UC Davis
UC Davis Previously Published Works

Title

Stabilized epoxygenated fatty acids regulate inflammation, pain, angiogenesis and cancer

Permalink

Journal

Progress in Lipid Research, 53(1)

ISSN

0163-7827

Authors

Zhang, Guodong
Kodani, Sean
Hammock, Bruce D

Publication Date

2014

DOI

10.1016/j.plipres.2013.11.003

Peer reviewed

Stabilized epoxygenated fatty acids regulate inflammation, pain, angiogenesis and cancer

Guodong Zhang[a,b], **Sean Kodani**[a], and **Bruce D. Hammock**[a,†]

[a]Department of Entomology and Comprehensive Cancer Center, University of California, Davis, CA, 95616

[b]Department of Food Science, University of Massachusetts, Amherst, MA, 01003

Abstract

Epoxygenated fatty acids (EpFAs), which are lipid mediators produced by cytochrome P450 epoxygenases from polyunsaturated fatty acids, are important signaling molecules known to regulate various biological processes including inflammation, pain and angiogenesis. The EpFAs are further metabolized by soluble epoxide hydrolase (sEH) to form fatty acid diols which are usually less-active. Pharmacological inhibitors of sEH that stabilize endogenous EpFAs are being considered for human clinical uses. Here we review the biology of ω-3 and ω-6 EpFAs on inflammation, pain, angiogenesis and tumorigenesis.

Keywords

Cytochrome P450 epoxygenase; soluble epoxide hydrolase; epoxyeicosatrienoic acids; epoxydocosapentaenoic acids

1. Introduction

Arachidonic acid (ARA, 20:4ω-6) compromises a major component in the membrane phospholipids and plays a critical role in cell signaling [1–3]. Upon cellular stimulation, the incorporated ARA is released by several enzymes including diacylglycerol lipase and phospholipase A2 (PLA$_2$) to generate free intracellular ARA, which is rapidly metabolized by a series of enzymes to generate lipid mediators (LMs) in a process collectively termed the ARA cascade [1–3]. The lipid signaling in the ARA cascade is important because the LMs regulate many fundamental biological processes from inflammation to blood flow, and therefore are important therapeutic targets for multiple human disorders [1–3]. There are three major branches in the ARA cascade: cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (CYP) pathways. The COX and LOX pathways generate predominately but not exclusively pro-inflammatory LMs and a variety of approved drugs target these two branches [2]. In contrast, our knowledge of the CYP pathway, which is usually regarded as the third branch of the ARA cascade, is rather limited and has not yet been exploited therapeutically [3–6]. Lipid amides and other endocannabinoids are important chemical

[†]To whom correspondence should be addressed: B.D.H (bdhammock@ucdavis.edu).

mediators [7]. However, they are usually not considered as part of the ARA cascade and only their epoxygenated metabolites are discussed here.

The CYP branch, which was first described in 1980s, converts ARA to two major classes of LMs: CYP ω/ω-1 hydroxylases (mainly CYP4A and CYP4F) catalyze the hydroxylation of ARA to generate 19-hydroxyeicosatetraenoic acid (19-HETE) and 20-HETE [8]. In the other branch of the CYP pathway, CYP epoxygenases (mainly CYP2C and CYP2J) catalyze the epoxidation of ARA to generate epoxygenated fatty acids (EpFAs) called epoxyeicosatrienoic acids (EETs) that include four regioisomers of 5,6-, 8,9-, 11,12- and 14,15-EET [3]. 20-HETE has been shown to have an array of largely detrimental effects, inducing hypertension, endothelial dysfunction, inflammation, cardiovascular diseases, angiogenesis and tumor growth [9–14]. EETs have been investigated as autocrine and paracrine signaling molecules which have anti-inflammatory, vasodilative, anti-hypertensive, cardio-protective, renal-protective, pro-angiogenic and analgesic effects [5]. As we simplistically discuss LMs with terms such as inflammatory or anti-inflammatory and suggest beneficial or detrimental effects, it is important to remember most LMs have multiple effects that maintain a critical balance in normal physiology. Although chemically stable (other than the 5,6-EET regioisomer), EETs are highly unstable *in vivo* mainly due to the rapid metabolism by soluble epoxide hydrolase (sEH, encoded by *EPHX2*) to the less-active fatty acid diols termed dihydroxyeicosatrienoic acids (DHETs) (Figure 1) [6]. Therefore, blocking the degradation of generally beneficial EETs by targeting sEH is pharmacologically attractive. During the past decade, pharmacological inhibitors of sEH (sEHIs) with IC_{50} values in nM-pM range and good pharmacokinetic (PK) profiles *in vivo* have been developed [4, 15]. The sEHIs, which stabilize endogenous EETs, are promising drug candidates for multiple human diseases and have been evaluated in phase II human trials [4, 16].

Linoleic acid (18:2, ω-6), which is a biosynthetic precursor to generate ARA and is highly abundant in the western diet [17], is also a substrate of the CYP/sEH pathway [6]. The metabolism of linoleic acid by CYP epoxygenases generates the linoleic epoxides including 9,10-epoxyoctadecenoic acid (9,10-EpOME) and 12,13-epoxyoctadecenoic acid (12,13-EpOME), which are further metabolized by sEH to form the linoleic diols including 9,10-dihydroxyoctadecenoic acid (9,10-DiHOME) and 12,13-dihydroxyoctadecenoic acid (12,13-DiHOME) [6]. EpOMEs have been associated with multiple organ failure and adult respiratory distress syndrome in some severe burn patients [18–21]. We have shown that the sEH-mediated conversion of EpOMEs to DiHOMEs plays a critical role in the cellular toxicity of EpOMEs [22]. With a high consumption of linoleic acid in the western diet, it is critical to investigate the effects of linoleic acid metabolites on human health, in particular EpOMEs and DiHOMEs which have been demonstrated to have toxic effects.

Besides ω-6 polyunsaturated fatty acids (PUFAs), ω-3 PUFAs such as eicosapentaenoic acid (EPA, 20:5ω-3) and docosahexaenoic acid (DHA, 22:6ω-3) are also substrates of the enzymes in the ARA cascade, which convert them to the ω-3-series LMs [23–25]. A major theory to explain the health-promoting effects of ω-3 PUFAs is that they compete with ARA for the enzymatic metabolism, decreasing the formation of ω-6-series LMs that are predominately pro-angiogenic and pro-inflammatory and increasing ω-3-series LMs that have less detrimental and possibly beneficial effects [23–25]. Indeed, the metabolism of ω-3 PUFAs by COX and LOX enzymes generates ω-3-series prostaglandins [26, 27] and leukotrienes [28], as well as unique ω-3 autacoids such as resolvins and protectins [25], which have anti-inflammatory or anti-angiogenic effects. EPA and DHA are believed to be poor substrates of COX and LOX enzymes [23], however they have been shown to be highly efficient alternative substrates of CYP epoxygenases, which convert them to the ω-3 EpFAs named epoxyeicosatetraenoic acids (EEQs) and epoxydocosapentaenoic acids

(EDPs) respectively [29] (Figure 2). Compared with EETs, the ω-3 EpFAs are generally better substrates of sEH which convert them to the corresponding ω-3-series fatty acid diols [30]. As expected from its structure, the 19,20-EDP is more slowly turned over by the sEH. Compared with EETs, the biological effects of the ω-3 EpFAs are less-studied. EEQs and EDPs have similar or more potent effects for vasodilation, anti-inflammation and analgesia than EETs [30, 31], while EDPs and EETs have opposite activities on angiogenesis, tumor growth and metastasis [32, 33]. This offers us additional opportunities to manipulate profiles of EpFAs to improve human health.

EpFAs have been demonstrated to be involved in many human diseases and hold promise as novel therapeutic targets [5]. This review discusses the biological activities and mechanisms of actions of the ω-6 and ω-3 EpFAs including EETs, EEQs and EDPs on inflammation, pain, angiogenesis and cancer. EpFAs have also been shown to have anti-hypertensive, cardio-protective and organ protective effects. These topics have been covered in several recent reviews [5, 34, 35] and will not be discussed here.

1.1. Overview of the CYP/sEH pathway

CYP epoxygenases catalyze epoxidation of the double bonds of ARA to generate EETs. The epoxidation can occur at all of the four double bonds of ARA, leading to formation of four regioisomers (5,6-, 8,9-, 11,12- and 14,15-EET) [3]. Among these regioisomers, 5,6-EET is chemically unstable and undergoes rapid cyclization and hydrolysis, the other isomers are chemically stable except under acidic conditions. The CYPs referred to as epoxygenases are by no means specific, for example, they also oxidize reactive methylenes in PUFAs. The biochemistry of CYP epoxygenases in EETs biosynthesis have been discussed in several reviews [3, 36, 37]. A series of CYP enzymes such as CYP1A, CYP2B, CYP2C, CYP2D, CYP2G, CYP2J, CYP2N, and CYP4A are capable of converting ARA to EETs [3]. Generally each enzyme produces EETs with different profiles of optical- and regioisomers. In mammals CYP2C and CYP2J isoforms appear to be the predominate epoxygenases. For human and rat, CYP2C isoforms are the most abundant in liver and kidney, and CYP2J are the major ones in heart [3]. CYP2C and CYP2J are also the major epoxygenases in endothelium [36]. Similar to other CYP enzymes, the expression of CYP epoxygenases can be modulated by environmental factors and cellular stimuli [3, 36, 38]. Among these, the most physiologically relevant stimulator of CYP epoxygenase expression is hypoxia [39, 40], which is a critical regulator to stimulate neovasculization [41], suggesting a role of CYP epoxygeanses in angiogenesis.

Once formed, EETs are rapidly metabolized by sEH to generate the corresponding fatty acid diols called DHETs [6]. Compared with EETs, DHETs are widely believed to be inactive or less-active [5]; a recent study shows that opposite to the anti-inflammatory effects of EETs, DHETs are pro-inflammatory in stimulating monocyte migration [42]. Therefore the sEH-mediated metabolic step is generally regarded as a loss of beneficial biological activities. The biochemistry, expression and regulation of sEH have been discussed in several reviews [4–6]. The mammalian sEH is a homodimer composed of two ~62 KDa monomers. Each monomer has a ~35 KDa C-terminal domain which displays epoxide hydrolase activity and a ~25 KDa N-terminal domain which appears to have phosphatase activity [43]. sEH is highly expressed in many tissues including liver, kidney, lung, heart, brain, spleen, endothelium and mammary gland [6]. The highest sEH activity was observed in liver, followed with kidney. Even in organs with relatively low sEH activity, the enzymatic activity can be high in individual cell types. The expression of sEH is inducible by peroxisome proliferator-activated receptor α (PPARα) and PPARγ agonists [6]. PPARγ agonists have been shown to inhibit tumor growth and metastasis by blocking angiogenesis [44, 45], the anti-cancer effect of these compounds could be partially mediated by reduction of the amount of EETs via stimulation of sEH [32]. The expression of sEH is also up-

regulated by angiotensin II [46] and homocysteine [47]. The sEH catalyzes hydrolysis of EpFAs by addition of water, in a two-step, base-catalyzed mechanism via the formation of a covalent intermediate [6]. Pharmacological inhibitors, many of which have a urea, amide, or carbamate group as the central pharmacophore to mimic the reaction transition states, were designed based on the catalytic mechanism of sEH enzyme [15]. A sEHI APAU has been evaluated in a Phase IIA trial targeting hypertension as the primary indication, recently more potent and metabolically stable sEHIs have been developed and are being considered for evaluation in human trials. FDA-approved anti-cancer drugs sorafenib (Nexavar®) and regorafenib (Stivarga®) are also potent sEH inhibitors with IC_{50} values in the low nM to pM range [48, 49].

Besides the hydrolysis catalyzed by sEH, EpFAs are also metabolized by other pathways including β-oxidation, chain shortening and chain elongation [3]. EpFAs can be reincorporated into the membrane phospholipids by acyl transferase causing a significant proportion of EpFAs to exist as esterified forms in the membrane phospholipids. In comparison, the fatty acid diols are less readily incorporated in the membrane phospholipids [3]. The biological significance of the membrane-incorporated EpFAs remains poorly studied. To address this question, more reliable analytical methods are needed to quantify the membrane-incorporated EpFAs and diols and particularly to evaluate the kinetics of this process. EpFAs could also be further metabolized by COX, LOX and CYP enzymes to generate novel series of LMs, though little is known about the chemical structures and biological activities of these lipid metabolites. Both 5,6- and 8,9-EET have been shown to be substrates of COX enzymes, which convert them to metabolites which may have potent effects of vasodilation or stimulation of cell proliferation [50–52]. A COX metabolite of 8,9-EET, 11-hydroxy-8,9-epoxyeicosatrienoic acid, has been shown to be >1000-fold more potent than 8,9-EET in stimulating cell proliferation and c-fos expression in rat glomerular mesangial cells [52]. Previous research from our laboratory has shown that sEHIs dramatically synergize with COX or LOX inhibitors to reduce inflammation [53, 54]. It would be interesting to investigate whether the observed synergistic interactions are in part mediated by the LOX and COX-derived metabolites of EETs. It was recently shown that EPA-derived 17,18-EEQ is further metabolized to generate 12-OH-17,18-EEQ, which inhibited LTB_4-induced neutrophil chemotaxis and polarization *in vitro* with $EC_{50} = 0.6$ nM [55]. Other pathways such as β-oxidation and chain elongation also participate in the metabolism of EETs. When sEH is inhibited, other metabolic pathways of EETs are up-regulated [56]. Since the fates of EETs are regulated by multiple metabolic pathways, pharmacological inhibition or genetic deletion of sEH only increases level of EETs in a limited range.

1.2. Metabolism of ω-3 PUFAs by CYP/sEH pathway

The ω-3 PUFAs including EPA and DHA were mainly derived from cold-water fish, and were of dramatically varying quality. Overall quality of fish oils is increasing, their composition is defined, and increasingly they may be derived from many sources including krill, tissues of marine mammals, algae and yeast. They are among the most popular dietary supplements in United States. In addition, major food companies are increasingly adding ω-3 PUFAs to food as value-added ingredients. Two ω-3 PUFA products have been approved by the FDA as prescription drugs to treat hypertriglyceridemia, including Lovaza® from GSK and Vascepa® from Amarin. Although epidemiological and pre-clinical data show a correlation between increases in ω-3 relative to ω-6 PUFAs in the diet and reduced risks of various chronic diseases such as cancers [57–63] and macular degeneration [64–67], the underlying mechanisms are largely unknown. A dominant theory to explain the effects of ω-3 PUFAs is that they suppress the metabolism of ARA which generates predominately pro-inflammatory and pro-angiogenic eicosanoids, or they serve as alternative substrates to

generate ω-3 LMs which have unique biological actions [24–26, 28]. Indeed, EPA effectively competes with ARA for metabolism by the COX enzymes, the COX-2 metabolite of EPA, prostaglandin E_3 (PGE_3), has less pro-angiogenic and pro-inflammatory effect than the ARA-derived metabolite PGE_2 [26, 27]. 5-LOX plays a critical role in the anti-angiogenic effect of DHA, the 5-LOX metabolite of DHA, 4-hydroxy-docosahexaenoic acid (4-HDHA), has potent anti-angiogenic effect and genetic deletion of 5-LOX significantly attenuated the anti-angiogenic effect of DHA in a murine retinopathy model [28]. In a human study, Dwyer et al. showed that a diet rich in ω-3 PUFAs decreased the risks while a diet rich in ω-6 PUAFs increased the risks of atherosclerosis only in the sub-population with high 5-LOX activity [68]. These studies suggest that there is a strong gene-diet interaction. Understanding the molecular mechanism of ω-3 PUFAs could help to design better therapeutic paradigms and human trials to clarify their health benefits.

Besides the well studied COX and LOX pathways, recent research showed that ω-3 PUFAs are good alternative substrates of CYP epoxygenases [29]. Cell-free enzymatic assays showed that CYP epoxygenases have similar activities toward ARA, EPA and DHA [29]. CYP epoxygenases selectively catalyze the epoxidation of the terminal double bond of ω-3 PUFAs, leading to predominate formation of 17,18-EEQ from EPA and 19,20-EDP from DHA, while no such selectivity was observed for biosynthesis of EETs from ARA [29, 69–71]. Most of the ω-3 EpFAs are turned over by sEH more rapidly than EETs except for 19,20-EDP from DHA [30]. The high biosynthesis and low degradation of 19,20-EDP make it among the most abundant ω-3 EpFAs *in vivo* [29, 72–74]. In endothelial cells, EPA has been shown to increase transcription of CYP2J2 in a time- and dose-dependent manner resulting in increased biosynthesis of EpFAs [75].

Because several PUFAs including linoleic acid, ARA, EPA and DHA are substrates of the CYP epoxygenases, the CYP pathway generates a large number of ω-6 and ω-3 EpFAs, whose levels are further regulated by the sEH enzyme [29, 72–74]. Inhibition of sEH is thus a 'shot-gun" approach, stabilizing multiple ω-3 and ω-6 EpFAs. Inhibition of sEH will stabilize EpFAs that are present in the tissue, but only will change the relative amounts of EpFAs based on the preference of the sEH for the EpFA substrates. In most tissues EETs are the major EpFAs because ARA is the most abundant PUFA in cell membrane, while DHA-derived EDPs could be the major EpFAs in DHA-rich tissues such as retina and brain [17]. In zebrafish which is DHA-rich, the most abundant EpFA is DHA-derived 19,20-EDP [76]. In mammals, the relative abundance of ω-6 ARA and ω-3 EPA and DHA in tissues are largely determined by dietary intake of PUFAs because mammals lack the enzymes for de novo biosynthesis of ω-3 PUFAs [17, 23]. Studies on man and other mammals demonstrate that ω-3 supplementation increases levels of EEQs and EDPs in plasma and tissues. A 3-week feeding of ω-3 PUFA ethyl ester in rats reduced levels of EETs and increased levels of EEQs and EDPs in plasma, brain, heart, kidney, liver and lung [29]. Supplementation of 4g/day of ω-3 PUFA ethyl ester (465 mg EPA and 375 mg DHA per 1 g capsule) in healthy volunteers for 4 weeks induced a respective ~5- and ~2-fold increase of EEQs and EDPs in human plasma, while the levels of EETs were not significantly changed [72, 73]. Zivkovic et al. reported that supplementation of 4g/d of fish oil (1.9 g/d EPA and 1.5 g/d DHA) in immunoglobulin A nephropathy patients for 24 months caused a ~2-fold increase of DHA epoxides and diols in plasma [74].

2. EETs on inflammation

Dysregulated inflammation is a common feature of most human diseases, therefore modulation or inhibition of inflammation has been proven to be an effective therapeutic strategy [77–79]. LMs play a central role to regulate inflammation. One of the most important LMs in inflammation is PGE_2 which is a COX-2 metabolite of ARA and has

predominantly but not exclusively pro-inflammatory activity [80]. Aspirin has been used to inhibit inflammatory pain and fever for over a century. It suppresses inflammation via its action on COX-2 to inhibit the formation of PGE_2 [81]. This discovery resulted in the Nobel Prize in Physiology or Medicine in 1982. Other drugs targeting COX enzymes include non-steroidal anti-inflammatory drugs (NSAIDs) and COX-2 selective inhibitors (coxibs), which are among the most widely used drugs in the world to treat inflammation and pain [82]. However, chronic and high dose use of COX inhibitors can cause life-threatening cardiovascular risks, jeopardizing their clinical applications [83, 84]. Most NSAIDs can also lead to organ specific damage and intestinal bleeding in addition to some apparently idiopathic problems in subpopulations. Novel therapeutic targets are urgently needed to treat inflammatory diseases. Recently EETs have been demonstrated to have potent anti-inflammatory effects *in vitro* and *in vivo* [85], suggesting that targeting sEH to stabilize EpFAs is a promising strategy to treat inflammatory disorders. In addition, dual inhibition of COX-2 and sEH synergistically inhibits inflammation with reduced cardiovascular and gastrointestinal toxicity [53, 54], indicating that sEHIs could be used as synergists with COX inhibitors to enhance the efficacy and reduce the adverse effects.

2.1. Biological activities of EETs on inflammation *in vitro*

Node et al. first reported the anti-inflammatory effects of EETs [85]. Synthetic EETs inhibited LPS-, IL-1α- or TNF-α-induced vascular cell adhesion molecule-1 (VCAM-1) expression in human endothelial cells. The anti-inflammatory effects of EETs are regio-selective: 11,12-EET showing the most potent effect, followed with 8,9- and 5,6-EET, while 14,15-EET was inactive. 11,12-EET also suppressed other endothelial cell adhesion molecules such as E-selectin and intercellular adhesion molecule 1 (ICAM-1) in TNF-α-stimulated endothelial cells. Over-expression of CYP2J2 produced similar anti-inflammatory response, which was abolished by co-treatment with SKF525A, a broad pharmacological inhibitor of CYP enzymes including epoxygenases [85]. A recent study indicated that 14,15-EET inhibited TNF-α-stimulated inflammation in human bronchi, suggesting this EET regioisomer is also biologically active to suppress inflammation in some systems [86]. In a recent study, Deng et al. studied LPS-induced inflammatory responses in primary lung endothelial cells isolated from transgenic mice with endothelial expression of human CYP2C8 and CYP2J2 [87]. Transgenic expression of CYP2C8 or CYP2J2, which has higher capacity to generate EETs, significantly inhibited LPS-induced expression of E-selectin and monocyte chemoattractant protein-1 (MCP-1) in isolated endothelial cells, and such anti-inflammatory effects were attenuated by a putative EET receptor antagonist 14,15-EEZE and a selective inhibitor of CYP epoxygenase MS-PPOH [87].

EETs were also shown to inhibit inflammation in inflammatory cells *in vitro*. The mRNA expression of CYP2J2 and CYP2C8 were detected in human peripheral leukocytes, monocytes (Mc) and the human monocyte THP-1 cell line, but not polymorphonuclear cells (PMNs) [88]. 11,12- and 8,9-EET inhibited basal TNF-α production in THP-1 cells; 11,12-EET abolished IL-1β + PMA-induced COX-2 expression, while the CYP inhibitor SKF525A dose-dependently increased basal COX-2 expression in THP-1 cells [88]. In LPS-stimulated rat monocytes, CYP epoxygenase inhibitors SKF525A and 1-aminobenzotriazole (ABT) increased production of PGE_2, while 11,12-EET dose-dependently suppressed LPS-induced PGE_2 formation via a mechanism of inhibiting the enzymatic activity but not the expression of COX-2 [89]. The sEHI *c*-TUCB reduced levels of LPS-induced MCP-1 and TNF-α, but not IL-6 and macrophage inflammatory protein-1α (MIP-1α), in human monocytes [90].

The sEH metabolites of EETs, DHETs, are generally believed to be inactive or less-active in many assays [5]. However a recent study showed that opposite to the anti-inflammatory effects of EETs, DHETs are pro-inflammatory in stimulating monocyte migration *in vitro* and *in vivo* [42]. Pharmacological inhibition of COX and LOX pathways had no effect on MCP-1-induced monocyte chemotaxis; while inhibition of CYP or sEH enzymes dose-dependently blocked this process, which was rescued by DHETs, suggesting the contribution of DHETs in MCP-1-induced chemotaxis [42]. This study indicates that pharmacological inhibition of sEH not only stabilizes and increases level of EETs which are anti-inflammatory, but also reduces the formation of DHETs which are pro-inflammatory.

2.2. Biological activities of EETs on inflammation *in vivo*

Animal studies have shown that EETs inhibited inflammation in various disease models. Continuous infusion of 11,12-EET, but not 14,15-EET, inhibited TNF-α-induced endothelial VCAM-1 expression and the resulting mononuclear cells adhesion and rolling in murine carotid artery [85]. Pharmacological inhibition of sEH, which increased level of EETs, reduced LPS-induced mortality, systemic hypotension and tissue injury, as well as elevation of inflammatory cytokines in a LPS-induced sepsis model in mice [91]. Inhibition of sEH reduced tobacco smoke-induced lung inflammation in spontaneously hypertensive rats [92]. Treatment with sEHIs decreased total bronchoalveolar lavage cell number with significant reductions noted in neutrophils, alveolar macrophages and lymphocytes in tobacco smoke-exposed rats. Co-administration of sEHIs with EETs further enhanced the anti-inflammatory effect of sEHIs [92]. In a streptozotocin-induced type 1 murine diabetic model, genetic deletion of sEH significantly reduced urinary MCP-1 excretion and NF-κB activation in diabetic mice [93]. Triclocarban (TCC) is a widely used antimicrobial component in personal care products, which has been shown to be a potent sEHI with $IC_{50} = 13$ nM for human sEH. In a LPS-induced acute inflammation model, treatment with TCC increased plasma levels of EETs and attenuated LPS-induced hypotension and elevation of pro-inflammatory cytokines in plasma [94]. Inhibition of sEH also reduced inflammation related to cardiovascular diseases. The mRNA expression of pro-inflammatory cytokines in liver (TNF-α and IL-6) and adipose tissues (TNF-α, IL-6, IL-1β and MCP-1) stimulated by high-fat diet were significantly reduced by treatment with the sEHI *t*-AUCB [95]. In a deoxycorticosterone acetate plus high salt (DOCA-salt)-induced hypertensive model in mice, genetic deletion of sEH or treatment with *t*-AUCB reduced DOCA-salt-induced hypertension, renal inflammation and renal injury [96]. Deletion of sEH reduced DOCA-salt-induced production of pro-inflammatory cytokines, macrophage infiltration, NF-κB signaling activation in renal tissues and elevation of urinary MCP-1 [96]. Inhibition or deletion of sEH reduces vascular remodeling and pro-inflammatory gene expression in an inflammatory model of neointima formation in mice [97]. IL-10 is an important anti-inflammatory cytokine, for example, genetic deletion of IL-10 induces inflammation in tissues and is used as a model for inflammatory bowel disease [98]. To study the role of sEH in inflammation caused by IL-10 deficiency, Zhang et al. created transgenic IL-10 (−/−) sEH (−/−) double knockout mice [99]. Deletion of sEH reduced the inflammatory cell infiltration, pro-inflammatory cytokine expression and NF-κB signaling activation caused by IL-10 deficiency [99, 100]. Beside manipulating sEH to modulate EETs, transgenic expression of CYP2C8 or CYP2J2 in mice significantly attenuated LPS-induced production of pro-inflammatory cytokines such as IL-6, MCP-1, E-selection, IL-1β and ENA-78, as well as NF-κB signaling activation and inflammatory cell infiltration in lung tissues [87].

Together, these studies provide strong evidence to support the anti-inflammatory effects of EETs in various inflammatory models, suggesting that inhibiting sEH to stabilize EETs is a promising therapeutic strategy to treat inflammatory disorders. However it should be noted that there are inconsistent results of sEH inhibition in inflammation [101, 102], more studies

are thus needed to characterize the effects and mechanisms of EETs in inflammatory diseases and to determine what animal models are indicative of human conditions.

2.3. Mechanisms of EETs on inflammation

EETs have been shown to inhibit inflammation via blocking NF-κB pathway, which is an important signaling cascade to regulate inflammation. Without cellular stimulation, the NF-κB complex is sequestered in the cytoplasm through binding to the inhibitory protein IκBα. When activated, IκBα is phosphorylated and then degraded, resulting in release of the NF-κB complex from IκBα. The released NF-κB translocates to the nucleus and activates the expression of multiple pro-inflammatory genes [103]. Node et al. showed that 11,12-EET, but not 14,15-EET, inhibited TNF-α-induced IκBα degradation and nuclear translocation of NF-κB in endothelial cells, suggesting that EETs inhibit inflammation via blocking NF-κB signaling activation [85]. Co-administration of the sEHI AUDA with individual EET regioisomers inhibited TNF-α-induced IκBα degradation in endothelial cells, which was attenuated by a PPAR-γ antagonist GW9662. These data suggest that EETs blocked NF-κB signaling via a PPAR-γ-dependent mechanism [104]. In human bronchi, 14,15-EET inhibited TNF-α-stimulated inflammation via inhibition of IκBα degradation [86]. In primary mouse neonatal cardiomyocytes, the sEHI AEPU suppressed ascending aortic constriction-induced IκBα phosphorylation and degradation, and nuclear accumulation of NF-κB subunit p65 [105]. In primary endothelial cells isolated from Tie2-CYP2J2-Tr mice, LPS-induced IκBα phosphorylation was attenuated compared with WT cells [87]. Animal experiments also support that EETs and CYP/sEH pathway modulate NF-κB signaling. LPS-induced IκBα phosphorylation in lung tissues was significantly attenuated in Tie2-CYP2C8-Tr, Tie2-CYP2J2-Tr and sEH-null mice compared with WT mice [87]. Genetic deletion of sEH suppressed activation of NF-κB signaling in IL-10-knockout mice [99, 100]. Inhibition or deletion of sEH suppressed DOCA-salt- and streptozotocin-induced NF-κB activation in renal tissues [93, 96].

Besides NF-κB, other signaling pathways have also been investigated. For example, treatment with the sEHI *c*-TUCB had no effect on LPS-induced NF-κB nuclear translocation, while it abolished LPS-induced phosphorylation of JNK in adherent human monocytes, suggesting that JNK pathway is a potential target of sEHIs or EETs in monocytes [90]. PPARs have also been shown to contribute to the anti-inflammatory effects of EETs [104, 106–108].

2.4. Synergistic interactions of sEHIs with other anti-inflammatory drugs

COX inhibitors such as NSAIDs and coxibs are the most widely used drugs in the world to treat inflammation. However, the dose-limiting cardiovascular side effects and gastrointestinal erosion of COX inhibitors have limited their clinical applications [83, 84]. We discovered that dual inhibition of sEH and COX-2 pathways synergistically inhibited inflammation with reduced cardiovascular toxicity [53, 54]. Co-administration of the sEHI AUDA-BE or *t*-AUCB synergized with indomethacin, celecoxib, rofecoxib and aspirin to suppress LPS-induced inflammation in mice [53, 54]. Genetic deletion of sEH also synergizes with aspirin to suppress inflammation [54]. Together, these results suggest a potent interaction of sEH and COX-2 pathways to regulate inflammation. The mechanisms for the synergistic interactions remain to be elucidated. Previous studies have shown that sEHIs inhibit transcription of COX-2 [109] and COX inhibitors inhibit the enzymatic activity of COX-2 [82], therefore, a combination of these two drugs synergistically inhibit PGE_2 formation as we have demonstrated in animal experiments [53, 54]. The synergistic reduction of PGE_2 in some tissues may partially explain the interaction of COX inhibitors with sEHIs. The synergistic interactions of these two drugs suggest that the required doses

of COX inhibitors could be reduced while producing equivalent effect, leading to attenuation of potential side effects.

A major theory to explain the cardiovascular risks of coxibs is that they block the formation of prostacyclin (PGI_2) which is a potent vasodilator, but not COX-1 derived thromboxane A_2 (TXA_2) which is a potent vasoconstrictor; therefore, a decrease of the PGI_2 to TAX_2 ratio may increase incidence of thrombotic cardiovascular events [110]. Therefore, the PGI_2-to-TXA_2 ratio is widely used as a biomarker to predict the COX-associated cardiovascular risks. Consistent with this theory, our previous study showed that a 6-h treatment of celecoxib or rofecoxib significantly reduced the ratio of PGI_2 (stable metabolite 6-keto-$PGF_{1\alpha}$) to TXA_2 (stable metabolite TXB_2) in plasma in mice. However, co-administration of coxibs with sEHIs did not perturb the balance of PGI_2-to-TXA_2 ratio, suggesting the co-treatment may reduce cardiovascular toxicity compared to coxibs [53].

Relying upon co-administration of synergistic drugs may introduce problems such as poor patient compliance, complicated drug-drug interactions, or patient-dependent differences in the PK profiles of each drug [111]. Therefore, we took a poly-pharmacology approach to design and synthesize the first-in-class COX-2/sEH dual inhibitors which simultaneously antagonize both COX-2 and sEH [112]. Surprisingly, a COX-2/sEH dual inhibitor, PTUPB, has been shown to be far more efficacious in attenuating inflammatory pain *in vivo* than a coxib celecoxib, a selective sEHI *t*-AUCB, or a combination of celecoxib and *t*-AUCB [112]. Presumably the co-treatment regime or the dual inhibitor acts both to reduce production of inflammatory eicosanoids such as PGE_2 and to stabilize anti-inflammatory and cardio-protective eicosanoids such as EETs. Besides COX inhibitors, our study showed that sEHIs also synergized with LOX inhibitors to inhibit inflammation [54]. The mechanisms for the synergistic interactions remain to be elucidated.

3. EETs on inflammatory and neuropathic pain

A potential therapeutic application for EpFAs, their mimics or sEHI is for alleviation of inflammatory and neuropathic pain. Based on a French survey, chronic pain persists within 30% of the population with 7% having characteristics of neuropathic pain [113]. The cost from this chronic pain, considering both health care costs and cost of lost productivity, is estimated to be up to 635 billion dollars for the United States [114]. Despite this expense, up to 40% of those with chronic pain say it is inadequately managed [115], indicating a need for new therapeutic strategies for treating pain. The use of sEHI and EpFAs as potential novel therapeutics for pain management has been previously reviewed by Inceoglu et al [116] and Wagner et al [117, 118].

Much of the current research investigating the relationship between pain and EpFAs has used sEHIs as the primary pharmacologic agent. The original basis for testing sEHIs on pain stemmed from observations that sEHIs reduce inflammation [91]. Based on this reduced inflammation, it was hypothesized that they may be effective at reducing inflammation-related pain. Consequently, it was shown that sEHIs could attenuate hyperalgesia (increased sensitivity to painful stimuli) and allodynia (sensitivity to normally innocuous stimuli) in rodents with inflammation [53]. Observations that direct administration of EETs in absence of sEHI produced the same effect and the structural diversity of sEHI tested indicate EpFAs are the primary mediators of pain relief, rather than an off-target effect of a particular sEHI [119]. Other EpFAs including metabolites of DHA and EPA have similar activity on reducing inflammatory sensitization; however the effect is less potent for EPA metabolites [30]. Additionally, this effect was regioisomer selective, as illustrated by 13,14-EDP being more potent than other DHA EpFAs.

In addition to effects on inflammatory pain, sEHIs have proven useful for decreasing diabetes-driven neuropathic pain. The efficacy of EpFAs on neuropathic pain was serendipitously discovered after models of neuropathic pain, used as a control for inflammatory pain, had an unexpected reduced sense of pain in response to noxious stimuli [109]. Although neuropathic pain has many etiologies, the model used for testing EpFAs on pain is destruction of peripheral nerves stemming from unregulated glucose toxicity seen in diabetics. In comparison to gabapentin, a currently prescribed treatment for neuropathic pain, sEHIs were more effective and more potent with a more favorable PK profile in a streptozocin model of type 1 diabetes [120]. Because many of the current therapies for treating chronic and neuropathic pain are associated with unwanted side effects, the development of an sEHI would provide relief for an unmet medical need.

3.1. Mechanisms of EETs on inflammatory pain

EpFA modulation of inflammatory pain has multiple effects including changes in COX-2 regulation and prostaglandin synthesis, interactions with TRP receptors and activity of CB_2 receptors. However, it is widely hypothesized that a yet undiscovered G-protein coupled receptor is the primary mechanism for modulating inflammatory pain [117].

Administration of sEHI during an inflamed state resulted in reduced COX-2 mRNA in the spinal cord [109] and protein [53] expression in the liver relative to inflamed controls. This change in COX-2 regulation is likely modulated by suppression of NF-κB stemming from EpFAs binding to PPARγ [53]. The combination of COX-2 and sEH inhibitors results in a synergistic effect on both reducing the pro-inflammatory prostaglandins and the nociception, perception of pain, associated with systemic inflammation [53]. From this synergistic effect, the development of COX-2/sEH dual inhibitors have resulted in particularly potent inhibitors of LPS-induced inflammatory pain with favorable PK profiles [112]. However, blocking pain is not completely dependent on modulation of the COX-2 pathway, as evidenced by sEHI-mediated analgesia during PGE_2 stimulated pain [121]. Interestingly, evidence indicates that EpFAs induce analgesia using a mechanism that requires prostaglandin activity, which explains why sEHI work only in an already inflamed state or a state of enhanced pain perception. The necessary component of this mechanism is cAMP, a downstream product of the prostanoid receptor, which can be mimicked by addition of phosphodiesterase inhibitors (PDEI) [121]. Thus co-administration of sEHI and PDEI results in reduction in pain-related response in the absence of an inflammatory state [121]. These data also suggest that a combination of sEHI and PDEI will enhance the potency, increase the therapeutic index, and broaden the activity of both drug classes. However, in the absence of inflammatory pain and upregulated cAMP, direct administration of ARA or EET increases sensitivity to pain briefly. A higher dose of EETs are required to enhance in a naive state than to control pain in a hyperalgesic state. The mechanism of this activity is not understood [119].

Paradoxically, when investigators have looked at sEH knockout mice, there does not seem to be the same regulation of COX-2 or modulation of pro-inflammatory prostaglandins seen with administration of sEHI [122]. This apparent discrepancy may be due to other genetic and physiological changes associated with strain of sEH knockout mice [123]. Alternatively each monomer of the sEH dimer has a catalytically active phosphatase domain active on lipophilic but not protein phosphatases [43]. The physiological role of this highly conserved domain is not known but is absent in the sEH knock out animals. In addition to *in vivo* administration to sEH knockout mice, adding 5,6-EET directly to dorsal root ganglia resulted in increased TRPA1-mediated sensitization [122]. Similar to the above results, direct addition of 5,6-EET to TRPV4 transfected cells resulted in generation of Ca^{2+} currents [124]. While these combined findings suggest that 5,6-EET could actually increase

sensitivity to pain, the rapid cyclization of this regioisomer indicate a secondary metabolite is the active sensitizing component.

In addition to direct effects on inflammation and pain, mounting evidence suggests a component relating epoxygenated endocannabinoids to pain and inflammation. The best understood endocannabinoids, anandamide (an ethanolamine derivative) and 2-arachidonylglycerol (a glycerol derivative), are derivatives of ARA that bind to the cannabinoid (CB_1 and CB_2) receptors. These receptors and ligands are named for their relation to the natural product Δ9-tetrahydrocannabinol – the active component of *Cannabis sativa* [125]. Changes in the endogenous cannabinoids are associated with a large spectrum of diseases including but not limited to obesity, inflammation, cardiovascular and metabolic disorders, cancer and pain [126]. Activation of CB_2 receptors inhibits nociception in multiple forms of sensitization [127, 128], mediated by both inflammatory and non-inflammatory mechanisms [129]. An epoxide of anandamide, 5,6-epoxyeicosatrienoic acid ethanolamide (5,6-EET-EA) binds to CB_2 receptor with high affinity (K_i = 8.9 nM) and increased stability relative to the parent anandamide. Compared to anandamide, which is hydrolyzed by fatty acid amide hydrolase (FAAH), 5,6-EET-EA can also be hydrolyzed by sEH to the respective diol, which can be inhibited by administration of sEHI [130]. Additionally, the 5,6-EET methyl ester is also a ligand for CB_2 receptor, although with significantly decreased potency (IC_{50} = 19 μM) [116]. To test the possibility that an epoxide may be directly effecting inflammatory pain through the CB_2 receptor, Wagner et al [118] used CB_2 antagonists to show the antinociceptive effects of sEHI were reduced when blocking CB_2 in an LPS model of localized inflammation. However, further experiments are necessary to determine the exact contribution of cannabinoid signaling to antinociception in both inflammatory and neuropathic pain.

3.2. Mechanisms of EETs on neuropathic pain

In addition to their effects on inflammatory pain, sEHI reduce neuropathic pain through multiple mechanisms that have still not been well characterized. In addition to the CB_2 receptor, a mixture of EETs can displace ligands for peripheral benzodiazapine receptor, neurokinin NK2 receptor and dopamine D_3 receptor with μM affinity [116]; however, the physiologic significance of this remains to be determined. Other pain pathways which have been well characterized are the regulation opioid neuropeptide synthesis, regulation neurosteroid synthesis and increased somatostatin synthesis.

Multiple lines of evidence suggest that EETs interact with components of opioid receptors resulting in analgesia. 14,15-EET injected directly into the ventrolateral periaqueductal gray region of the brain produced effects similar to morphine [131]. 14,15-EET did not directly compete against radioligands for opioid receptors, but the effects of EETs were inhibited by antisera targeting the β-endorphin neuropeptides, Met-enkephalin and either μ-opioid or δ-opioid receptor antagonists. Furthermore, attenuated analgesic responses from morphine administration in CYP-null mice (which lack the ability to generate high levels of EETs) suggest this effect is essential for normal function of opioid receptor systems and is required for the analgesic effects of opioids [132]. Interestingly, inhibition of fatty acid oxidizing CYPs results in inhibited antinociception mediated by both morphine, an opioid, and improgan, a nonopioid analgesic with a poorly characterized mechanism of action [133, 134]. It has been proposed that the EpFA mediates signaling by suppressing GABA terminals which mediate antinociception associated with the rostral ventromedial medulla, blocking convergent signals of the unknown improgan receptor, the opioid receptor and CB_1 receptor [133]. However, this hypothesis has not been confirmed and as of yet, still requires validation.

In addition to effects on opioid receptors, EETs are known to interact with neurohormone synthesis. As previously mentioned, EETs bind weakly to the peripheral benzodiazepine receptor, also known as TSPO, which is involved in the synthesis of analgesic neurosteroids in the central nervous system. Blocking the synthesis of these hormones with inhibitors resulted in abatement of sEHI-mediated analgesia, indicating that this weak binding is relevant physiologically [109]. Additionally, increased availability of either sEHI or 5,6-, 8,9- or 11,12-EETs resulted in a cAMP dependent upregulation of steroidogenic acute regulatory protein (StARD1), a protein involved with and a general biomarker of neurosteroid synthesis, and progesterone, an analgesic compound and precursor for neurosteroids [109, 135].

4. EETs on angiogenesis

Angiogenesis, the formation of new blood vessels from pre-existing vessels, is critical for multiple physiological and pathological processes [136]. Blocking angiogenesis is a promising strategy to treat cancers and FDA has approved multiple anti-angiogenic drugs for cancer treatment [137]. Anti-angiogenic drugs are also used to treat macular degeneration [138]. On the other hand, pro-angiogenic drugs, which stimulate neovascularization to increase blood flow to tissues, could be useful for some human disorders such as hepatic insufficiency, immature lung development, burn and post surgical treatment and diabetic wound healing [139].

The process of angiogenesis is orchestrated by an array of angiogenic stimulators and inhibitors. While current research of angiogenic mediators has mainly focused on proteins such as VEGF, the non-proteinaceous LMs have received less attention. Emerging evidence demonstrates that the LMs potently regulate angiogenesis, tumor growth and metastasis and are thus therapeutic targets to treat cancers and angiogenic diseases [140]. COX- and LOX-derived LMs have been shown to stimulate angiogenesis and inflammation, playing critical roles in tumorigenesis. Epidemiological and pre-clinical evidence support that the COX and LOX inhibitors are effective to treat or prevent cancers [140]. In comparison, the roles of the CYP-derived EpFAs on angiogenesis and cancer are largely unknown [141].

4.1. Biological activities of EETs on angiogenesis *in vitro*

The first data to suggest the pro-angiogenic effect of EETs was that endogenous EETs released from astrocytes stimulated cellular proliferation and tube formation of co-cultured endothelial cells [142]. Further studies showed that treatment with synthetic EETs stimulated angiogenesis in endothelial cells [36, 143, 144]. 11,12- and 14,15-EET are the most studied EET regioisomers and they have been shown to increase endothelial cell proliferation, migration and invasion, which are critical cellular steps involved in angiogenesis [145–149]. 11,12-EET also increased activity of matrix metalloproteases (MMPs), though the identity of the MMP enzymes involved remain to be confirmed [39]. Besides 11,12- and 14,15-EET, 5,6- and 8,9-EET have also been shown to stimulate cellular proliferation and tube formation in pulmonary murine microvascular endothelial cells, while the diols (5,6-, 8,9-, 11,12- and 14,15-DHET) lacked such effects [150]. Overexpression of CYP epoxygenases or pharmacological inhibition of sEH produced similar pro-angiogenic effects in endothelial cells [39, 40, 148, 150–152], while pharmacological inhibition of CYP epoxygenases suppressed angiogenesis [148]. Over-expression of CYP2C9 or treatment with 11,12-EET increased the expression of COX-2 in endothelial cells, consistent with the pro-angiogenic effect of EETs [153]. CYP2C in endothelial cells is inducible by hypoxia which is a key regulator to stimulate angiogenesis, suggesting that EETs are involved in the hypoxia-induced angiogenic responses [39]. Together, these results support the pro-angiogenic effects of EETs *in vitro*.

4.2. Biological activities of EETs on angiogenesis *in vivo*

Animal studies were followed up to support the pro-angiogenic effects of EETs *in vivo*. iTreatment with 11,12- or 14,15-EET stimulated neovascularization in a Matrigel plug assay and a chck chorioallantoic membrane (CAM) assay [148, 151, 154]. 5,6- and 8,9-EET stimulated angiogenesis in a subcutaneous sponge assay in mice [150]. Local treatment with 11,12-EET, 14,15-EET or the sEHI *t*-AUCB accelerated wound epithelialization and neovascularization in mice [155]. Recently Panigrahy et al. demonstrated that endothelium-derived EETs accelerated organ and tissue regeneration by stimulation of angiogenesis [156]. This study used three transgenic mouse models which have high level of EETs: endothelial expression of human CYP2C8 and human CYP2J2 (Tie2-CYP2C8-Tr and Tie2-CYP2J2-Tr) and genetic knockout of sEH (sEH-null). In these high-EETs transgenic mouse models, accelerated liver regeneration, kidney compensatory growth, lung compensatory growth, wound healing, corneal neovascularization and retinal vascularization were observed. Continuous infusion of 14,15-EET or administration of sEHI also stimulated tissue regeneration, while transgenic over-expression of sEH which reduced EETs levels delayed normal organ and tissue growth. This study suggests that sEHIs could be novel therapeutics for a number of human diseases which requires stimulation of angiogenesis [156].

4.3. Mechanisms of EETs on angiogenesis

VEGF is an important signal protein that stimulates angiogenesis [157] and EETs have been shown to stimulate angiogenesis via a VEGF-dependent mechanism. 14,15-EET stimulated mRNA and protein expression of VEGF in human dermal microvascular endothelial cells [154]. EETs also increased production of VEGF *in vivo*, the plasma level of VEGF was significantly elevated in tumor-bearing Tie2-CYP2C8-Tr and sEH-null mice compared with WT mice [32]. Interestingly, VEGF, which can be stimulated by EETs, can also increase the expression of CYP epoxygenases and EETs biosynthesis [158, 159]. These results suggest that there is a positive feedback loop between EETs and VEGF. Depletion of VEGF using VEGF antibody abolished the pro-angiogenic effects of 14,15-EET *in vitro* and *in vivo* [154]. Neither the sEHI *t*-AUCB nor TUPS stimulated endothelial cell migration in endothelial basal medium without VEGF, while they significantly increased endothelial cell migration with the presence of VEGF [32]. These results indicate that the pro-angiogenic effects of EET require VEGF. The biology of VEGF is largely mediated by its receptor VEGF receptor 2 (VEGFR2) [157]. The putative EET receptor antagonist 14,15-EEZE suppressed VEGF-mediated angiogenesis via a VEGFR2-independent mechanism [158]. Our previous study showed that 11,12-EET had no effect on VEGF-induced VEGFR2 phosphorylation, while DHA-derived 19,20-EDP abolished VEGFR2 activation in endothelial cells [33]. More studies are needed to characterize the biology of EETs on VEGFR2.

Besides VEGF signaling, another major pathway involved in the pro-angiogenic effects of EETs involves activation of the epidermal growth factor (EGF) receptor. EETs were first shown to activate EGF receptor in renal epithelial cells [160, 161], such effects were also observed later in endothelial cells [148, 149] and cancer cells [162]. 11,12-EET increased the supernatant level of EGF/heparin binding EGF (HB-EGF), via a mechanism involving activation of MMPs to liberate cell surface bound EGF/HB-EGF, resulting in activation of the EGF receptor [148]. Inhibition of the EGF receptor using a pharmacological inhibitor or EGF receptor-neutralizing antibody attenuated 11,12-EET-induced angiogenesis in a CAM assay *in vivo* [148]. These results support that 11,12-EET stimulates angiogenesis via an EGF receptor-dependent mechanism. Besides EGF receptor signaling, EETs activated multiple signaling pathways related with angiogenesis, including Src-STAT-3 [154], sphingosine kinase-1 [149] and PI3K/Akt [147, 163].

5. EETs on cancer

5.1. Biological activities of EETs on tumorigenesis *in vitro*

Angiogenesis is required for tumor growth and metastasis of almost all types of cancers. The pro-angiogenic effects of EETs indicate their potential roles in tumor progression. The Wang group first showed that over-expression of CYP2J2 or treatment with synthetic EETs in cancer cells stimulated cancer cell proliferation, migration and invasion [164–166]. Follow-up studies showed that EETs stimulated proliferation, migration and invasion in certain cancer cell lines. Nithipatikom et al. showed that 11,12-EET stimulated cancer cell migration and invasion in human prostate cancer cells (PC-3, LnCaP and Du145), while pharmacological inhibitors of CYP epoxygenase or EET antagonist 14,15-EEZE inhibited such effects [162]. 11,12-EET induced cell stretching and myosin-actin microfilament formation via a mechanism involving activation of EGF receptor and Akt in prostate cancer cells [162]. Our recent study also repeated that 11,12-EET stimulated PC-3 invasion through Matrigel [33]. Mitra et al. showed that 14,15-EET, but not other EET regioisomers, increased cell proliferation of the breast cancer cell line MCF7 with a high threshold of 1–3 & mu;M [167]. 14(S),15(R)- and 14(R),15(S)-EET showed similar efficacy to induce MCF7 proliferation, indicating the effect of EETs on MCF7 cell proliferation was regio- but not stereospecific [167]. PPARα and PPARα agonists induce sEH expression [6], which could contribute to the anti-cancer effects of these compounds [44, 150].

5.2. Biological activities of EETs on tumorigenesis *in vivo*

Over-expression of CYP2J2 in cancer cells has been shown to stimulate tumor growth and metastasis *in vivo* [164–166]. The endothelium is a major site for biosynthesis of EETs. Recently Panigrahy et al. demonstrated that endothelium-derived EETs stimulated tumor growth and metastasis in multiple tumor models [32]. High levels of EETs in transgenic Tie2-CYP2C8-Tr, Tie2-CYP2J2-Tr and sEH-null mice slightly increased primary tumor growth but dramatically stimulated tumor metastasis. Continuous infusion with 14,15-EET via osmotic mini-pumps or treatment with a high-dose sEHI *t*-AUCB (10 mg/kg/day) also increased tumor progression, while the corresponding diols DHETs have no such effects. Lowering EETs by transgenic over-expression of sEH or using the EET antagonist 14,15-EEZE reduced tumor progression. The biological effects of EETs are at least partially mediated by VEGF. The plasma levels of EETs were significantly elevated in Tie2-CYP2C8-Tr and sEH-null mice compared with WT mice. Continuous infusion of 14,15-EET also increased VEGF expression in tumor endothelium and stromal fibroblasts of LLC tumors. Depletion of VEGF using Ad-sFIt dramatically suppressed B16F10 tumor growth in Tie2-CYP2J2-Tr and sEH-null but not in WT mice [32]. This study demonstrates that EETs play a central role in tumor growth and metastasis by stimulation of tumor angiogenesis.

This study raises questions on the biological implication from the sEH inhibition regarding cancer risks. Currently sEHIs are considered for human clinical trials for multiple disorders such as pain and inflammation [5], thus this study raised potential safety concerns of sEHIs in cancer patients. However it should be noted that in animal experiments the doses of sEHIs to treat hypertension, pain, and inflammation are in low mg/kg range (0.1–1 mg/kg), while the active dose used in the tumor experiment was as high as 10 mg/kg/day [32]. Our study showed that at 1 mg/kg/day, *t*-AUCB had no effect on primary tumor growth and metastasis in mice [33]; while at 10 mg/kg/day, *t*-AUCB significantly increased tumor growth and metastasis [32]. These results indicate that the biological effects of sEHIs (or EETs) on tumorigenesis have a high threshold. This still leaves the question if the therapeutic index of sEHI is sufficiently high to justify long term chronic use as pharmaceuticals.

5.3. Expression of CYP and sEH in tumor tissues

CYP2J2 expression was increased in carcinoma cell lines (LS-174, ScaBER, SiHa, U251, A549, Tca-8113, Ncl-H446 and HepG2) compared with non-cancer cell lines (HT-1080 and HEk293) [164]. The mRNA and proteins of CYP2C8, CYP2C9 and CYP2J2 were detected in human prostate cancer cells, producing predominately 11,12-EET *in vitro* [162]. CYP3A4 is up-regulated in 80% of breast cancer tissues and is correlated with poor prognosis [168]. Recently CYP3A4 was shown to catalyze epoxidation of ARA to generate EETs in the breast cancer MCF7 line [167]. CYP epoxygenases such as CYP2J2 have been reported to be highly expressed in human tumor tissues [164, 166]. Guengerich and Turvy analyzed expression of CYP in a group of 100 liver samples and discovered that the expression of CYP2C was elevated in samples from metastatic cancer patients, though the expression levels varied by two orders of magnitude [169]. However, other studies demonstrate a more complicated expression pattern of CYP epoxygenase in tumor tissues. CYP epoxygenases were underexpressed or undetectable in adenocarcinoma and squamous cell carcinoma tissues [170], breast tumor tissues [168] and renal tumor tissues [171]. The expression of sEH also varied greatly in tumor tissues [143]. Compared with matched benign tissues, the sEH expression was elevated in colon, renal and testicular cancer, but decreased in liver and pancreatic cancer [172]. The complicated expression pattern of CYP epoxygenases and sEH makes it difficult to investigate the significance of CYP/sEH pathway in tumorigenesis. Besides CYP2C and CYP2J, a large number of other CYP isoforms could also contribute to biosynthesis of EETs, which are metabolized by multiple pathways beside sEH [3]. Therefore, analyzing the tissue or plasma level of EETs, rather than just the expression levels of selected CYP epoxygenases or sEH, may provide more consistent predictions regarding the roles of EETs and other EpFAs in tumorigenesis.

6. Biological activities of ω-3 EpFAs

6.1. Biological activities of ω-3 EpFAs on vascular tone and inflammation

EDPs have been shown to be the most potent EpFAs in the dilation of blood vessels [31]. EDP regioisomers (except 5,6-EDP which is chemically unstable) had EC_{50} values ranging from 0.5 to 24 pM for dilation of porcine coronary arterioles precontracted with endothelin, while the corresponding diol 13,14-DiHDPA was >1000-fold less active with an EC_{50} value of 30 ± 22 nM, and the parent fatty acid DHA only dilated vessels at 1 μM. EDPs also potently activated BKca channels with a 1000-fold higher potency than EETs [31]. Animal experiments also support the anti-hypertensive effects of ω-3 EpFAs. A combination of the sEHI TPPU and a ω-3-rich diet caused a more potent reduction of Ang-II-induced hypertension than treatment with sEHI or ω-3 diet alone. Stabilized 19,20-EDP also suppressed hypertension, suggesting that the anti-hypertensive effect of the co-treatment was at least partially mediated by the formation of EDPs [173].

The EPA epoxide 17,18-EEQ has been shown to inhibit TNF-α-induced inflammation in human bronchi via NF-κB- and PPAR-γ-related mechanisms [174]. 17,18-EEQ reduced TNF-α-induced hyperresponsiveness, COX-2 expression, IκBα degradation and phosphorylation of p38 mitogen–activated protein kinase (p38-MAPK) in human bronchi [174]. In a previous study, 14,15-EET has also been shown to inhibit TNF-α-induced inflammation in a similar system [86]. In a carrageenan-induced inflammatory pain model in rats, EDPs and EETs had similar efficacy on reduction of inflammatory pain, while the effects of EEQs were less potent [30]. The parent fatty acids and the corresponding fatty acid diols lacked such effects. The effects of EDPs on pain were region-specific, 13,14-EDP was the most potent regioisomer, followed with 16,17- and 19,20-EDP [30]. These studies demonstrate that similar to EETs, the ω-3 EpFAs also have potent effects to reduce inflammation and pain.

6.2. Biological activities of ω-3 EpFAs on angiogenesis

Opposite to the effects of EETs in stimulation of angiogenesis, our recent study demonstrated that DHA-derived EDPs are strongly anti-angiogenic [33]. In a Matrigel plug assay in mice, treatment with synthetic EDP regioisomers (7,8-, 10,11-, 13,14-, 16,17- and 19,20-EDP) inhibited VEGF-induced neovascularization in a dose-dependent manner with $EC_{50} = 0.3$ μg per mouse. The 5,6-EDP regioisomer was chemically unstable, thus it was not tested. 19,20-EDP also inhibited fibroblast growth factor-2 (FGF-2)-induced angiogenesis using the Matrigel plug assay, supporting the potential broad-spectrum anti-angiogenic effects of EDPs [33]. In contrary, previous studies in Matrigel plug assays showed that EETs stimulated angiogenesis when treated alone [148, 154], and further up-regulated VEGF-induced angiogenesis in the presence of VEGF [156]. Together, these results support the opposite effects of ω-6 and ω-3 EpFAs on angiogenesis. To test whether EDPs inhibits angiogenesis via targeting endothelial cells, we tested the effects of EDPs on angiogenesis in the primary endothelial cell line HUVECs. 19,20-EDP inhibited endothelial tube formation after 6-h treatment in HUVECs, supporting its anti-angiogenic effect *in vitro*. The process of angiogenesis composes of multiple cellular steps including cell proliferation, migration and production of MMPs. We found that 19,20-EDP potently inhibited VEGF-stimulated endothelial cell migration, with no effect on cell proliferation and weak inhibitory effect on MMP-2 activity in HUVECs, suggesting that EDPs inhibited angiogenesis primarily via suppressing endothelial cell migration [33]. The detailed mechanism by which EDPs suppressed endothelial cell migration requires more study.

19,20-EDP at 1 μM almost abolished VEGF-induced VEGFR2 activation after a 10-min treatment in HUVECs, supporting that 19,20-EDP inhibits angiogenesis via a VEGFR2-dependent mechanism [33]. VEGFR2 is a critical mediator of angiogenesis, regulating all known biology of VEGF [157]. Most anti-angiogenic drugs on the market target VEGFR2/VEGF pathway to inhibit angiogenesis. However, VEGF is also critical for cardiovascular system [157]; therefore, all of currently available anti-angiogenic drugs cause cardiovascular side effects such as hypertension [175]. Human studies support that ω-3 PUFA supplementation reduced hypertension [176–178], and DHA-derived EDPs are among the most potent vessel dilators ever discovered [31]. In this aspect, pharmacological or nutritional manipulation of tissue level of EDPs may have unique advantages in anti-angiogenic therapy with reduced adverse effects on pain, blood pressure and other cardiovascular functions than current therapies.

Interestingly, our study also suggested potential roles of EDPs in lymphangiogenesis, an important process involved in tumor metastasis and other human diseases [179]. An angiogenesis assay (>80 genes correlated with angiogenesis) showed that the most down-regulated gene by 19,20-EDP is VEGF-C. RT-PCR analysis further confirmed that treatment of 19,20-EDP in HUVECs inhibited transcription of VEGF-C in a dose-dependent manner, while it has no effect on mRNA expression of VEGF-A [33]. VEGF-C is a critical mediator to regulate formation of new lymphatic vessels [179], suggesting a potential role of EDPs in inhibiting lymphangiogenesis. Currently no lymphangiogenesis inhibitors have been approved in therapy and a VEGF-C antibody is in Phase I human trials to treat cancers. More studies are needed to characterize the effects of EDPs on lymphangiogenesis and associated diseases.

The opposite effect of EETs and EDPs may explain away some inconsistent results in previous studies. Substantial studies have demonstrated that pharmacological inhibition or genetic deletion of sEH increases angiogenesis. For example, Panigrahy et al. showed that genetic deletion of sEH increased tissue regenerations in liver, lung and kidney via stimulation of angiogenesis [156]. However, recent studies showed that inhibition or deletion of sEH suppresses angiogenesis in zebrafish [76] and retina [180]. These

"inconsistent" results could be partially explained by our findings of the opposite effects of EETs and EDPs on angiogenesis (Figure 3). The ω-6 ARA is the most abundant PUFA in membrane phospholipids in most tissues, pharmacological inhibition or genetic deletion of sEH would thus mainly accumulate ARA-derived EETs to stimulate angiogenesis. On the other hand, the ω-3 DHA is highly enriched in retina and brain tissues [17], inhibition or deletion of sEH is likely to mainly accumulate DHA-derived EDPs, leading to suppression of angiogenesis in these tissues. Currently there is no report of the tissue levels of EpFAs in retinal or brain tissues, while previous study has shown that EDPs are the most abundant EpFAs in zebrafish which is rich in ω-3 PUFAs, inhibition of sEH causes a 5-fold increase of the level of EDPs in zebrafish [76]. Since endogenous DHA is highly enriched in retinal tissues [17], pharmacological inhibition of sEH may be a potential strategy to suppress retinal angiogenesis and associated diseases such as macular degeneration. These studies emphasize that sEH inhibition is a 'shot-gun' approach, modulating the levels of multiple EpFAs from several ω-3 and ω-6 PUFAs including ARA, EPA and DHA. The profiles of the EpFAs are tissue specific and can be modulated by nutrition. This modulation of lipid stores through nutrition offers more therapeutic opportunities by modulating sEH.

Besides our study, Cui et al. showed that 17,18-EEQ, but not other EEQ regioisomers derived from EPA, inhibited cell proliferation in the immortalized endothelial cell line bEND.3 at a dose of 10 μM, while EETs at the same dose showed opposite effects to increase cell proliferation in bEND.3 cells [181]. This study suggests that EPA epoxides may also suppress angiogenesis.

6.3. Biological activities of ω-3 EpFAs on cancer

Cell culture experiments showed that EDPs, treated alone or combined with a sEHI, had no effect on cancer cell proliferation; while they potently inhibited cancer cell (prostate cancer PC-3 cells) invasion, opposite to the effect of 11,12-EET [33, 162]. Animal experiments support that EDPs inhibit tumor growth and metastasis by suppressing tumor angiogenesis [33]. EDPs are highly unstable *in vivo* due to their rapid metabolism by sEH, continuous infusion of even the relatively stable 19,20-EDP (toward sEH metabolism) by osmotic mini pumps failed to elevate the level of 19,20-EDP in plasma and tumor tissues. We found that co-administration of 19,20-EDP with a low-dose sEHI *t*-AUCB caused a ~2-fold increase of 19,20-EDP level in circulation, while treatment with 19,20-EDP or sEHI alone had no effect on the systematic level of 19,20-EDP. The elevated level of 19,20-EDP by the co-treatment resulted in ~70% inhibition of Met-1 breast tumor growth (a highly aggressive triple-negative breast cancer model) in FVB female mice by blocking tumor angiogenesis, while treatment with 19,20-EDP or sEHI alone had no such effect. In addition, the sEH metabolite of 19,20-EDP, 19,20-DiHDPA, had no effect on Met-1 tumor progression. Together, these results support that 19,20-EDP had potent anti-cancer effect, which was stabilized by co-administration of a sEHI to block the sEH-mediated metabolism. The co-treatment reduced vessel density in Met-1 tumors, supporting anti-cancer effect was in part mediated by inhibition of tumor angiogenesis. In an angiogenesis-dependent LLC metastasis model [182], the co-treatment inhibited ~70% of LLC metastasis toward lung tissues, supporting that EDPs had potent anti-metastatic effects. These data suggest utility for stabilized mimics of EpFAs in medicine as well as a combination of sEHI with careful nutritional intervention.

As far as we know, EDPs are the first class of lipid mediators which have such potent anti-cancer and anti-metastatic effects. Tumor metastasis results in 90% of human cancer death [183], therefore, drugs which inhibit tumor metastasis and increase survival are useful in cancer therapy. It is likely that EDPs also target other pathways or targets related with metastasis, more studies are thus needed to characterize the effects and mechanisms of EDPs on metastasis. Due to the potent effects of EDPs on tumor growth and metastasis, EDPs are

potential structural targets that can be used to develop stable analogs as anticancer agents. Previous research has shown medicinal chemistry approaches to design and synthesize stable mimics of ω-3 EpFAs, similar approaches could be used to generate EDP mimics to treat cancers [184].

The opposing effects between EpFAs from ω-3 and ω-6 PUFAs on angiogenesis and tumorigenesis suggest the biological effects of sEH inhibition could be diet-dependent. In mammals, the relative abundance of ω-6 ARA and ω-3 fatty acids (EPA and DHA) in tissues is largely determined by dietary intake of PUFAs [17]. DHA is the most abundant ω-3 fatty acid in most tissues, the levels of EPA in tissues are usually very low [17, 23]. It is expected that under a ω-3-rich diet, sEH inhibition would mainly accumulate DHA-derived EDPs that are anti-angiogenic, while under a ω-6-rich diet, sEH inhibition would mainly accumulate pro-angiogenic EETs from ARA. Co-administration of sEHI and ω-3-rich diet could be beneficial for cancer treatment, while a combination of sEHI and ω-6-rich diet could be useful for some human diseases or injuries which require stimulation of angiogenesis. For example, a stimulation of tissue repair and angiogenesis with ω-6 PUFAs and sEHI could be of therapeutic benefit following severe burns, surgery or other dermal injuries. The stimulation of tissue repair and angiogenesis probably contributes to the success of sEHI in treating equine laminitis [185]. The sEHIs are promising therapeutics to various human disorders, while the pro-angiogenic and pro-metastatic side effects have raised some safety concerns of sEHIs in cancer patients. The discovery of the anti-angiogenic effect of EDPs suggests that a combination of a ω-3-rich diet and sEHI could enhance the efficacy with reduced or eliminated safety concerns for cancers with sEHIs.

Sorafenib (Nexavar®) is a multikinase inhibitor of tumor cell proliferation and angiogenesis approved to treat advanced renal and liver cancers [186, 187]. A similar compound, regorafenib (Stivarga®), has recently been approved to treat advanced gastrointestinal stromal tumors and metastatic colorectal cancer [188, 189]. However, both drugs are reported to cause severe adverse effects such as fatigue, hypertension and hand-foot skin reaction, thus limiting their therapeutic applications [186–189]. In addition to sEH inhibition, we have found that both drugs are also potent inhibitors of sEH (sorafenib IC_{50}=12 nM, regorafenib IC_{50}=0.5 nM) [48, 49], much more potent than their kinase inhibitory effects [190]. The hypothesis has been advanced that sorafenib is tolerated by cancer patients due, in part, to its inhibition of sEH and the resulting increase in analgesic, anti-hypertensive and anti-inflammatory EpFAs such as EETs. Therefore, based on our data of the anti-angiogenic, anti-cancer and anti-metastatic effects of EDPs, a combination of sorafenib or regorafenib with a diet high in ω-3 PUFAs (particularly DHA) and low in ω-6 PUFAs could boost EDPs and reduce EETs, leading to enhanced anti-cancer efficacy and reduced adverse effects [33]. EDPs have potent vasodilative and analgesic effects [30, 31], thus the co-administration could also reduce the side effects, such as hypertension, that are associated with this type of drugs.

7. Conclusion and future work

Multiple approaches were carried out to investigate the biological activities of EpFAs in cell cultures and animal experiments. These approaches include (1) treatment with synthetic EpFAs, (2) pharmacological inhibition of CYP epoxygenases, (3) inhibition of sEH using sEHIs with diverse structural features, (4) transgenic expression of CYP epoxygenases, and (5) transgenic over-expression or knockout of sEH. Substantial evidence obtained from these studies support that EpFAs are important signaling molecules to regulate inflammation, pain, angiogenesis and cancer [5]. The sEHIs have been shown to have beneficial effects in various *in vivo* disease models [4, 5, 15]. These compounds have been in Phase II human clinical trials targeting hypertension and novel classes of sEHIs are being

considered for human trials targeting other disorders [4, 15, 16]. In addition, the sEHIs have been shown to synergize with various drugs to enhance the efficacy and reduce the adverse effects [53, 54, 191, 192]; therefore, these compounds hold promise as therapeutics for disorders in humans and companion animals.

The discovery of the receptor(s) for EpFAs could help to clarify some inconsistent results [101, 102]. Many classes of LMs such as prostaglandins and leukotrienes exert their biological activities via G-protein coupled receptors (GPCRs) [2]. EETs have been shown to specifically bind specific membrane proteins, supporting the presence of putative EET receptor(s) [193, 194]. Until now the identities of the EET receptors remain unknown. The biological activities of EETs appear to be cell type- and tissue-selective. For example, EETs stimulated VEGF expression in endothelial cells, but not in smooth muscle cells [154]. EETs have been consistently shown to stimulate cellular proliferation in primary endothelial cells, but the effects in cancer cells or other cells were greatly varied [164, 167]. The discovery of the receptors could help to explain away the contradictions in biological and cell signaling of EETs. Other classes of EpFAs such as EEQs and EDPs likely also act through cellular receptor(s). Certainly an understanding of how these lipid mediators regulate so many diverse biological processes would advance the field.

Compared with EETs, the ω-3 EpFAs (EEQs and EDPs) are even less-studied. Until now several studies have suggested that the ω-3 EpFAs, in particular DHA-derived EDPs, inhibit inflammation, hypertension, pain, angiogenesis, tumor growth and metastasis [30, 31, 33, 173]. More studies are needed to investigate their biological activities and mechanisms of actions. These promising discoveries suggest that stabilizing or mimicking ω-3 EpFAs represent therapeutic targets. On the basis of our research, sEHIs (including anti-cancer drugs sorafenib and regorafenib) may synergize with ω-3 PUFAs to boost the levels of EDPs, leading to enhanced anti-angiogenic and anti-cancer effects. Investigation of the drug-nutrition interactions in pre-clinical models could lead to rapid human clinical trials in cancer patients. Endogenous DHA is highly enriched in retinal and brain tissues, pharmacological inhibition of sEH is thus promising to suppress pathological angiogenesis in these tissues.

Pre-clinical and epidemiological studies support the potential health benefits of ω-3 PUFAs on various chronic diseases, including but not limited to cancers [57–62, 195], macular degeneration [64–67], hypertension [176–178], cardiovascular diseases [196, 197] and inflammatory diseases [198]. There are large pools of fatty acids in man, and some of these pools are refractory to turnover. Thus, the effects of altering dietary intake of PUFAs may be delayed. Thus, we can anticipate that altering lipid composition and relative flux can be a long term flux effect relative to the relatively rapid effects from many pharmaceuticals. However, the underlying molecular mechanisms are largely unknown and the biological efficacy of ω-3 PUFAs in humans remains controversial [199–201], which greatly limits the effective utilization of ω-3 PUFAs for disease prevention. A major theory for health-promoting effects of ω-3 PUFAs is that they suppress the formation of ARA-derived LMs and generate ω-3-series of LMs with beneficial actions [24, 25]. It is likely that people with different genetic or biochemical background will metabolize ω-3 PUFAs to bioactive lipid mediators in distinct manners, leading to different biological responses. Indeed, it was recently shown that there is a high degree of inter-individual variability in lipid metabolism upon supplementation of ω-3 PUFAs [202]. Understanding the specific metabolic pathways and the metabolites involved in the bioactivities of ω-3 PUFAs is thus critical to develop effective therapeutic paradigms and human trials to clarify their health benefits [24, 28, 68, 203]. The opposite effects of EETs and EDPs on tumor growth and metastasis suggest a novel mechanistic linkage between ω-3 and ω-6 PUFAs and cancer. Our findings demonstrate that the previously unappreciated CYP/sEH pathway could play a critical role

to mediate the opposite effects of ω-3 and ω-6 PUFAs on cancer [32, 33]. The polymorphisms in the genes encoding CYP [204, 205] and sEH [206–210] could affect the metabolism of DHA and alter the response to DHA supplementation. For example, based on our findings, people carrying Lys55Arg and Cys154Tyr mutations of sEH, which lead to higher sEH enzymatic activities [209], are expected to have lower tissue levels of EDPs and thus poorer anti-cancer responses upon dietary DHA supplementation. Such knowledge, together with utilization of nutrigenomic and metabolomic methods, could lead to targeted human trials to better understand the metabolic individuality and nutrition effects of ω-3 PUFAs on human health [211].

Acknowledgments

References

1. Buczynski MW, Dumlao DS, Dennis EA. Thematic Review Series: Proteomics. An integrated omics analysis of eicosanoid biology. J Lipid Res. 2009; 50:1015–38. [PubMed: 19244215]

2. Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. Science. 2001; 294:1871–5. [PubMed: 11729303]

3. Zeldin DC. Epoxygenase pathways of arachidonic acid metabolism. J Biol Chem. 2001; 276:36059–62. [PubMed: 11451964]

4. Imig JD, Hammock BD. Soluble epoxide hydrolase as a therapeutic target for cardiovascular diseases. Nat Rev Drug Discov. 2009; 8:794–805. [PubMed: 19794443]

5. Morisseau C, Hammock BD. Impact of soluble epoxide hydrolase and epoxyeicosanoids on human health. Annu Rev Pharmacol Toxicol. 2013; 53:37–58. [PubMed: 23020295]

6. Newman JW, Morisseau C, Hammock BD. Epoxide hydrolases: their roles and interactions with lipid metabolism. Prog Lipid Res. 2005; 44:1–51. [PubMed: 15748653]

7. Blankman JL, Cravatt BF. Chemical probes of endocannabinoid metabolism. Pharmacol Rev. 2013; 65:849–71. [PubMed: 23512546]

8. Kroetz DL, Xu F. Regulation and inhibition of arachidonic acid omega-hydroxylases and 20-HETE formation. Annu Rev Pharmacol Toxicol. 2005; 45:413–38. [PubMed: 15822183]

9. Williams JM, Murphy S, Burke M, Roman RJ. 20-hydroxyeicosatetraenoic acid: a new target for the treatment of hypertension. J Cardiovasc Pharmacol. 2010; 56:336–44. [PubMed: 20930591]

10. Cheng J, Ou JS, Singh H, Falck JR, Narsimhaswamy D, Pritchard KA Jr, et al. 20-hydroxyeicosatetraenoic acid causes endothelial dysfunction via eNOS uncoupling. Am J Physiol Heart Circ Physiol. 2008; 294:H1018–26. [PubMed: 18156192]

11. Ishizuka T, Cheng J, Singh H, Vitto MD, Manthati VL, Falck JR, et al. 20-Hydroxyeicosatetraenoic acid stimulates nuclear factor-kappaB activation and the production of inflammatory cytokines in human endothelial cells. J Pharmacol Exp Ther. 2008; 324:103–10. [PubMed: 17947496]

12. Miyata N, Roman RJ. Role of 20-hydroxyeicosatetraenoic acid (20-HETE) in vascular system. J Smooth Muscle Res. 2005; 41:175–93. [PubMed: 16258232]

13. Guo AM, Arbab AS, Falck JR, Chen P, Edwards PA, Roman RJ, et al. Activation of Vascular Endothelial Growth Factor through Reactive Oxygen Species Mediates 20-Hydroxyeicosatetraenoic Acid-Induced Endothelial Cell Proliferation. J Pharmacol Exp Ther. 2007; 321:18–27. [PubMed: 17210799]

14. Guo AM, Sheng J, Scicli GM, Arbab AS, Lehman NL, Edwards PA, et al. Expression of CYP4A1 in U251 human glioma cell induces hyperproliferative phenotype in vitro and rapidly growing tumors in vivo. J Pharmacol Exp Ther. 2008; 327:10–9. [PubMed: 18591218]

15. Shen HC, Hammock BD. Discovery of inhibitors of soluble epoxide hydrolase: a target with multiple potential therapeutic indications. J Med Chem. 2012; 55:1789–808. [PubMed: 22168898]

16. Shen HC. Soluble epoxide hydrolase inhibitors: a patent review. Expert Opin Ther Pat. 2010; 20:941–56. [PubMed: 20429668]

17. Arterburn LM, Hall EB, Oken H. Distribution, interconversion, and dose response of n-3 fatty acids in humans. Am J Clin Nutr. 2006; 83:1467S–76S. [PubMed: 16841856]

18. Kosaka K, Suzuki K, Hayakawa M, Sugiyama S, Ozawa T. Leukotoxin, a linoleate epoxide: its implication in the late death of patients with extensive burns. Mol Cell Biochem. 1994; 139:141–8. [PubMed: 7862104]

19. Hanaki Y, Kamiya H, Ohno M, Hayakawa M, Sugiyama S, Ozawa T. Leukotoxin, 9, 10-epoxy-12-octadecenoate: a possible responsible factor in circulatory shock and disseminated intravascular coagulation. Jpn J Med. 1991; 30:224–8. [PubMed: 1920962]

20. Hayakawa M, Kosaka K, Sugiyama S, Yokoo K, Aoyama H, Izawa Y, et al. Proposal of leukotoxin, 9,10-epoxy-12-octadecenoate, as a burn toxin. Biochem Int. 1990; 21:573–9. [PubMed: 2222499]

21. Ozawa T, Hayakawa M, Kosaka K, Sugiyama S, Ogawa T, Yokoo K, et al. Leukotoxin, 9,10-epoxy-12-octadecenoate, as a burn toxin causing adult respiratory distress syndrome. Adv Prostaglandin Thromboxane Leukot Res. 1991; 21B:569–72. [PubMed: 1825379]

22. Moghaddam MF, Grant DF, Cheek JM, Greene JF, Williamson KC, Hammock BD. Bioactivation of leukotoxins to their toxic diols by epoxide hydrolase. Nat Med. 1997; 3:562–6. [PubMed: 9142128]

23. Jump DB. The biochemistry of n-3 polyunsaturated fatty acids. J Biol Chem. 2002; 277:8755–8. [PubMed: 11748246]

24. Rose DP, Connolly JM. Omega-3 fatty acids as cancer chemopreventive agents. Pharmacol Ther. 1999; 83:217–44. [PubMed: 10576293]

25. Serhan CN, Petasis NA. Resolvins and protectins in inflammation resolution. Chem Rev. 2011; 111:5922–43. [PubMed: 21766791]

26. Bagga D, Wang L, Farias-Eisner R, Glaspy JA, Reddy ST. Differential effects of prostaglandin derived from omega-6 and omega-3 polyunsaturated fatty acids on COX-2 expression and IL-6 secretion. Proc Natl Acad Sci U S A. 2003; 100:1751–6. [PubMed: 12578976]

27. Szymczak M, Murray M, Petrovic N. Modulation of angiogenesis by omega-3 polyunsaturated fatty acids is mediated by cyclooxygenases. Blood. 2008; 111:3514–21. [PubMed: 18216296]

28. Sapieha P, Stahl A, Chen J, Seaward MR, Willett KL, Krah NM, et al. 5-Lipoxygenase metabolite 4-HDHA is a mediator of the antiangiogenic effect of omega-3 polyunsaturated fatty acids. Sci Transl Med. 2011; 3:69ra12.

29. Arnold C, Markovic M, Blossey K, Wallukat G, Fischer R, Dechend R, et al. Arachidonic acid-metabolizing cytochrome P450 enzymes are targets of {omega}-3 fatty acids. J Biol Chem. 2010; 285:32720–33. [PubMed: 20732876]

30. Morisseau C, Inceoglu B, Schmelzer K, Tsai HJ, Jinks SL, Hegedus CM, et al. Naturally occurring monoepoxides of eicosapentaenoic acid and docosahexaenoic acid are bioactive antihyperalgesic lipids. J Lipid Res. 2010; 51:3481–90. [PubMed: 20664072]

31. Ye D, Zhang D, Oltman C, Dellsperger K, Lee HC, VanRollins M. Cytochrome p-450 epoxygenase metabolites of docosahexaenoate potently dilate coronary arterioles by activating large-conductance calcium-activated potassium channels. J Pharmacol Exp Ther. 2002; 303:768–76. [PubMed: 12388664]

32. Panigrahy D, Edin ML, Lee CR, Huang S, Bielenberg DR, Butterfield CE, et al. Epoxyeicosanoids stimulate multiorgan metastasis and tumor dormancy escape in mice. J Clin Invest. 2012; 122:178–91. [PubMed: 22182838]

33. Zhang G, Panigrahy D, Mahakian LM, Yang J, Liu JY, Stephen Lee KS, et al. Epoxy metabolites of docosahexaenoic acid (DHA) inhibit angiogenesis, tumor growth, and metastasis. Proc Natl Acad Sci U S A. 2013; 110:6530–5. [PubMed: 23553837]

34. Imig JD. Epoxides and soluble epoxide hydrolase in cardiovascular physiology. Physiol Rev. 2012; 92:101–30. [PubMed: 22298653]

35. Imig JD. Targeting epoxides for organ damage in hypertension. J Cardiovasc Pharmacol. 2010; 56:329–35. [PubMed: 20531214]

36. Fleming I. Cytochrome p450 and vascular homeostasis. Circ Res. 2001; 89:753–62. [PubMed: 11679404]

37. Capdevila JH, Falck JR, Estabrook RW. Cytochrome P450 and the arachidonate cascade. FASEB J. 1992; 6:731–6. [PubMed: 1537463]

38. Lee AC, Murray M. Up-regulation of human CYP2J2 in HepG2 cells by butylated hydroxyanisole is mediated by c-Jun and Nrf2. Mol Pharmacol. 2010; 77:987–94. [PubMed: 20194533]

39. Michaelis UR, Fisslthaler B, Barbosa-Sicard E, Falck JR, Fleming I, Busse R. Cytochrome P450 epoxygenases 2C8 and 2C9 are implicated in hypoxia-induced endothelial cell migration and angiogenesis. J Cell Sci. 2005; 118:5489–98. [PubMed: 16291720]

40. Michaelis UR, Xia N, Barbosa-Sicard E, Falck JR, Fleming I. Role of cytochrome P450 2C epoxygenases in hypoxia-induced cell migration and angiogenesis in retinal endothelial cells. Invest Ophthalmol Vis Sci. 2008; 49:1242–7. [PubMed: 18326754]

41. Pugh CW, Ratcliffe PJ. Regulation of angiogenesis by hypoxia: role of the HIF system. Nat Med. 2003; 9:677–84. [PubMed: 12778166]

42. Kundu S, Roome T, Bhattacharjee A, Carnevale KA, Yakubenko VP, Zhang R, et al. Metabolic products of soluble epoxide hydrolase are essential for monocyte chemotaxis to MCP-1 in vitro and in vivo. J Lipid Res. 2013; 54:436–47. [PubMed: 23160182]

43. Newman JW, Morisseau C, Harris TR, Hammock BD. The soluble epoxide hydrolase encoded by EPXH2 is a bifunctional enzyme with novel lipid phosphate phosphatase activity. Proc Natl Acad Sci U S A. 2003; 100:1558–63. [PubMed: 12574510]

44. Panigrahy D, Singer S, Shen LQ, Butterfield CE, Freedman DA, Chen EJ, et al. PPARgamma ligands inhibit primary tumor growth and metastasis by inhibiting angiogenesis. J Clin Invest. 2002; 110:923–32. [PubMed: 12370270]

45. Pozzi A, Ibanez MR, Gatica AE, Yang S, Wei S, Mei S, et al. Peroxisomal proliferator-activated receptor-alpha-dependent inhibition of endothelial cell proliferation and tumorigenesis. J Biol Chem. 2007; 282:17685–95. [PubMed: 17405874]

46. Ai D, Fu Y, Guo D, Tanaka H, Wang N, Tang C, et al. Angiotensin II up-regulates soluble epoxide hydrolase in vascular endothelium in vitro and in vivo. Proc Natl Acad Sci U S A. 2007; 104:9018–23. [PubMed: 17495027]

47. Zhang D, Xie X, Chen Y, Hammock BD, Kong W, Zhu Y. Homocysteine upregulates soluble epoxide hydrolase in vascular endothelium in vitro and in vivo. Circ Res. 2012; 110:808–17. [PubMed: 22354938]

48. Liu JY, Park SH, Morisseau C, Hwang SH, Hammock BD, Weiss RH. Sorafenib has soluble epoxide hydrolase inhibitory activity, which contributes to its effect profile in vivo. Mol Cancer Ther. 2009; 8:2193–203. [PubMed: 19671760]

49. Hwang SH, Wecksler AT, Zhang G, Morisseau C, Nguyen LV, Fu SH, et al. Synthesis and biological evaluation of sorafenib- and regorafenib-like sEH inhibitors. Bioorg Med Chem Lett. 2013; 23:3732–7. [PubMed: 23726028]

50. Moreland KT, Procknow JD, Sprague RS, Iverson JL, Lonigro AJ, Stephenson AH. Cyclooxygenase (COX)-1 and COX-2 participate in 5,6-epoxyeicosatrienoic acid-induced contraction of rabbit intralobar pulmonary arteries. J Pharmacol Exp Ther. 2007; 321:446–54. [PubMed: 17267581]

51. Zhang JY, Prakash C, Yamashita K, Blair IA. Regiospecific and enantioselective metabolism of 8,9-epoxyeicosatrienoic acid by cyclooxygenase. Biochem Biophys Res Commun. 1992; 183:138–43. [PubMed: 1543485]

52. Homma T, Zhang JY, Shimizu T, Prakash C, Blair IA, Harris RC. Cyclooxygenase-derived metabolites of 8,9-epoxyeicosatrienoic acid are potent mitogens for cultured rat glomerular mesangial cells. Biochem Biophys Res Commun. 1993; 191:282–8. [PubMed: 8447831]

53. Schmelzer KR, Inceoglu B, Kubala L, Kim IH, Jinks SL, Eiserich JP, et al. Enhancement of antinociception by coadministration of nonsteroidal anti-inflammatory drugs and soluble epoxide hydrolase inhibitors. Proc Natl Acad Sci U S A. 2006; 103:13646–51. [PubMed: 16950874]

54. Liu JY, Yang J, Inceoglu B, Qiu H, Ulu A, Hwang SH, et al. Inhibition of soluble epoxide hydrolase enhances the anti-inflammatory effects of aspirin and 5-lipoxygenase activation protein inhibitor in a murine model. Biochem Pharmacol. 2010; 79:880–7. [PubMed: 19896470]

55. Kubota T, Arita M, Isobe Y, Iwamoto R, Goto T, Yoshioka T, et al. Eicosapentaenoic acid is converted via omega-3 epoxygenation to the anti-inflammatory metabolite 12-hydroxy-17,18-epoxyeicosatetraenoic acid. FASEB J. 2013

56. Fang X, Kaduce TL, Weintraub NL, Harmon S, Teesch LM, Morisseau C, et al. Pathways of epoxyeicosatrienoic acid metabolism in endothelial cells. Implications for the vascular effects of soluble epoxide hydrolase inhibition. J Biol Chem. 2001; 276:14867–74. [PubMed: 11278979]

57. Wolk A, Larsson SC, Johansson JE, Ekman P. Long-term fatty fish consumption and renal cell carcinoma incidence in women. JAMA. 2006; 296:1371–6. [PubMed: 16985229]

58. Hall MN, Chavarro JE, Lee IM, Willett WC, Ma J. A 22-year prospective study of fish, n-3 fatty acid intake, and colorectal cancer risk in men. Cancer Epidemiol Biomarkers Prev. 2008; 17:1136–43. [PubMed: 18483335]

59. Brasky TM, Lampe JW, Potter JD, Patterson RE, White E. Specialty supplements and breast cancer risk in the VITamins And Lifestyle (VITAL) Cohort. Cancer Epidemiol Biomarkers Prev. 2010; 19:1696–708. [PubMed: 20615886]

60. Kim S, Sandler DP, Galanko J, Martin C, Sandler RS. Intake of polyunsaturated fatty acids and distal large bowel cancer risk in whites and African Americans. Am J Epidemiol. 2010; 171:969–79. [PubMed: 20392864]

61. Sawada N, Inoue M, Iwasaki M, Sasazuki S, Shimazu T, Yamaji T, et al. Consumption of n-3 fatty acids and fish reduces risk of hepatocellular carcinoma. Gastroenterology. 2012; 142:1468–75. [PubMed: 22342990]

62. Simonsen N, van't Veer P, Strain JJ, Martin-Moreno JM, Huttunen JK, Navajas JF, et al. Adipose tissue omega-3 and omega-6 fatty acid content and breast cancer in the EURAMIC study. European Community Multicenter Study on Antioxidants, Myocardial Infarction, and Breast Cancer. Am J Epidemiol. 1998; 147:342–52. [PubMed: 9508101]

63. Alfano CM, Imayama I, Neuhouser ML, Kiecolt-Glaser JK, Smith AW, Meeske K, et al. Fatigue, inflammation, and omega-3 and omega-6 fatty acid intake among breast cancer survivors. J Clin Oncol. 2012; 30:1280–7. [PubMed: 22412148]

64. Cho E, Hung S, Willett WC, Spiegelman D, Rimm EB, Seddon JM, et al. Prospective study of dietary fat and the risk of age-related macular degeneration. Am J Clin Nutr. 2001; 73:209–18. [PubMed: 11157315]

65. Seddon JM, George S, Rosner B. Cigarette Smoking, Fish Consumption, Omega-3 Fatty Acid Intake, and Associations With Age-Related Macular Degeneration: The US Twin Study of Age-Related Macular Degeneration. Arch Ophthalmol. 2006; 124:995–1001. [PubMed: 16832023]

66. SanGiovanni JP, Chew EY, Clemons TE, Davis MD, Ferris FL 3rd, Gensler GR, et al. The relationship of dietary lipid intake and age-related macular degeneration in a case-control study: AREDS Report No. 20. Arch Ophthalmol. 2007; 125:671–9. [PubMed: 17502507]

67. Connor KM, SanGiovanni JP, Lofqvist C, Aderman CM, Chen J, Higuchi A, et al. Increased dietary intake of omega-3-polyunsaturated fatty acids reduces pathological retinal angiogenesis. Nat Med. 2007; 13:868–73. [PubMed: 17589522]

68. Dwyer JH, Allayee H, Dwyer KM, Fan J, Wu H, Mar R, et al. Arachidonate 5-lipoxygenase promoter genotype, dietary arachidonic acid, and atherosclerosis. N Engl J Med. 2004; 350:29–37. [PubMed: 14702425]

69. Lucas D, Goulitquer S, Marienhagen J, Fer M, Dreano Y, Schwaneberg U, et al. Stereoselective epoxidation of the last double bond of polyunsaturated fatty acids by human cytochromes P450. J Lipid Res. 2010; 51:1125–33. [PubMed: 19965576]

70. Schwarz D, Kisselev P, Ericksen SS, Szklarz GD, Chernogolov A, Honeck H, et al. Arachidonic and eicosapentaenoic acid metabolism by human CYP1A1: highly stereoselective formation of 17(R),18(S)-epoxyeicosatetraenoic acid. Biochemical Pharmacology. 2004; 67:1445–57. [PubMed: 15041462]

71. Barbosa-Sicard E, Markovic M, Honeck H, Christ B, Muller DN, Schunck WH. Eicosapentaenoic acid metabolism by cytochrome P450 enzymes of the CYP2C subfamily. Biochem Biophys Res Commun. 2005; 329:1275–81. [PubMed: 15766564]

72. Shearer GC, Harris WS, Pedersen TL, Newman JW. Detection of omega-3 oxylipins in human plasma and response to treatment with omega-3 acid ethyl esters. J Lipid Res. 2010; 51:2074–81. [PubMed: 19671931]

73. Keenan AH, Pedersen TL, Fillaus K, Larson MK, Shearer GC, Newman JW. Basal omega-3 fatty acid status affects fatty acid and oxylipin responses to high-dose n3-HUFA in healthy volunteers. J Lipid Res. 2012; 53:1662–9. [PubMed: 22628615]

74. Zivkovic A, Yang J, Georgi K, Hegedus C, Nording M, O'Sullivan A, et al. Serum oxylipin profiles in IgA nephropathy patients reflect kidney functional alterations. Metabolomics. 2012; 8:1102–13. [PubMed: 23833568]

75. Wang D, Hirase T, Nitto T, Soma M, Node K. Eicosapentaenoic acid increases cytochrome P-450 2J2 gene expression and epoxyeicosatrienoic acid production via peroxisome proliferator-activated receptor gamma in endothelial cells. J Cardiol. 2009; 54:368–74. [PubMed: 19944311]

76. Fromel T, Jungblut B, Hu J, Trouvain C, Barbosa-Sicard E, Popp R, et al. Soluble epoxide hydrolase regulates hematopoietic progenitor cell function via generation of fatty acid diols. Proc Natl Acad Sci U S A. 2012; 109:9995–10000. [PubMed: 22665795]

77. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med. 2005; 352:1685–95. [PubMed: 15843671]

78. Libby P. Inflammation in atherosclerosis. Nature. 2002; 420:868–74. [PubMed: 12490960]

79. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002; 420:860–7. [PubMed: 12490959]

80. Kuehl FA Jr, Egan RW. Prostaglandins, arachidonic acid, and inflammation. Science. 1980; 210:978–84. [PubMed: 6254151]

81. Vane JR, Bakhle YS, Botting RM. Cyclooxygenases 1 and 2. Annu Rev Pharmacol Toxicol. 1998; 38:97–120. [PubMed: 9597150]

82. Marnett LJ. The COXIB experience: a look in the rearview mirror. Annu Rev Pharmacol Toxicol. 2009; 49:265–90. [PubMed: 18851701]

83. Bresalier RS, Sandler RS, Quan H, Bolognese JA, Oxenius B, Horgan K, et al. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. N Engl J Med. 2005; 352:1092–102. [PubMed: 15713943]

84. FitzGerald GA. Coxibs and cardiovascular disease. N Engl J Med. 2004; 351:1709–11. [PubMed: 15470192]

85. Node K, Huo Y, Ruan X, Yang B, Spiecker M, Ley K, et al. Anti-inflammatory Properties of Cytochrome P450 Epoxygenase-Derived Eicosanoids. Science. 1999; 285:1276–9. [PubMed: 10455056]

86. Morin C, Sirois M, Echave V, Gomes MM, Rousseau E. EET displays anti-inflammatory effects in TNF-alpha stimulated human bronchi: putative role of CPI-17. Am J Respir Cell Mol Biol. 2008; 38:192–201. [PubMed: 17872494]

87. Deng Y, Edin ML, Theken KN, Schuck RN, Flake GP, Kannon MA, et al. Endothelial CYP epoxygenase overexpression and soluble epoxide hydrolase disruption attenuate acute vascular inflammatory responses in mice. Faseb J. 2011; 25:703–13. [PubMed: 21059750]

88. Bystrom J, Wray JA, Sugden MC, Holness MJ, Swales KE, Warner TD, et al. Endogenous epoxygenases are modulators of monocyte/macrophage activity. PLoS One. 2011; 6:e26591. [PubMed: 22028915]

89. Kozak W, Aronoff DM, Boutaud O, Kozak A. 11,12-epoxyeicosatrienoic acid attenuates synthesis of prostaglandin E2 in rat monocytes stimulated with lipopolysaccharide. Exp Biol Med (Maywood). 2003; 228:786–94. [PubMed: 12876297]

90. Sanders WG, Morisseau C, Hammock BD, Cheung AK, Terry CM. Soluble epoxide hydrolase expression in a porcine model of arteriovenous graft stenosis and anti-inflammatory effects of a soluble epoxide hydrolase inhibitor. Am J Physiol Cell Physiol. 2012; 303:C278–90. [PubMed: 22621785]

91. Schmelzer KR, Kubala L, Newman JW, Kim IH, Eiserich JP, Hammock BD. Soluble epoxide hydrolase is a therapeutic target for acute inflammation. Proc Natl Acad Sci U S A. 2005; 102:9772–7. [PubMed: 15994227]

92. Smith KR, Pinkerton KE, Watanabe T, Pedersen TL, Ma SJ, Hammock BD. Attenuation of tobacco smoke-induced lung inflammation by treatment with a soluble epoxide hydrolase inhibitor. Proc Natl Acad Sci U S A. 2005; 102:2186–91. [PubMed: 15684051]

93. Elmarakby AA, Faulkner J, Al-Shabrawey M, Wang MH, Maddipati KR, Imig JD. Deletion of soluble epoxide hydrolase gene improves renal endothelial function and reduces renal inflammation and injury in streptozotocin-induced type 1 diabetes. Am J Physiol Regul Integr Comp Physiol. 2011; 301:R1307–17. [PubMed: 21832210]

94. Liu JY, Qiu H, Morisseau C, Hwang SH, Tsai HJ, Ulu A, et al. Inhibition of soluble epoxide hydrolase contributes to the anti-inflammatory effect of antimicrobial triclocarban in a murine model. Toxicol Appl Pharmacol. 2011; 255:200–6. [PubMed: 21741984]

95. Liu Y, Dang H, Li D, Pang W, Hammock BD, Zhu Y. Inhibition of soluble epoxide hydrolase attenuates high-fat-diet-induced hepatic steatosis by reduced systemic inflammatory status in mice. PLoS One. 2012; 7:e39165. [PubMed: 22720061]

96. Manhiani M, Quigley JE, Knight SF, Tasoobshirazi S, Moore T, Brands MW, et al. Soluble epoxide hydrolase gene deletion attenuates renal injury and inflammation with DOCA-salt hypertension. Am J Physiol Renal Physiol. 2009; 297:F740–8. [PubMed: 19553349]

97. Revermann M, Schloss M, Barbosa-Sicard E, Mieth A, Liebner S, Morisseau C, et al. Soluble epoxide hydrolase deficiency attenuates neointima formation in the femoral cuff model of hyperlipidemic mice. Arterioscler Thromb Vasc Biol. 2010; 30:909–14. [PubMed: 20224052]

98. Sturlan S, Oberhuber G, Beinhauer BG, Tichy B, Kappel S, Wang J, et al. Interleukin-10-deficient mice and inflammatory bowel disease associated cancer development. Carcinogenesis. 2001; 22:665–71. [PubMed: 11285204]

99. Zhang W, Yang AL, Liao J, Li H, Dong H, Chung YT, et al. Soluble epoxide hydrolase gene deficiency or inhibition attenuates chronic active inflammatory bowel disease in IL-10(−/−) mice. Dig Dis Sci. 2012; 57:2580–91. [PubMed: 22588244]

100. Zhang W, Liao J, Li H, Dong H, Bai H, Yang A, et al. Reduction of inflammatory bowel disease-induced tumor development in IL-10 knockout mice with soluble epoxide hydrolase gene deficiency. Mol Carcinog. 2013; 52:726–38. [PubMed: 22517541]

101. Davis BB, Liu JY, Tancredi DJ, Wang L, Simon SI, Hammock BD, et al. The anti-inflammatory effects of soluble epoxide hydrolase inhibitors are independent of leukocyte recruitment. Biochem Biophys Res Commun. 2011; 410:494–500. [PubMed: 21683067]

102. Fife KL, Liu Y, Schmelzer KR, Tsai HJ, Kim IH, Morisseau C, et al. Inhibition of soluble epoxide hydrolase does not protect against endotoxin-mediated hepatic inflammation. J Pharmacol Exp Ther. 2008; 327:707–15. [PubMed: 18815352]

103. Baeuerle PA, Henkel T. Function and activation of NF-kappa B in the immune system. Annu Rev Immunol. 1994; 12:141–79. [PubMed: 8011280]

104. Liu Y, Zhang Y, Schmelzer K, Lee TS, Fang X, Zhu Y, et al. The antiinflammatory effect of laminar flow: the role of PPARgamma, epoxyeicosatrienoic acids, and soluble epoxide hydrolase. Proc Natl Acad Sci U S A. 2005; 102:16747–52. [PubMed: 16267130]

105. Xu D, Li N, He Y, Timofeyev V, Lu L, Tsai HJ, et al. Prevention and reversal of cardiac hypertrophy by soluble epoxide hydrolase inhibitors. Proc Natl Acad Sci U S A. 2006; 103:18733–8. [PubMed: 17130447]

106. Ng VY, Huang Y, Reddy LM, Falck JR, Lin ET, Kroetz DL. Cytochrome P450 eicosanoids are activators of peroxisome proliferator-activated receptor alpha. Drug Metab Dispos. 2007; 35:1126–34. [PubMed: 17431031]

107. Wray J, Bishop-Bailey D. Epoxygenases and peroxisome proliferator-activated receptors in mammalian vascular biology. Exp Physiol. 2008; 93:148–54. [PubMed: 17872966]

108. Wray JA, Sugden MC, Zeldin DC, Greenwood GK, Samsuddin S, Miller-Degraff L, et al. The epoxygenases CYP2J2 activates the nuclear receptor PPARalpha in vitro and in vivo. PLoS One. 2009; 4:e7421. [PubMed: 19823578]

109. Inceoglu B, Jinks SL, Ulu A, Hegedus CM, Georgi K, Schmelzer KR, et al. Soluble epoxide hydrolase and epoxyeicosatrienoic acids modulate two distinct analgesic pathways. Proc Natl Acad Sci U S A. 2008; 105:18901–6. [PubMed: 19028872]

110. Cheng Y, Austin SC, Rocca B, Koller BH, Coffman TM, Grosser T, et al. Role of prostacyclin in the cardiovascular response to thromboxane A(2). Science. 2002; 296:539–41. [PubMed: 11964481]

111. Morphy R, Rankovic Z. Designed Multiple Ligands. An Emerging Drug Discovery Paradigm. J Med Chem. 2005; 48:6523–43. [PubMed: 16220969]

112. Hwang SH, Wagner KM, Morisseau C, Liu JY, Dong H, Wecksler AT, et al. Synthesis and structure-activity relationship studies of urea-containing pyrazoles as dual inhibitors of cyclooxygenase-2 and soluble epoxide hydrolase. J Med Chem. 2011; 54:3037–50. [PubMed: 21434686]

113. Bouhassira D, Lanteri-Minet M, Attal N, Laurent B, Touboul C. Prevalence of chronic pain with neuropathic characteristics in the general population. Pain. 2008; 136:380–7. [PubMed: 17888574]

114. Gaskin DJ, Richard P. The economic costs of pain in the United States. J Pain. 2012; 13:715–24. [PubMed: 22607834]

115. Breivik H, Collett B, Ventafridda V, Cohen R, Gallacher D. Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. Eur J Pain. 2006; 10:287–333. [PubMed: 16095934]

116. Inceoglu B, Schmelzer K, Morisseau C, Jinks SL, Hammock BD. Soluble epoxide hydrolase inhibition reveals novel biological functions of epoxyeicosatrienoic acids (EETs). Prostaglandins Other Lipid Mediat. 2007; 82:42–9. [PubMed: 17164131]

117. Wagner K, Inceoglu B, Gill SS, Hammock BD. Epoxygenated fatty acids and soluble epoxide hydrolase inhibition: novel mediators of pain reduction. J Agric Food Chem. 2011; 59:2816–24. [PubMed: 20958046]

118. Wagner K, Inceoglu B, Hammock BD. Soluble epoxide hydrolase inhibition, epoxygenated fatty acids and nociception. Prostaglandins Other Lipid Mediat. 2011; 96:76–83. [PubMed: 21854866]

119. Inceoglu B, Jinks SL, Schmelzer KR, Waite T, Kim IH, Hammock BD. Inhibition of soluble epoxide hydrolase reduces LPS-induced thermal hyperalgesia and mechanical allodynia in a rat model of inflammatory pain. Life Sci. 2006; 79:2311–9. [PubMed: 16962614]

120. Inceoglu B, Wagner K, Yang J, Bettaieb A, Schebb NH, Hwang SH, et al. Acute augmentation of epoxygenated fatty acid levels rapidly reduces pain-related behavior in a rat model of type I diabetes. Proc Natl Acad Sci U S A. 2012; 109:11390–5. [PubMed: 22733772]

121. Inceoglu B, Wagner K, Schebb NH, Morisseau C, Jinks SL, Ulu A, et al. Analgesia mediated by soluble epoxide hydrolase inhibitors is dependent on cAMP. Proc Natl Acad Sci U S A. 2011; 108:5093–7. [PubMed: 21383170]

122. Brenneis C, Sisignano M, Coste O, Altenrath K, Fischer MJ, Angioni C, et al. Soluble epoxide hydrolase limits mechanical hyperalgesia during inflammation. Mol Pain. 2011; 7:78. [PubMed: 21970373]

123. Luria A, Bettaieb A, Xi Y, Shieh G-J, Liu H-C, Inoue H, et al. Soluble epoxide hydrolase deficiency alters pancreatic islet size and improves glucose homeostasis in a model of insulin resistance. Proc Natl Acad Sci U S A. 2011; 108:9038–43. [PubMed: 21571638]

124. Watanabe H, Vriens J, Prenen J, Droogmans G, Voets T, Nilius B. Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels. Nature. 2003; 424:434–8. [PubMed: 12879072]

125. Demuth DG, Molleman A. Cannabinoid signalling. Life Sci. 2006; 78:549–63. [PubMed: 16109430]

126. Di Marzo V. Targeting the endocannabinoid system: to enhance or reduce? Nat Rev Drug Discov. 2008; 7:438–55. [PubMed: 18446159]

127. Ibrahim MM, Deng H, Zvonok A, Cockayne DA, Kwan J, Mata HP, et al. Activation of CB2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: pain inhibition by receptors not present in the CNS. Proc Natl Acad Sci U S A. 2003; 100:10529–33. [PubMed: 12917492]

128. Hohmann AG, Farthing JN, Zvonok AM, Makriyannis A. Selective activation of cannabinoid CB2 receptors suppresses hyperalgesia evoked by intradermal capsaicin. J Pharmacol Exp Ther. 2004; 308:446–53. [PubMed: 14610224]

129. Reis GM, Pacheco D, Perez AC, Klein A, Ramos MA, Duarte ID. Opioid receptor and NO/cGMP pathway as a mechanism of peripheral antinociceptive action of the cannabinoid receptor agonist anandamide. Life Sci. 2009; 85:351–6. [PubMed: 19576231]

130. Snider NT, Nast JA, Tesmer LA, Hollenberg PF. A cytochrome P450-derived epoxygenated metabolite of anandamide is a potent cannabinoid receptor 2-selective agonist. Mol Pharmacol. 2009; 75:965–72. [PubMed: 19171674]

131. Terashvili M, Tseng LF, Wu HE, Narayanan J, Hart LM, Falck JR, et al. Antinociception produced by 14,15-epoxyeicosatrienoic acid is mediated by the activation of beta-endorphin and met-enkephalin in the rat ventrolateral periaqueductal gray. J Pharmacol Exp Ther. 2008; 326:614–22. [PubMed: 18492947]

132. Conroy JL, Fang C, Gu J, Zeitlin SO, Yang W, Yang J, et al. Opioids activate brain analgesic circuits through cytochrome P450/epoxygenase signaling. Nat Neurosci. 2010; 13:284–6. [PubMed: 20139973]

133. Heinricher MM, Maire JJ, Lee D, Nalwalk JW, Hough LB. Physiological basis for inhibition of morphine and improgan antinociception by CC12, a P450 epoxygenase inhibitor. J Neurophysiol. 2010; 104:3222–30. [PubMed: 20926616]

134. Hough LB, Nalwalk JW, Yang J, Conroy JL, VanAlstine MA, Yang W, et al. Brain P450 epoxygenase activity is required for the antinociceptive effects of improgan, a nonopioid analgesic. Pain. 2011; 152:878–87. [PubMed: 21316152]

135. Wang X, Shen CL, Dyson MT, Yin X, Schiffer RB, Grammas P, et al. The involvement of epoxygenase metabolites of arachidonic acid in cAMP-stimulated steroidogenesis and steroidogenic acute regulatory protein gene expression. J Endocrinol. 2006; 190:871–8. [PubMed: 17003287]

136. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med. 1995; 1:27–31. [PubMed: 7584949]

137. Folkman J. Angiogenesis: an organizing principle for drug discovery? Nat Rev Drug Discov. 2007; 6:273–86. [PubMed: 17396134]

138. Rosenfeld PJ, Brown DM, Heier JS, Boyer DS, Kaiser PK, Chung CY, et al. Ranibizumab for neovascular age-related macular degeneration. N Engl J Med. 2006; 355:1419–31. [PubMed: 17021318]

139. Griffioen AW, Molema G. Angiogenesis: potentials for pharmacologic intervention in the treatment of cancer, cardiovascular diseases, and chronic inflammation. Pharmacol Rev. 2000; 52:237–68. [PubMed: 10835101]

140. Wang D, Dubois RN. Eicosanoids and cancer. Nat Rev Cancer. 2010; 10:181–93. [PubMed: 20168319]

141. Panigrahy D, Kaipainen A, Greene ER, Huang S. Cytochrome P450-derived eicosanoids: the neglected pathway in cancer. Cancer Metastasis Rev. 2010; 29:723–35. [PubMed: 20941528]

142. Munzenmaier DH, Harder DR. Cerebral microvascular endothelial cell tube formation: role of astrocytic epoxyeicosatrienoic acid release. Am J Physiol Heart Circ Physiol. 2000; 278:H1163–7. [PubMed: 10749710]

143. Panigrahy D, Greene ER, Pozzi A, Wang DW, Zeldin DC. EET signaling in cancer. Cancer Metastasis Rev. 2011; 30:525–40. [PubMed: 22009066]

144. Fleming I. Epoxyeicosatrienoic acids, cell signaling and angiogenesis. Prostaglandins Other Lipid Mediat. 2007; 82:60–7. [PubMed: 17164133]

145. Potente M, Fisslthaler B, Busse R, Fleming I. 11,12-Epoxyeicosatrienoic acid-induced inhibition of FOXO factors promotes endothelial proliferation by down-regulating p27Kip1. J Biol Chem. 2003; 278:29619–25. [PubMed: 12773534]

146. Ma J, Zhang L, Han W, Shen T, Ma C, Liu Y, et al. Activation of JNK/c-Jun is required for the proliferation, survival, and angiogenesis induced by EET in pulmonary artery endothelial cells. J Lipid Res. 2012; 53:1093–105. [PubMed: 22493087]

147. Zhang B, Cao H, Rao GN. Fibroblast growth factor-2 is a downstream mediator of phosphatidylinositol 3-kinase-Akt signaling in 14,15-epoxyeicosatrienoic acid-induced angiogenesis. J Biol Chem. 2006; 281:905–14. [PubMed: 16286479]

148. Michaelis UR, Fisslthaler B, Medhora M, Harder D, Fleming I, Busse R. Cytochrome P450 2C9-derived epoxyeicosatrienoic acids induce angiogenesis via cross-talk with the epidermal growth factor receptor (EGFR). FASEB J. 2003; 17:770–2. [PubMed: 12586744]

149. Yan G, Chen S, You B, Sun J. Activation of sphingosine kinase-1 mediates induction of endothelial cell proliferation and angiogenesis by epoxyeicosatrienoic acids. Cardiovasc Res. 2008; 78:308–14. [PubMed: 18192241]

150. Pozzi A, Macias-Perez I, Abair T, Wei S, Su Y, Zent R, et al. Characterization of 5,6- and 8,9-epoxyeicosatrienoic acids (5,6- and 8,9-EET) as potent in vivo angiogenic lipids. J Biol Chem. 2005; 280:27138–46. [PubMed: 15917237]

151. Medhora M, Daniels J, Mundey K, Fisslthaler B, Busse R, Jacobs ER, et al. Epoxygenase-driven angiogenesis in human lung microvascular endothelial cells. Am J Physiol Heart Circ Physiol. 2003; 284:H215–24. [PubMed: 12388259]

152. Webler AC, Popp R, Korff T, Michaelis UR, Urbich C, Busse R, et al. Cytochrome P450 2C9-induced angiogenesis is dependent on EphB4. Arterioscler Thromb Vasc Biol. 2008; 28:1123–9. [PubMed: 18340006]

153. Michaelis UR, Falck JR, Schmidt R, Busse R, Fleming I. Cytochrome P4502C9-derived epoxyeicosatrienoic acids induce the expression of cyclooxygenase-2 in endothelial cells. Arterioscler Thromb Vasc Biol. 2005; 25:321–6. [PubMed: 15569819]

154. Cheranov SY, Karpurapu M, Wang D, Zhang B, Venema RC, Rao GN. An essential role for SRC-activated STAT-3 in 14,15-EET-induced VEGF expression and angiogenesis. Blood. 2008; 111:5581–91. [PubMed: 18408167]

155. Sander AL, Jakob H, Sommer K, Sadler C, Fleming I, Marzi I, et al. Cytochrome P450-derived epoxyeicosatrienoic acids accelerate wound epithelialization and neovascularization in the hairless mouse ear wound model. Langenbecks Arch Surg. 2011; 396:1245–53. [PubMed: 21887579]

156. Panigrahy D, Kalish BT, Huang S, Bielenberg DR, Le HD, Yang J, et al. Epoxyeicosanoids promote organ and tissue regeneration. Proc Natl Acad Sci U S A. 2013; 110:13528–33. [PubMed: 23898174]

157. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med. 2003; 9:669–76. [PubMed: 12778165]

158. Webler AC, Michaelis UR, Popp R, Barbosa-Sicard E, Murugan A, Falck JR, et al. Epoxyeicosatrienoic acids are part of the VEGF-activated signaling cascade leading to angiogenesis. Am J Physiol Cell Physiol. 2008; 295:C1292–301. [PubMed: 18787075]

159. Yang S, Wei S, Pozzi A, Capdevila JH. The arachidonic acid epoxygenase is a component of the signaling mechanisms responsible for VEGF-stimulated angiogenesis. Arch Biochem Biophys. 2009; 489:82–91. [PubMed: 19464254]

160. Chen JK, Falck JR, Reddy KM, Capdevila J, Harris RC. Epoxyeicosatrienoic acids and their sulfonimide derivatives stimulate tyrosine phosphorylation and induce mitogenesis in renal epithelial cells. J Biol Chem. 1998; 273:29254–61. [PubMed: 9786938]

161. Chen JK, Capdevila J, Harris RC. Heparin-binding EGF-like growth factor mediates the biological effects of P450 arachidonate epoxygenase metabolites in epithelial cells. Proc Natl Acad Sci U S A. 2002; 99:6029–34. [PubMed: 11983897]

162. Nithipatikom K, Brody DM, Tang AT, Manthati VL, Falck JR, Williams CL, et al. Inhibition of carcinoma cell motility by epoxyeicosatrienoic acid (EET) antagonists. Cancer Sci. 2010; 101:2629–36. [PubMed: 20804500]

163. Yang S, Lin L, Chen JX, Lee CR, Seubert JM, Wang Y, et al. Cytochrome P-450 epoxygenases protect endothelial cells from apoptosis induced by tumor necrosis factor-alpha via MAPK and PI3K/Akt signaling pathways. Am J Physiol Heart Circ Physiol. 2007; 293:H142–51. [PubMed: 17322420]

164. Jiang JG, Chen CL, Card JW, Yang S, Chen JX, Fu XN, et al. Cytochrome P450 2J2 promotes the neoplastic phenotype of carcinoma cells and is up-regulated in human tumors. Cancer Res. 2005; 65:4707–15. [PubMed: 15930289]

165. Jiang JG, Ning YG, Chen C, Ma D, Liu ZJ, Yang S, et al. Cytochrome p450 epoxygenase promotes human cancer metastasis. Cancer Res. 2007; 67:6665–74. [PubMed: 17638876]

166. Chen C, Wei X, Rao X, Wu J, Yang S, Chen F, et al. Cytochrome P450 2J2 is highly expressed in hematologic malignant diseases and promotes tumor cell growth. J Pharmacol Exp Ther. 2011; 336:344–55. [PubMed: 21030485]

167. Mitra R, Guo Z, Milani M, Mesaros C, Rodriguez M, Nguyen J, et al. 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). J Biol Chem. 2011; 286:17543–59. [PubMed: 21402692]

168. Murray GI, Patimalla S, Stewart KN, Miller ID, Heys SD. Profiling the expression of cytochrome P450 in breast cancer. Histopathology. 2010; 57:202–11. [PubMed: 20716162]

169. Guengerich FP, Turvy CG. Comparison of levels of several human microsomal cytochrome P-450 enzymes and epoxide hydrolase in normal and disease states using immunochemical analysis of surgical liver samples. J Pharmacol Exp Ther. 1991; 256:1189–94. [PubMed: 2005581]

170. Leclerc J, Tournel G, Courcot-Ngoubo Ngangue E, Pottier N, Lafitte JJ, Jaillard S, et al. Profiling gene expression of whole cytochrome P450 superfamily in human bronchial and peripheral lung tissues: Differential expression in non-small cell lung cancers. Biochimie. 2010; 92:292–306. [PubMed: 20034539]

171. Goodman AI, Choudhury M, da Silva JL, Schwartzman ML, Abraham NG. Overexpression of the heme oxygenase gene in renal cell carcinoma. Proc Soc Exp Biol Med. 1997; 214:54–61. [PubMed: 9012361]

172. Enayetallah AE, French RA, Grant DF. Distribution of soluble epoxide hydrolase, cytochrome P450 2C8, 2C9 and 2J2 in human malignant neoplasms. J Mol Histol. 2006; 37:133–41. [PubMed: 16957870]

173. Ulu A, Harris TR, Morisseau C, Miyabe C, Inoue H, Schuster G, et al. Anti-inflammatory Effects of Omega-3 Polyunsaturated Fatty Acids and Soluble Epoxide Hydrolase Inhibitors in Angiotensin-II Dependent Hypertension. J Cardiovasc Pharmacol. 2013

174. Morin C, Sirois M, Echave V, Albadine R, Rousseau E. 17,18-epoxyeicosatetraenoic acid targets PPARgamma and p38 mitogen-activated protein kinase to mediate its anti-inflammatory effects in the lung: role of soluble epoxide hydrolase. Am J Respir Cell Mol Biol. 2010; 43:564–75. [PubMed: 20008283]

175. Chen HX, Cleck JN. Adverse effects of anticancer agents that target the VEGF pathway. Nat Rev Clin Oncol. 2009; 6:465–77. [PubMed: 19581909]

176. Morris MC, Sacks F, Rosner B. Does fish oil lower blood pressure? A meta-analysis of controlled trials. Circulation. 1993; 88:523–33. [PubMed: 8339414]

177. Bonaa KH, Bjerve KS, Straume B, Gram IT, Thelle D. Effect of eicosapentaenoic and docosahexaenoic acids on blood pressure in hypertension. A population-based intervention trial from the Tromso study. N Engl J Med. 1990; 322:795–801. [PubMed: 2137901]

178. Hoshi T, Wissuwa B, Tian Y, Tajima N, Xu R, Bauer M, et al. Omega-3 fatty acids lower blood pressure by directly activating large-conductance Ca(2)(+)-dependent K(+) channels. Proc Natl Acad Sci U S A. 2013; 110:4816–21. [PubMed: 23487785]

179. Alitalo K, Tammela T, Petrova TV. Lymphangiogenesis in development and human disease. Nature. 2005; 438:946–53. [PubMed: 16355212]

180. Hu J, Popp R, fleming I. Genetic deletion and pharmacological inhibition of the soluble epoxide hydrolase attenuate angiogenesis in the murine retina. Acta Physiologica. 2011; 201(Supplement 682):P045.

181. Cui PH, Petrovic N, Murray M. The omega-3 epoxide of eicosapentaenoic acid inhibits endothelial cell proliferation by p38 MAP kinase activation and cyclin D1/CDK4 down-regulation. Br J Pharmacol. 2011; 162:1143–55. [PubMed: 21077851]

182. O'Reilly MS, Holmgren L, Shing Y, Chen C, Rosenthal RA, Moses M, et al. Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. Cell. 1994; 79:315–28. [PubMed: 7525077]

183. Steeg PS. Tumor metastasis: mechanistic insights and clinical challenges. Nat Med. 2006; 12:895–904. [PubMed: 16892035]

184. Falck JR, Wallukat G, Puli N, Goli M, Arnold C, Konkel A, et al. 17(R),18(S)-epoxyeicosatetraenoic acid, a potent eicosapentaenoic acid (EPA) derived regulator of cardiomyocyte contraction: structure-activity relationships and stable analogues. J Med Chem. 2011; 54:4109–18. [PubMed: 21591683]

185. Guedes AG, Morisseau C, Sole A, Soares JH, Ulu A, Dong H, et al. Use of a soluble epoxide hydrolase inhibitor as an adjunctive analgesic in a horse with laminitis. Vet Anaesth Analg. 2013; 40:440–8. [PubMed: 23463912]

186. Escudier B, Eisen T, Stadler WM, Szczylik C, Oudard S, Siebels M, et al. Sorafenib in advanced clear-cell renal-cell carcinoma. N Engl J Med. 2007; 356:125–34. [PubMed: 17215530]

187. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med. 2008; 359:378–90. [PubMed: 18650514]

188. Demetri GD, Reichardt P, Kang YK, Blay JY, Rutkowski P, Gelderblom H, et al. Efficacy and safety of regorafenib for advanced gastrointestinal stromal tumours after failure of imatinib and sunitinib (GRID): an international, multicentre, randomised, placebo-controlled, phase 3 trial. Lancet. 2013; 381:295–302. [PubMed: 23177515]

189. Grothey A, Van Cutsem E, Sobrero A, Siena S, Falcone A, Ychou M, et al. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. Lancet. 2013; 381:303–12. [PubMed: 23177514]

190. Wilhelm SM, Dumas J, Adnane L, Lynch M, Carter CA, Schutz G, et al. Regorafenib (BAY 73-4506): a new oral multikinase inhibitor of angiogenic, stromal and oncogenic receptor tyrosine kinases with potent preclinical antitumor activity. Int J Cancer. 2011; 129:245–55. [PubMed: 21170960]

191. Parrish AR, Chen G, Burghardt RC, Watanabe T, Morisseau C, Hammock BD. Attenuation of cisplatin nephrotoxicity by inhibition of soluble epoxide hydrolase. Cell Biol Toxicol. 2009; 25:217–25. [PubMed: 18386137]

192. Liu Y, Webb HK, Fukushima H, Micheli J, Markova S, Olson JL, et al. Attenuation of cisplatin-induced renal injury by inhibition of soluble epoxide hydrolase involves nuclear factor kappaB signaling. J Pharmacol Exp Ther. 2012; 341:725–34. [PubMed: 22414856]

193. Wong PY, Lin KT, Yan YT, Ahern D, Iles J, Shen SY, et al. 14(R),15(S)-epoxyeicosatrienoic acid (14(R),15(S)-EET) receptor in guinea pig mononuclear cell membranes. J Lipid Mediat. 1993; 6:199–208. [PubMed: 8395243]

194. Yang W, Tuniki VR, Anjaiah S, Falck JR, Hillard CJ, Campbell WB. Characterization of epoxyeicosatrienoic acid binding site in U937 membranes using a novel radiolabeled agonist, 20-125i-14,15-epoxyeicosa-8(Z)-enoic acid. J Pharmacol Exp Ther. 2008; 324:1019–27. [PubMed: 18171909]

195. Alfano CM, Imayama I, Neuhouser ML, Kiecolt-Glaser JK, Smith AW, Meeske K, et al. Fatigue, Inflammation, and omega-3 and omega-6 Fatty Acid Intake Among Breast Cancer Survivors. Journal of Clinical Oncology. 30:1280–7. [PubMed: 22412148]

196. Kris-Etherton PM, Harris WS, Appel LJ. American Heart Association. Nutrition C. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation. 2002; 106:2747–57. [PubMed: 12438303]

197. Albert CM, Campos H, Stampfer MJ, Ridker PM, Manson JE, Willett WC, et al. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. N Engl J Med. 2002; 346:1113–8. [PubMed: 11948270]

198. Simopoulos AP. Omega-3 fatty acids in inflammation and autoimmune diseases. J Am Coll Nutr. 2002; 21:495–505. [PubMed: 12480795]

199. MacLean CH, Newberry SJ, Mojica WA, Khanna P, Issa AM, Suttorp MJ, et al. Effects of omega-3 fatty acids on cancer risk: a systematic review. JAMA. 2006; 295:403–15. [PubMed: 16434631]

200. Rizos EC, Ntzani EE, Bika E, Kostapanos MS, Elisaf MS. Association between omega-3 fatty acid supplementation and risk of major cardiovascular disease events: a systematic review and meta-analysis. JAMA. 2012; 308:1024–33. [PubMed: 22968891]

201. Brasky TM, Darke AK, Song X, Tangen CM, Goodman PJ, Thompson IM, et al. Plasma Phospholipid Fatty Acids and Prostate Cancer Risk in the SELECT Trial. J Natl Cancer Inst. 2013; 105:1132–41. [PubMed: 23843441]

202. Nording ML, Yang J, Georgi K, Hegedus Karbowski C, German JB, Weiss RH, et al. Individual variation in lipidomic profiles of healthy subjects in response to omega-3 Fatty acids. PLoS One. 2013; 8:e76575. [PubMed: 24204640]

203. Berquin IM, Min Y, Wu R, Wu J, Perry D, Cline JM, et al. Modulation of prostate cancer genetic risk by omega-3 and omega-6 fatty acids. J Clin Invest. 2007; 117:1866–75. [PubMed: 17607361]

204. Dreisbach AW, Japa S, Sigel A, Parenti MB, Hess AE, Srinouanprachanh SL, et al. The Prevalence of CYP2C8, 2C9, 2J2, and soluble epoxide hydrolase polymorphisms in African Americans with hypertension. Am J Hypertens. 2005; 18:1276–81. [PubMed: 16202848]

205. Spiecker M, Darius H, Hankeln T, Soufi M, Sattler AM, Schaefer JR, et al. Risk of coronary artery disease associated with polymorphism of the cytochrome P450 epoxygenase CYP2J2. Circulation. 2004; 110:2132–6. [PubMed: 15466638]

206. Fornage M, Boerwinkle E, Doris PA, Jacobs D, Liu K, Wong ND. Polymorphism of the soluble epoxide hydrolase is associated with coronary artery calcification in African-American subjects: The Coronary Artery Risk Development in Young Adults (CARDIA) study. Circulation. 2004; 109:335–9. [PubMed: 14732757]

207. Lee CR, North KE, Bray MS, Fornage M, Seubert JM, Newman JW, et al. Genetic variation in soluble epoxide hydrolase (EPHX2) and risk of coronary heart disease: The Atherosclerosis Risk in Communities (ARIC) study. Hum Mol Genet. 2006; 15:1640–9. [PubMed: 16595607]

208. Wei Q, Doris PA, Pollizotto MV, Boerwinkle E, Jacobs DR Jr, Siscovick DS, et al. Sequence variation in the soluble epoxide hydrolase gene and subclinical coronary atherosclerosis: interaction with cigarette smoking. Atherosclerosis. 2007; 190:26–34. [PubMed: 16545818]

209. Przybyla-Zawislak BD, Srivastava PK, Vazquez-Matias J, Mohrenweiser HW, Maxwell JE, Hammock BD, et al. Polymorphisms in human soluble epoxide hydrolase. Mol Pharmacol. 2003; 64:482–90. [PubMed: 12869654]

210. Srivastava PK, Sharma VK, Kalonia DS, Grant DF. Polymorphisms in human soluble epoxide hydrolase: effects on enzyme activity, enzyme stability, and quaternary structure. Arch Biochem Biophys. 2004; 427:164–9. [PubMed: 15196990]

211. Zeisel SH, Waterland RA, Ordovas JM, Muoio DM, Jia W, Fodor A. Highlights of the 2012 Research Workshop: Using nutrigenomics and metabolomics in clinical nutrition research. JPEN J Parenter Enteral Nutr. 2013; 37:190–200. [PubMed: 23042849]
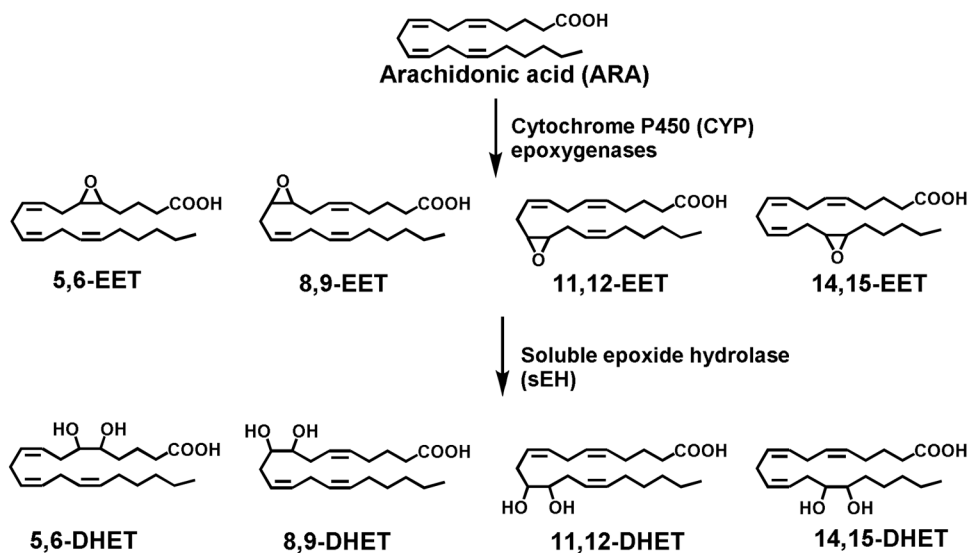
Fig. 1.
The metabolism of arachidonic acid by cytochrome P450 (CYP) epoxygenases (largely CYP2C and CYP2J) leads to the formation of epoxyeicosatrienoic acids (EETs) including four regioisomers of 5,6-, 8,9-, 11,12- and 14,15-EET. EETs are further metabolized by soluble epoxide hydrolase (sEH) to form the fatty acid diols termed dihydroxyeicosatrienoic acids (DHETs) which are usually less-active or inactive.
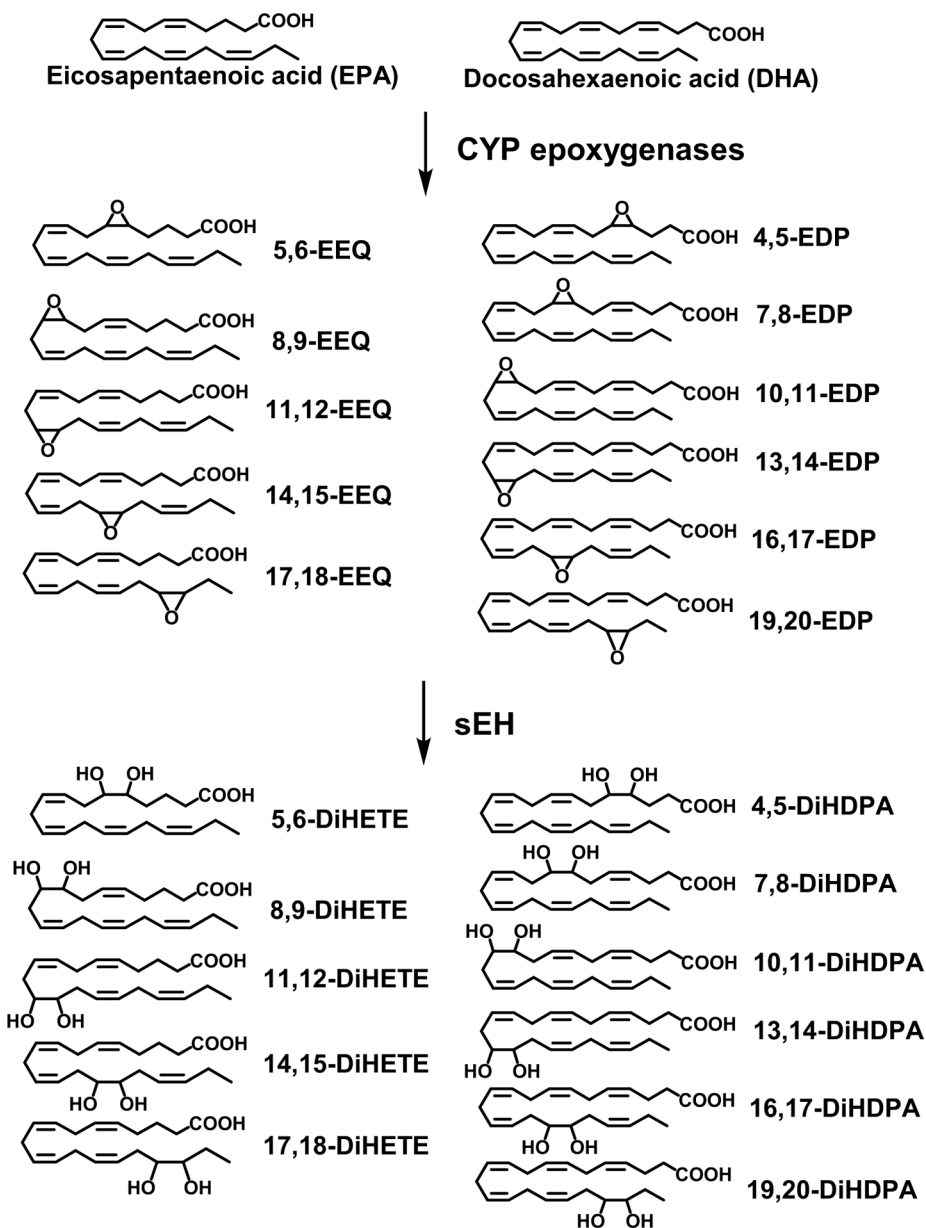
Fig. 2.
The ω-3 PUFAs including EPA and DHA are highly efficient alternative substrates of the CYP/sEH pathway. The metabolism of EPA and DHA by CYP epoxygenases generates ω-3-series epoxygenated fatty acids (EpFAs), including 5 regioisomers of EEQs from EPA and 6 EDP isomers from DHA. Compared with ARA, EPA and DHA showed higher or similar activities toward the metabolism by CYP epoxygenases. Compared with EETs, EEQs and EDPs are also better substrates of sEH to be metabolized to the corresponding fatty acid diols (DiHETE and DiHDPA respectively) with the exception of 19,20-EDP.
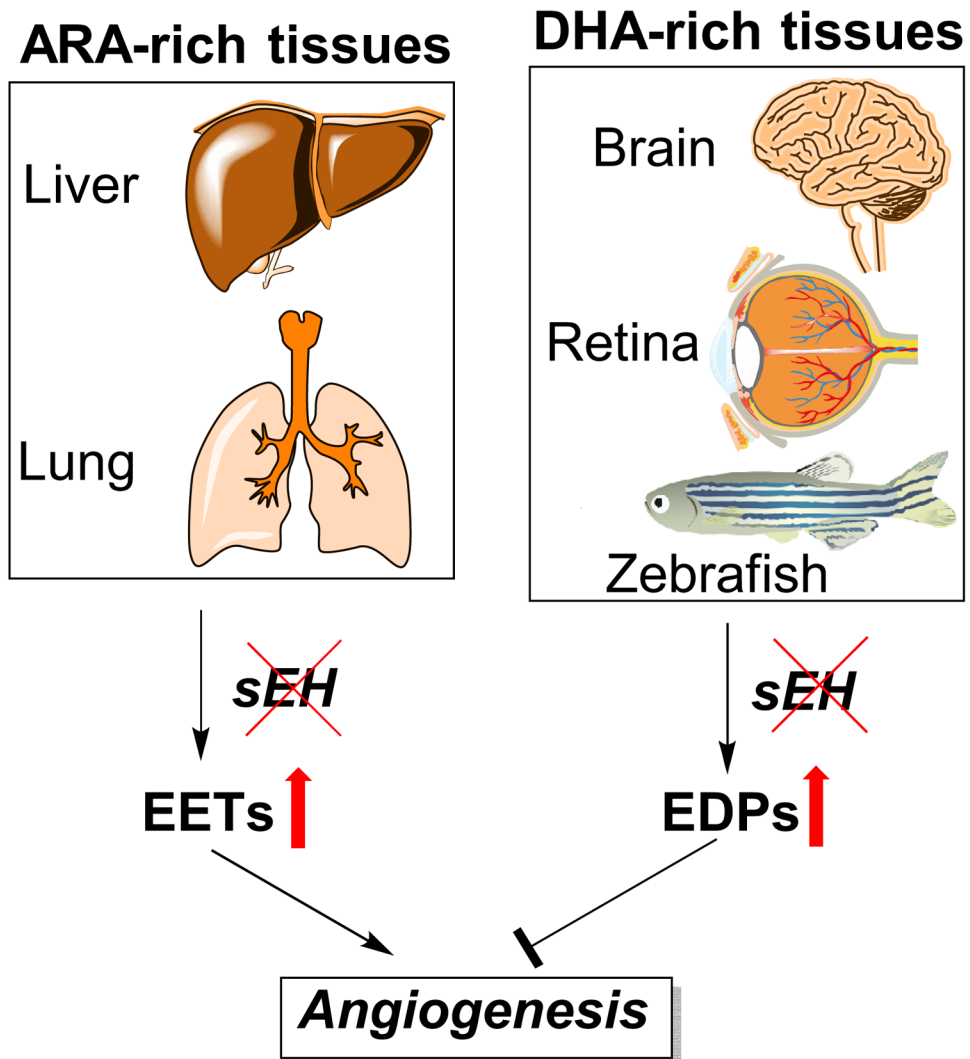
Fig. 3.
Genetic deletion or pharmacological inhibition of sEH in ω-6-rich and ω-3-rich tissues leads to accumulation of EETs and EDPs respectively, leading to different effects on angiogenesis.