301.

Wei, X., Zhang, D., Dou, X., Niu, N., Huang, W., Bai, J., & Zhang, G. (2014). Elevated 14,15-epoxyeicosatrienoic acid by increasing of cytochrome P450 2C8, 2C9 and 2J2 and decreasing of soluble epoxide hydrolase associated with aggressiveness of human breast cancer. *BMC Cancer 14*, 841.

Westphal, C., Konkel, A., & Schunck, W. H. (2011). CYP-eicosanoids–a new link between omega-3 fatty acids and cardiac disease? *Prostaglandins & Other Lipid Mediators 96*, 99–108.

White, K. L., Vierkant, R. A., Fogarty, Z. C., Charbonneau, B., Block, M. S., Pharoah, P. D., ... Goode, E. L. (2013). Analysis of over 10,000 Cases finds no association between previously reported candidate polymorphisms and ovarian cancer outcome. *Cancer Epidemiology, Biomarkers & Prevention 22*, 987–992.

Wong, P. Y., Lai, P. S., & Falck, J. R. (2000). Mechanism and signal transduction of 14 (R), 15 (S)-epoxyeicosatrienoic acid (14,15-EET) binding in guinea pig monocytes. *Prostaglandins & Other Lipid Mediators 62*, 321–333.

Wong, P. Y., Lai, P. S., Shen, S. Y., Belosludtsev, Y. Y., & Falck, J. R. (1997). Post-receptor signal transduction and regulation of 14(R),15(S)-epoxyeicosatrienoic acid (14,15-EET) binding in U-937 cells. *Journal of Lipid Mediators and Cell Signalling 16*, 155–169.

Wong, P. Y., Lin, K. T., Yan, Y. T., Ahern, D., Iles, J., Shen, S. Y., ... Falck, J. R. (1993). 14(R),15(S)-epoxyeicosatrienoic acid (14(R),15(S)-EET) receptor in guinea pig mononuclear cell membranes. *Journal of Lipid Mediators 6*, 199–208.

Wu, C. T., Cheng, Y. H., Chen, F. N., Chen, D. R., Wei, M. F., & Chang, N. W. (2012). Combined effects of peroxisome proliferator-activated receptor alpha and apolipoprotein E polymorphisms on risk of breast cancer in a Taiwanese population. *Journal of Investigative Medicine 60*, 1209–1213.

Wu, S., Moomaw, C. R., Tomer, K. B., Falck, J. R., & Zeldin, D. C. (1996). Molecular cloning and expression of CYP2J2, a human cytochrome P450 arachidonic acid epoxygenase highly expressed in heart. *The Journal of Biological Chemistry 271*, 3460–3468.

Yadav, S. S., Ruwali, M., Pant, M. C., Shukla, P., Singh, R. L., & Parmar, D. (2010). Interaction of drug metabolizing cytochrome P450 2D6 poor metabolizers with cytochrome P450 2C9 and 2C19 genotypes modify the susceptibility to head and neck cancer and treatment response. *Mutation Research 684*, 49–55.

Yadav, S. S., Seth, S., Khan, A. J., Maurya, S. S., Dhawan, A., Pant, S., ... Parmar, D. (2014). Association of polymorphism in cytochrome P450 2C9 with susceptibility to head and neck cancer and treatment outcome. *Applied & Translational Genomics 3*, 8–13.

Yan, G., Chen, S., You, B., & Sun, J. (2008). Activation of sphingosine kinase-1 mediates induction of endothelial cell proliferation and angiogenesis by epoxyeicosatrienoic acids. *Cardiovascular Research 78*, 308–314.

Yanai, R., Mulki, L., Hasegawa, E., Takeuchi, K., Sweigard, H., Suzuki, J., ... Connor, K. M. (2014). Cytochrome P450-generated metabolites derived from omega-3 fatty acids attenuate neovascularization. *Proceedings of the National Academy of Sciences of the United States of America 111*, 9603–9608.

Yang, S., Wei, S., Pozzi, A., & Capdevila, J. H. (2009). The arachidonic acid epoxygenase is a component of the signaling mechanisms responsible for VEGF-stimulated angiogenesis. *Archives of Biochemistry and Biophysics 489*, 82–91.

Ye, D., Zhang, D., Oltman, C., Dellsperger, K., Lee, H. C., & Vanrollins, M. (2002). Cytochrome p-450 epoxygenase metabolites of docosahexaenoate potently dilate coronary arterioles by activating large-conductance calcium-activated potassium channels. *The Journal of Pharmacology and Experimental Therapeutics 303*, 768–776.

Yu, Z., Xu, F., Huse, L. M., Morisseau, C., Draper, A. J., Newman, J. W., ... Kroetz, D. L. (2000). Soluble epoxide hydrolase regulates hydrolysis of vasoactive epoxyeicosatrienoic acids. *Circulation Research 87*, 992–998.

Zeldin, D. C. (2001). Epoxygenase pathways of arachidonic acid metabolism. *The Journal of Biological Chemistry 276*, 36059–36062.

Zeldin, D. C., Dubois, R. N., Falck, J. R., & Capdevila, J. H. (1995). Molecular cloning, expression and characterization of an endogenous human cytochrome P450 arachidonic acid epoxygenase isoform. *Archives of Biochemistry and Biophysics 322*, 76–86.

Zeldin, D. C., Kobayashi, J., Falck, J. R., Winder, B. S., Hammock, B. D., Snapper, J. R., & Capdevila, J. H. (1993). Regio- and enantiofacial selectivity of epoxyeicosatrienoic acid hydration by cytosolic epoxide hydrolase. *The Journal of Biological Chemistry 268*, 6402–6407.

Zhang, B., Cao, H., & Rao, G. N. (2006). Fibroblast growth factor-2 is a downstream mediator of phosphatidylinositol 3-kinase-Akt signaling in 14,15-epoxyeicosatrienoic acid-induced angiogenesis. *The Journal of Biological Chemistry 281*, 905–914.

Zhang, G., Panigrahy, D., Hwang, S. H., Yang, J., Mahakian, L. M., Wettersten, H. I., ... Hammock, B. D. (2014). Dual inhibition of cyclooxygenase-2 and soluble epoxide hydrolase synergistically suppresses primary tumor growth and metastasis. *Proceedings of the National Academy of Sciences of the United States of America 111*, 11127–11132.

Zhang, G., Panigrahy, D., Mahakian, L. M., Yang, J., Liu, J. Y., Stephen Lee, K. S., ... Hammock, B. D. (2013). Epoxy metabolites of docosahexaenoic acid (DHA) inhibit angiogenesis, tumor growth, and metastasis. *Proceedings of the National Academy of Sciences of the United States of America 110*, 6530–6535.

Zhang, J. Y., Prakash, C., Yamashita, K., & Blair, I. A. (1992). Regiospecific and enantioselective metabolism of 8,9-epoxyeicosatrienoic acid by cyclooxygenase. *Biochemical and Biophysical Research Communications 183*, 138–143.

Zhao, J., Leemann, T., & Dayer, P. (1992). In vitro oxidation of oxicam NSAIDS by a human liver cytochrome P450. *Life Sciences 51*, 575–581.

Zhou, Y., Ingelman-Sundberg, M., & Lauschke, V. M. (2017). Worldwide distribution of cytochrome P450 alleles: A meta-analysis of population-scale sequencing projects. *Clinical Pharmacology and Therapeutics 102*, 688–700.