

Cytochrome P450-derived eicosanoids: the neglected pathway in cancer

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Abstract Endogenously produced lipid autacoids are locally acting small molecule mediators that play a central role in the regulation of inflammation and tissue homeostasis. A well-studied group of autacoids are the products of arachidonic acid metabolism, among which the prostaglandins and leukotrienes are the best known. They are generated by two pathways controlled by the enzyme systems cyclooxygenase and lipoxygenase, respectively. However, arachidonic acid is also substrate for a third enzymatic pathway, the cytochrome P450 (CYP) system. This third eicosanoid pathway consists of two main branches: ω -hydroxylases convert arachidonic acid to hydroxyeicosatetraenoic acids (HETEs) and epoxygenases convert it to epoxyeicosatrienoic acids (EETs). This third CYP pathway was originally studied in conjunction with inflammatory and cardiovascular disease. Arachidonic acid and its metabolites have recently stimulated great interest in cancer biology; but, unlike

prostaglandins and leukotrienes the link between cytochrome P450 metabolites and cancer has received little attention. In this review, the emerging role in cancer of cytochrome P450 metabolites, notably 20-HETE and EETs, are discussed.

Keywords Cytochrome P450 · Arachidonic acid · HETEs · EETs · Cancer · Metastasis

Abbreviations

CYP and P450	Cytochrome P450
COX	Cyclooxygenase
LOX	Lipoxygenase
EET	Epoxyeicosatrienoic acid
HETE	Hydroxyeicosatetraenoic acid
sEH	Soluble epoxide hydrolase
DHET	Dihydroxyeicosatrienoic acid
14,15-EEZE	14,15-epoxyeicosa-5(Z)-enoic acid
PGE ₂	Prostaglandin E ₂
LTB ₄	Leukotriene B ₄
VEGF	Vascular endothelial growth factor
FGF-2	Fibroblast growth factor-2
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
MAPK	Mitogen-activated protein kinase
NF- κ B	Nuclear factor-kappaB
HIF-1 α	Hypoxia-inducible factor-1 α
NO	Nitric oxide
eNOS	Endothelial nitric oxide synthase
PI3K/Akt	Phosphatidylinositol-3-kinase/Akt
PPAR	Peroxisome-proliferator-activated receptor

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1 Introduction

Products of arachidonic acid metabolism, including prostaglandins and leukotrienes are potent mediators of inflammation [1]. These lipid mediators, collectively called

eicosanoids, play critical roles in diverse physiological and pathological processes such as pulmonary fibrosis and cancer (Fig. 1). The first two pathways of arachidonic acid metabolism are controlled by the enzyme families cyclooxygenase (COX) and lipoxygenase (LOX). These enzymes are the target of approved drugs for the treatment of pain, inflammation, asthma, and allergies [2]. Both of these pathways produce prostaglandins and leukotrienes, respectively, and have been implicated in cancer [3]. However, a third eicosanoid pathway, in which cytochrome P450 (CYP) enzymes convert arachidonic acid into hydroxyeicosatetraenoic acids (HETEs) or epoxyeicosatrienoic acids (EETs), appears to have a role in tumor growth. COX- and LOX-derived eicosanoids have been intensely studied in tumor biology, while the study of cytochrome P450-derived eicosanoids has focused on inflammation, angiogenesis, and cardiovascular function rather than cancer pathways [1–6].

1.1 Overview of the CYP pathway

Cytochrome P450-dependent metabolism of arachidonic acid occurs in several tissues including liver, kidney, and the cardiovascular system. The CYP enzymes relevant to arachidonic acid metabolism include two distinct pathways: the ω -hydroxylase and epoxygenase pathways. The ω -hydroxylases of the 4A and 4F gene families of cytochrome P450 (CYP4A and CYP4F) convert arachidonic acid to autacoids such as hydroxyeicosatetraenoic acids. 20-hydroxyeicosatetraenoic acid is the principal isoform of this pathway and has shown vasoconstrictory activity [7–9]. The epoxygenase pathway is encoded predominantly by the CYP2C and CYP2J genes and generates epoxyeicosatrienoic acids, which have demonstrated vasodilatory activity [1, 10, 11]. EETs are then metabolized mainly by soluble epoxide hydrolase (sEH) to the dihydroxyeicosatrienoic acids (DHETs), which have traditionally been considered to be less active than EETs [12, 13]. The biology of both the epoxygenase and ω -hydroxylase pathways of cytochrome P450 enzymes has been extensively reviewed [1, 2, 4–6].

1.2 History of the CYP eicosanoids

Based on the pioneering work of Estabrook, both Capdevila and Falck found and characterized a third pathway, microsomal cytochrome P450 arachidonic acid metabolism [14, 15]. In 1981, metabolites separate from the prostanoids and leukotrienes were identified by the oxidative metabolism of arachidonic acid through microsomal cytochrome P450 systems [16–19]. In 1996, EETs were identified by Campbell and colleagues as endothelium-derived substances that hyperpolarize vascular smooth muscle [20]. This discovery sparked interest in the newly developing field of CYP eicosanoids. Within this field, Zeldin and colleagues identi-

fied the EET regioselectivity of sEH and were the first to identify and clone the CYP2J2 gene. Over the past decade, the Falck laboratory has synthesized agonists and antagonists of CYP 450 metabolites, including EETs and 20-HETE. However, the rapid metabolism of EETs and other epoxy-lipids has made it difficult to study the biological relevance of these metabolites. To address this challenge, the Hammock laboratory pioneered a series of sEH inhibitors which further stabilized EETs [2, 21]. sEH inhibitors, which increase EET levels, have been evaluated in the clinic for cardiovascular diseases, such as hypertension [2]. In addition, EET and HETE levels are now quantifiable by liquid chromatography–tandem mass spectrometry [22].

In this review, we survey the largely unexplored field of cytochrome P450 metabolites of arachidonic acid in tumorigenesis. We will focus on their roles in cancer as well as in angiogenesis and inflammation; two interdependent processes in the tumor stroma that play pivotal roles in tumor growth and metastasis.

2 CYP P450 genes, enzymes, and current role in pharmacology

This CYP superfamily is a complex group of enzymes that consist of upwards of 102 putatively functional genes in mice, and as few as 57 in humans [23, 24]. These CYP enzymes differ greatly from mouse to man, presenting challenges in the characterization of CYPs in this field [25, 26].

The best known function of the CYP enzymes is the detoxification of compounds, such as anti-cancer drugs and xenobiotics in the liver. Blocking these enzymes improves the half-life of the cytotoxic drugs—a strategy that is currently under evaluation to improve the efficacy of cancer drug delivery [23, 27, 28]. Conversely, prodrugs activated by cytochrome P450 enzymes are being used to inhibit tumor growth by targeting the tumor cells and tumor-associated endothelial cells [29, 30].

Therapeutic success has already been obtained using cytochrome P450 inhibitors to treat breast cancer [31]. This has prompted investigators to determine whether cytochrome P450 inhibitors can be utilized to treat other hormonally responsive cancers including prostate cancer [29, 32, 33]. While the field of directly targeting cytochrome P450 enzymes in cancer has rapidly expanded, the biological role of CYP-derived lipid autacoids in cancer has been largely neglected.

3 Synthesis and degradation of hydroxyeicosatetraenoic acids and epoxyeicosatrienoic acids

Arachidonic acid is an essential component of mammalian cell membranes and plays a critical role in the synthesis of

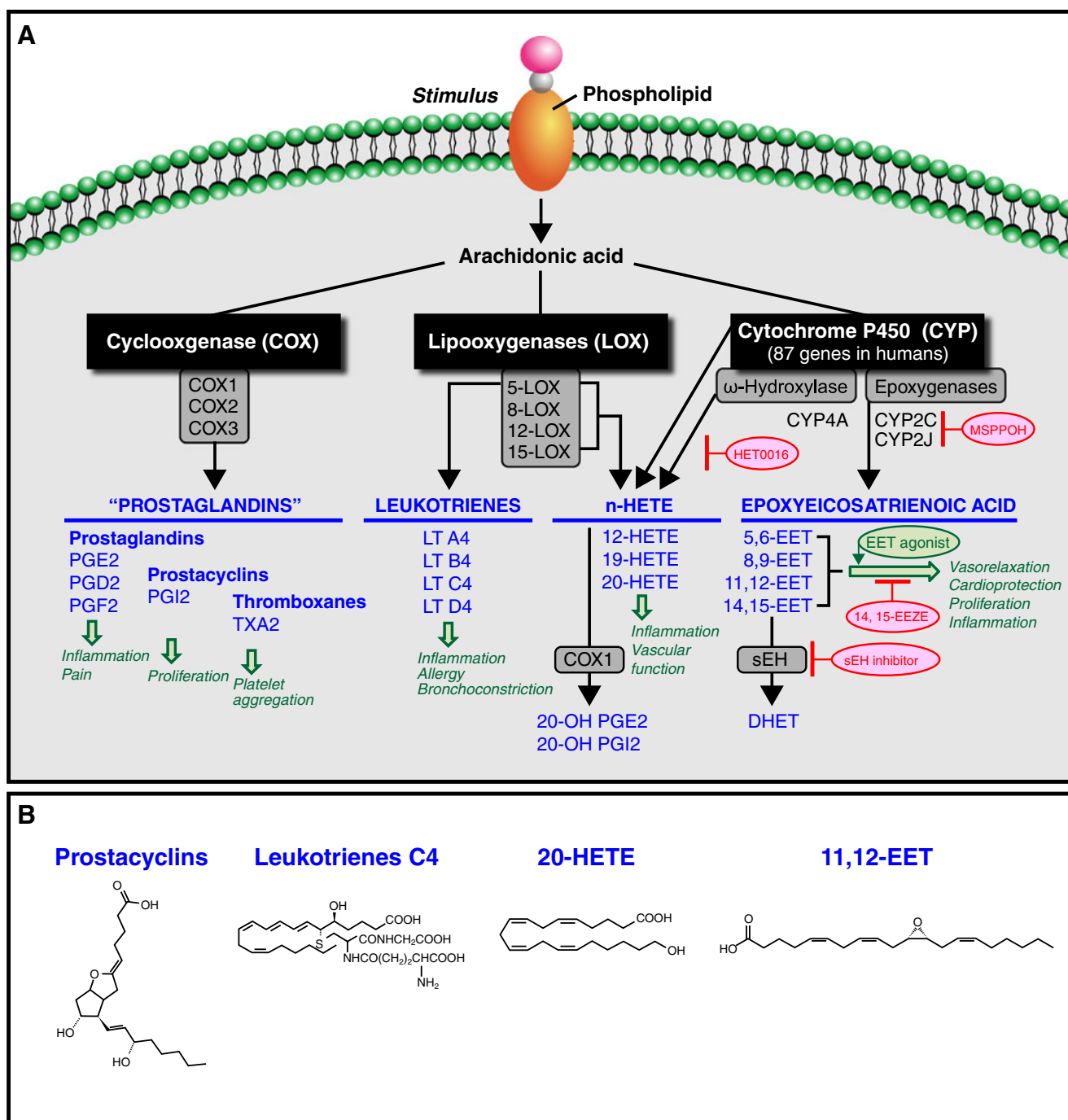


Fig. 1 a, b Bioactive eicosanoids derived from the arachidonic acid cascade. Arachidonic acid is metabolized by three pathways—the cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP) pathways. Schematic overview of major mediators and their metabolites (blue); enzymes (black, boxed) and biological role (green). Inhibitors (red ovals) and agonists (green ovals). HETEs Hydroxyeicosatetraenoic acids, EETs epoxyeicosatrienoic acids, CYP cytochrome

P450 enzymes. MS-PPHOH is a selective inhibitor of a subset of epoxygenases. HET0016 is a selective inhibitor of the ω -hydroxylase CYP4A. The sEH inhibitor (soluble epoxide hydrolase inhibitor) increases EET levels by acting as an agonist of the EET pathway. 14,15-EEZE is a putative EET receptor antagonist. PGE₂ prostaglandin E₂, PGI₂ prostacyclin, LTA₄ leukotriene A₄, DHET dihydroxyeicosatrienoic acid, 20-OH PGE₂ 20-hydroxy-prostaglandin E₂

bioactive eicosanoids [1]. Eicosanoids are generated via the oxidation of the 20-carbon chain present on arachidonic acid or other related fatty acids [2]. During processes, such as inflammation, arachidonic acid is released from the cell

membrane through the activation of phospholipase A₂ [1]. Arachidonic acid is metabolized by the CYP ω -hydroxylases to 7-, 10-, 12-, 13-, 15-, 16-, 17-, 18-, 19-, and 20-HETEs, the principal metabolite being the pro-inflammatory 20-HETE [4].

The epoxygenase CYP enzymes metabolize arachidonic acid by olefin epoxidation, resulting in four regioisomeric epoxyeicosatrienoic acids (EETs): 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET [1]. Each regioisomer can be formed as either an R,S or S,R enantiomer as the epoxide group can attach at each of the double bonds in two separate configurations, resulting in a total of eight EETs (reviewed by Zeldin [1]).

EETs are primarily synthesized in endothelial cells which express isoforms of CYP2C and CYP2J (e.g., CYP2C9 and CYP2J2) [10, 34–37]. EETs are also produced in other cell types such as astrocytes and cardiac myocytes [38–42]. Additionally, monocytic leukocytes have recently been shown to express CYP2J2 and therefore, may generate EETs [43].

The synthesis of 20-HETE and 12-HETE occurs in vascular smooth muscle cells and fibroblasts respectively through the cytochrome P-450 (CYP450) pathway [4, 44]. 20-HETE synthesis can be controlled by a positive feedback mechanism by activating calcium/calmodulin-dependent protein kinase-induced mitogen-activated protein kinase (MAPK) in smooth muscle cells [45]. This Ras/MAPK pathway then amplifies cytosolic phospholipase A₂. This mechanism results in the release of additional arachidonic acid substrates that can then be converted to 20-HETE [45].

Degradation of 20-HETE occurs via multiple pathways. For example, in endothelial cells 20-HETE can be metabolized by cyclooxygenase to 20-hydroxy-prostaglandin G₂ and H₂ [46]. 20-HETE may also be oxidized by ω -oxidation or β -oxidation [47]. In contrast, the degradation of EETs appears to be more uniform and is exerted mainly by sEH resulting in DHETs [12, 13].

The synthesis pathways for HETEs and EETs are complex exhibiting multiple routes leading to the same compound. HETEs can be generated through the three arachidonic acid metabolic pathways, COX, LOX, and CYP 450 [48]. While EETs are mainly formed by CYP2C and CYP2J, other EET-producing CYPs such as CYP4X1 and CYP2U1 have been characterized [11, 49, 50]. These two cytochrome P450s (CYP4X1 and CYP2U1) metabolize arachidonic acid to 8,9- and 14,15-EETs as well as 19- and 20-HETE, respectively [49, 50].

4 Targets of EETs

The molecular mechanism(s) of EETs is poorly understood, but continues to be fiercely studied. A series of agonists have been developed to help characterize the binding and metabolism of 14,15-EET [51]. While the EET receptor(s) has not yet been identified, intracellular signals by G protein pathways have been implicated [2,

51, 52]. Over 90% of circulating EETs are incorporated into phospholipids of the cell membrane, mainly as low-density lipoproteins [53]. EETs can act as long-chain fatty acids and bind to fatty-acid-binding proteins and nuclear peroxisome-proliferator-activated receptors (PPAR γ and PPAR α). These actions suggest an intracellular mechanism [52, 54–56]. In fact, all four EETs and their metabolite DHET can stimulate PPAR/RXR heterodimer binding to a peroxisome proliferator response element [56–60].

5 The role of tumor stroma in tumorigenesis: angiogenesis and inflammation

Prior to examining a potential role of 20-HETE and EETs in cancer, we need to review the various cellular components of a tumor that could serve as sources and targets of these lipid autacoids. A paradigm shift has taken place over the past decade in cancer research. The simple notion, unquestioned for decades, was that cancer is a cell-autonomous disease driven by mutations for fast growing and increasingly malignant cell clones. Now it is accepted that tumor growth is a non-cell-autonomous process, requiring support from the “tissue microenvironment” in the “tumor bed” [61–63]. Non-cell-autonomous contribution to tumorigenesis from the “host-tissue”, most clearly epitomized by tumor vasculature, is crucial for tumor expansion and progression [64]. This hypothesis, developed by Folkman in 1971, stated that tumor growth requires neovascularization, and that such “tumor angiogenesis” is induced by tumor-derived soluble factors [65]. Since then, the contributions of non-cancerous cells to the growth of tumors has extended beyond endothelial cells to pericytes, inflammatory cells, immune cells, fibroblasts, myofibroblasts, and adipocytes; for example, carcinoma-associated fibroblasts can promote the growth of invasive breast cancer [66]. Non-local cells, including bone-marrow-derived macrophages, neutrophils, mast cells, and mesenchymal stem cells are also recruited, contributing to the invasiveness and metastatic ability of neoplastic epithelial cells [67].

Both angiogenesis and inflammation are interdependent stromal processes that exert substantial influence on tumor growth and metastasis. Pro-inflammatory enzymes and cytokines act to promote tumors; increased infiltration of macrophages and neutrophils can increase angiogenesis and correlates with a poor prognosis [68, 69]. In other cases, inflammatory infiltration of lymphocytic/monocytic cells can actually inhibit tumor growth [70]. Conversely, blocking inflammation can be associated with the stimulation of cancer [3, 71, 72]. Both inhibition and activation of the nuclear factor-kappaB (NF- κ B) protein complex can promote carcinogenesis [73–75]. Inflammation in the tumor bed can

then either stimulate or inhibit tumor growth [72, 76, 77]. Thus, pharmacological modulation of inflammation in cancer treatments must be evaluated with the notion that inflammation may be a double-edged sword in tumor growth.

6 Lipid autacoids in cancer

It has been recognized that tumor growth is a complex process involving many cell types. The intercellular communication that takes place between these cells is conducted by an array of soluble factors such as: proteinaceous growth factors and chemokines, vascular endothelial growth factor (VEGF), FGF-2, TGF- β , TNF- α , interleukin (IL)-1, and oxygen radicals [78].

Little attention has been paid to small molecule mediators, such as lipid autacoids, whose role in cancer has only recently emerged. Given that a tumor consists of both cancerous and non-cancerous cells, the role of autacoids in tumor growth can be separated into their direct effects on neoplastic growth and their effects on inflammation, angiogenesis, and stromal cells.

The pro-inflammatory prostaglandins and leukotrienes directly induce epithelial tumor cell proliferation, survival, migration, and invasion in an autocrine and paracrine manner [3]. Lipid autacoids, such as prostaglandin E₂ (PGE₂) and leukotriene B₄ (LTB₄), stimulate both epithelial cells and stromal cells to produce VEGF and FGF-2. These angiogenic growth factors induce COX2 and in turn produce PGE₂ and PGI₂ in endothelial cells [3, 79]. Other studies have linked eicosanoids to stroma inflammation in epithelial ovarian cancer [80]. Levels of eicosanoid metabolites, such as PGE₂, 5-HETE, and 12-HETE, increase progressively in patients with benign pelvic disease to those with epithelial ovarian cancer. This demonstrates the involvement of lipid autacoids in the inflammatory environment of cancer [80]. However, the role of lipid autacoids derived from the third eicosanoid pathway of arachidonic acid remains poorly characterized in cancer.

7 HETEs effects on inflammation and the vasculature

Lipoxygenase-derived HETEs inhibit apoptosis, stimulate angiogenesis, and enhance proliferation and migration of cancer cells [48]. 20-HETE, the principal metabolite of the ω -hydroxylation pathway, is a pro-inflammatory mediator that markedly stimulates the production of inflammatory cytokines/chemokines in endothelial cells, including IL-8, IL-13, IL-4, and prostaglandin E₂ [81]. 20-HETE stimulates NF- κ B activation and MAPK/ERK pathways, which suggests that HETE's pro-inflammatory effect may be mediated by the central inflammatory pathway of NF- κ B [81].

In addition to its pro-inflammatory activity, 20-HETE has pro-angiogenic activity including the stimulation of endothelial cell proliferation, migration, and cell survival [82–85]. 20-HETE has an important role in VEGF-dependent angiogenesis [86] (reviewed in [85]). While VEGF seems to be the primary mediator of 20-HETE-induced endothelial cell proliferation, inhibition with a VEGF antibody does not completely abrogate the mitogenic effect of 20-HETE [82]. This suggests other pathways are involved in 20-HETE-mediated angiogenesis [82].

The pro-angiogenic factor fibroblast growth factor-2 (FGF-2) can activate cytosolic phospholipase A₂ (the enzyme which releases arachidonic acid from cell membranes) in endothelial cells [87]. FGF-2 increases arachidonic acid production, potentially stimulating CYP4A and production of 20-HETE [85]. The overexpression of CYP4A1, which increases 20-HETE production, results in increased neovessel formation [88].

HET0016, a selective inhibitor of CYP4A, suppresses the formation of 20-HETE at a concentration <10 nM, and has no effect on epoxygenase, cyclooxygenase, or lipoxygenase activity at concentrations up to 1 μ M [4, 89]. HET0016 inhibits VEGF-induced endothelial cell proliferation *in vitro* and corneal neovascularization *in vivo* when administered locally with pellets containing VEGF [84]. When administered locally into the cornea, HET0016 inhibited tumor-induced (U251 glioblastoma cells) angiogenesis by 70% [84]. Furthermore, the administration of the stable 20-HETE agonist, 20-hydroxyeicosa-6(Z) 15(Z)-dienoic acid (WIT003), induced mitogenesis in endothelial cells and corneal neovascularization *in vivo* [84]. These studies provide experimental evidence that inhibiting 20-HETE may offer a strategy to reduce pathological angiogenesis not only in tumors but in angiogenic diseases such as diabetic retinopathy, macular degeneration and chronic inflammatory diseases, such as psoriasis [84]. However, these studies did not determine whether 20-HETE was produced by the cornea or endothelial cells and, therefore, further studies are needed [90].

In the systemic circulation, 20-HETE produced by vascular smooth muscle cells acts as a vasoconstrictor [4]. However, in pulmonary arteries, 20-HETE contributes to VEGF-induced relaxation of the lungs [91]. VEGF, a nitric oxide (NO)-dependent dilator of systemic arteries, plays a key role in maintaining the integrity of the pulmonary vasculature [91].

8 20-HETE effects in cancer

In 2008, U251 glioblastoma cells were genetically altered (transfected with rat CYP4A1 cDNA) to increase the formation of 20-HETE [92]. This stimulated proliferation

in culture. When these transfected U251 glioblastoma cells were implanted into the brain of rats, a tenfold increase in tumor volume was observed when compared to animals receiving mock-transfected U251 cells [92].

Conversely, Guo et al. demonstrated that HET0016 significantly inhibited human U251 glioblastoma cell proliferation in a dose-dependent manner [90]. HET0016 inhibited the phosphorylation of the epidermal growth factor receptor (EGFR) and the subsequent phosphorylation of p42/p44 MAPK [90]. While U251 cells expressed CYP4A11 mRNA and protein, HPLC and mass spectrometry analysis of U251 cell extracts revealed that they did not appear to synthesize 20-HETE [90]. Thus, HET0016 has other effects independent of suppressing 20-HETE. Subsequently, the same group demonstrated that 9L gliosarcoma proliferation and tumor growth in rats are suppressed by HET0016 [93]. Systemic administration of HET0016 inhibited the tumor growth of 9L gliosarcomas by 80%, and tumor angiogenesis by roughly 50%. In a separate study, HET0016 and a 20-HETE antagonist (WIT002) both inhibited the proliferation of a renal adenocarcinoma. This cell type expressed CYP4F isoforms and produced 20-HETE [94].

Little is known about 20-HETE in cancer patients. In one study, 12-HETE and 20-HETE concentrations were shown to be elevated in the urine of patients with benign prostatic hypertrophy and prostate cancer patients as compared to normal subjects [95]. Further analysis did not establish a correlation between the concentrations of HETEs and prostatic specific antigen level, gland size, or tumor grade [95].

9 EETs and angiogenesis

EETs are mainly secreted by endothelial cells and play critical roles in cellular proliferation, migration, and inflammation; their major target is blood vessels [6, 37]. EETs may act in an autocrine fashion on the endothelium inducing vasodilatory and anti-inflammatory effects in blood vessels [96]. As a result of these effects, EETs lower blood pressure and protect the myocardium and brain from ischemia [56, 97–99].

The initial finding that linked EETs to angiogenesis was shown by an increase in proliferation of cerebral capillary endothelial cells by astrocyte conditioned media [40]. In contrast, an inhibitor of cytochrome P450, 17-octadecynoic acid (17-ODYA), suppressed the formation of capillary tubes in a co-culture of astrocytes and endothelial cells. Both EETs secreted by astrocytes and synthetic EETs stimulated endothelial cell proliferation, tube formation, and angiogenesis in a matrigel plug *in vivo* [40, 100, 101].

Angiogenesis is critically dependent on endothelial cell migration [102]. The development of synthetic EETs has

provided insight into the angiogenic functions and pathways of the various EETs. For instance, EETs have been shown to promote endothelial cell migration via endothelial NO synthase, MEK/MAPK, and PI3K [103]. Another assay to evaluate angiogenesis is the chick chorioallantoic membrane assay, which uses the chorioallantoic membrane (CAM) of a chicken embryo [104]. Michaelis et al. employed this assay to demonstrate that 11,12-EET stimulates vessel formation [105]. Importantly, this CAM-mediated angiogenesis was suppressed by either an EGF receptor-neutralizing antibody or an inhibitor of the EGF receptor. Thus, 11,12-EET may stimulate angiogenesis through the activation of the EGF receptor [105].

Several other pathways have been implicated in 11,12-EET- and 14,15-EET-mediated angiogenesis. Sphingosine kinase-1 (SK1) is one important mediator of 11,12-EET-induced angiogenic effects [106]. The expression of a dominant-negative SK1 or knockdown of SK1 by siRNA, inhibited 11,12-EET-induced endothelial cell proliferation, migration, tube formation, and matrigel plug vessel formation [106]. In other studies, EphB4 is a critical component of the CYP2C9-activated signaling cascade [107]. Both CYP2C9 overexpression or the administration of 11,12-EET showed increased expression of EphB4 in endothelial cells. The availability of these synthetic EETs has made it possible to evaluate another regioisomer, 14,15-EET. 14,15-EET was shown to induce angiogenesis via several pathways including: Src, phosphatidylinositol-3-kinase/Akt (PI3K/Akt) signaling in parallel with mTOR-S6K1 activation and Src-dependent STAT-3-mediated VEGF expression [108, 109].

Other groups have studied CYP 450-derived metabolites, utilizing the strategy of overexpressing CYP epoxygenases. In lieu of EETs, this system inhibited endothelial cell apoptosis through activation of the PI3K/Akt pathway [110]. The overexpression of CYP epoxygenases, including CYP2J2, also increased muscle capillary density in a rat ischemic hind limb model [103]. Thus, CYP 450-derived metabolites may stimulate the development of collateral circulation in ischemic tissue [103].

While most investigators have focused on 11,12-EET and 14,15-EET, Pozzi et al. identified 5,6- and 8,9-EET as pro-angiogenic lipids [36]. These regioisomers increased blood vessel density and formed functionally intact vessels in a subcutaneous sponge model in mice. This neovascularization was enhanced by the co-administration of an epoxide hydrolase inhibitor, which elevates the levels of EETs [36]. This study corroborates the critical role that EETs plays in angiogenesis.

It is known that hypoxia stimulates angiogenesis via transcriptional VEGF induction, a response that is mediated by the hypoxia-inducible factor-1 α (HIF-1 α) [111]. It was shown by the Fleming laboratory that hypoxia also

stimulates CYP 2C8 and 2C9 expression [5, 112, 113]. Consistently, the CYP inhibitor (MS-PPOH) and the putative EET receptor antagonist (14,15-EEZE), inhibited hypoxia-induced endothelial tube formation [112]. Furthermore, the angiogenic effect of EETs is partially dependent on HIF-1 α -mediated VEGF induction [114]. This may have implications in cancer beyond angiogenesis, since HIF-1 α can provide a growth and survival advantage to tumor cells, especially under metabolic stress [72].

The effects of EETs and VEGF regulation are closely intertwined. EETs can enhance the effects of VEGF-induced angiogenesis [115]. In turn, VEGF can increase CYP2C promoter activity in endothelial cells and induce the expression of CYP2C8, resulting in increased intracellular EET levels [115]. The putative EET receptor antagonist, 14,15-EEZE, inhibits VEGF-induced endothelial cell tube formation. However, 14,15-EEZE does not affect VEGF-induced phosphorylation of its receptor or FGF-2-stimulated tube formation [115]. In a parallel study, CYP2C44 epoxygenase appears to be an important component in the VEGF signaling pathway [116]. For example, in cultured lung endothelial cells that express VEGF-inducible CYP2C44 epoxygenase, resulting in increased levels of 11,12- and 14,15-EET, angiogenesis was stimulated *in vitro*. Taken together, these studies suggest that the pro-angiogenic activity of EETs is mediated at least, in part, by VEGF [115, 116].

10 CYP 450 epoxygenases and cancer

While the pro-angiogenic activity of EETs has extensively been investigated [36, 103, 117], the role of EETs in cancer remains poorly characterized. Although two decades ago, 14,15-EET was shown to stimulate mesangial and renal epithelial cell proliferation [118, 119], only in the last 5 years has evidence, supporting cytochrome P450 epoxygenases as a potential tumor-promoting enzyme, begun to emerge [120]. The role of CYP2J2 epoxygenase in cancer was first shown by Jiang et al. In this study, CYP2J2 was upregulated in 77% of human carcinoma tissues and eight different human carcinoma cell lines [120]. Furthermore, the transfection of tumor cells with cytochrome epoxygenase 2J2 enhanced tumor formation [120]. Subsequent studies, in which CYP epoxygenase levels were manipulated, by either overexpression of CYP2J2 or antisense in the xenotransplanted tumor cell, suggest EETs may play a role in cancer metastasis [121].

EETs also appear to be important for cancer cell survival. Specific CYP2J2 inhibitors suppress human tumor cell proliferation [122]. These inhibitors activate caspase-3, which leads to reduced tumor cell adhesion, migration, invasion, and suppressed murine xenograft tumor growth

[122]. It is often difficult to distinguish a direct effect on the tumor cells or the stromal processes. It is likely that both mechanisms synergize to account for the potential pro-tumorigenic activity of EETs.

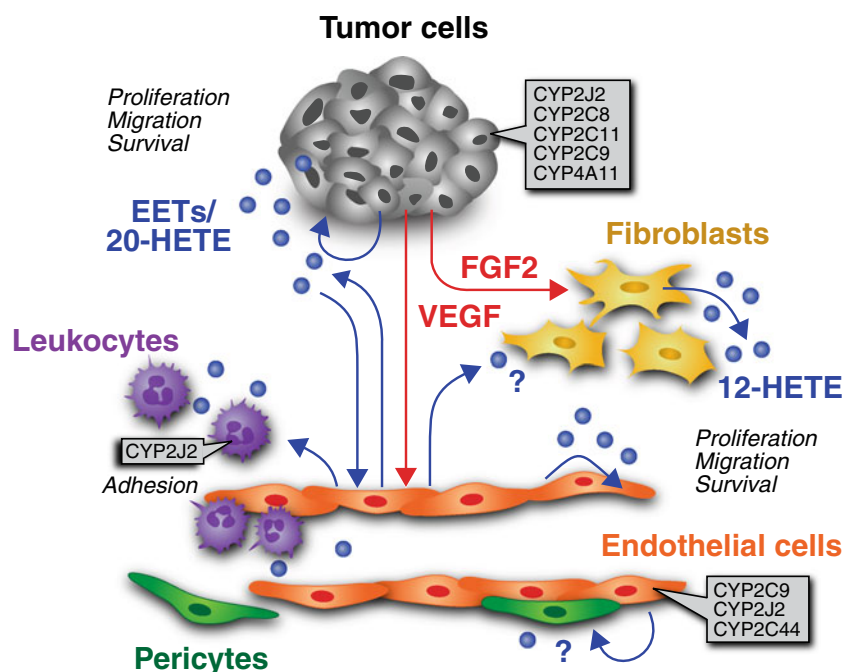
There are few pharmacological studies using drugs which can non-specifically affect EETs. In one study conducted by Pozzi et al., mice treated with PPAR α ligands exhibited a reduction of tumor growth, vascularization, and plasma EETs [123]. In a separate study, two mechanistically different synthetic inhibitors of cytochrome P450, 17-ODYA, and miconazole significantly reduced tumor size and capillary formation in intracranial glial tumors, and prolonged survival of treated rats [124]. Interestingly, these inhibitors had no effect on EETs in the tumor tissue suggesting that the tumor endothelium may be the target of these CYP inhibitors [124]. It has recently been reported that EET antagonists inhibit prostate carcinoma cell motility [125]. This may represent a novel mechanism of EET antagonists acting directly on the tumor cell [125].

Several CYP epoxygenases have been detected in tumor cells *in vitro* and *in vivo*, supporting the potential role of EETs in cancer (Fig. 2). For instance, CYP2C8, CYP2C9, and CYP2J2 were recently shown to be expressed in three prostate carcinoma cell lines (PC3, DU-145, and LNCaP) [125]. In these studies no consistent correlation between mRNA expression, protein expression, or EET concentrations was found [125]. Another epoxygenase, CYP2C11, was shown by Zagorac et al. to be upregulated in cerebral brain tumors of rats [124]. In addition to tumor cells, CYP epoxygenases are also expressed in the tumor stroma. For example, CYP2C44 epoxygenase is expressed in the tumor vessels of a xenograft model of human non-small cell lung cancer in mice [36]. Furthermore, in human renal tumors, CYP2C9 epoxygenase was recently found to be selectively expressed in the vasculature [126]. These findings open the possibility that EETs may act as a trophic factor for both tumor stroma and parenchyma.

In earlier clinical studies, 14,15-EET levels were detected in the urine samples of patients with benign prostatic hypertrophy and prostate cancer in comparison to normal volunteers [95]. Interestingly, after the removal of the prostate gland in prostate cancer patients, the urinary concentration of 14,15-EET did not decrease. This data suggests that the origin of the 14,15-EET was not the prostate gland but another source [95].

Whether the levels of CYP epoxygenases are dependent on isoenzyme or tumor type remains up for debate. Enayetallah et al. analyzed three cytochrome P450 epoxygenases (CYP2C8, CYP2C9, and CYP2J2) and soluble epoxide hydrolase in human malignant neoplasms [127]. CYP2C9 was the most abundantly expressed epoxygenase in several human malignant neoplasms [127]. In contrast CYP2J2 staining was not detected in pancreatic or prostate

Fig. 2 Cytochrome P450 epoxygenase expression in tumor cell compartments and their potential role in cell–cell communication in the tumor stroma. Note that both tumor cells and endothelial cell can produce EETs (*blue circles*), establishing an autocatalytic loop. EETs may act in an autocrine or paracrine fashion



adenocarcinoma. Furthermore, CYP2J2 expression was detected in less than 50% of lung squamous cell carcinoma samples and in less than 15% of lung adenocarcinoma samples [127]. These results showing that CYP2J2 can be suppressed in tumors were confirmed by Leclerc et al. [128]. It has been suggested that this decrease in arachidonic acid epoxidation in certain tumors may allow arachidonic acid to be metabolized to the other eicosanoids [128]. Consequently, the inconsistent overexpression and underexpression of CYP epoxygenases in tumors makes it difficult to understand the biological significance of these enzymes in cancer. This variability must be examined in the wider context of the complex metabolic pathways of lipid autacoids.

In addition to the study of CYP epoxygenases, the expression of sEH, the main metabolizing enzyme of EETs, has been investigated in cancer. The loss of sEH has been reported in hepatocellular carcinoma and hepatoma cells [129, 130]. Enayetallah et al. further confirmed that sEH can be downregulated in renal and hepatic tumors, in principle increasing the levels of EETs in the tumor tissue [127]. These studies support a potential role for EETs in cancer due to downregulation of its metabolizing enzyme. Mirroring the expression of CYP epoxygenases in tumors, the expression of sEH can also be upregulated. sEH expression was increased in seminoma, cholangiocarcinoma, and advanced ovarian cancer when compared to normal tissues or early stage cancer [127]. As in the case of CYP, there is no consistent finding in the expression of sEH in tumors. According to the rationale that the elevation of EETs in tissues promotes tumor growth, sEH would be expected to be downregulated in tumors—which has been

observed only in certain tumors, to date. More studies will be needed to reveal whether a potential increase of EETs is associated with a particular tumor type or cancer stage. Whether another biochemical component can compensate for a lack of EET increase in tumors remains unknown.

There is another aspect to consider when discussing CYP expression in cancer. Since cytochrome P450 (CYP) 2C8 metabolizes drugs, such as paclitaxel (Taxol), tamoxifen, and other chemotherapeutic agents, genetic polymorphisms in the CYP2C8/9 gene may affect cancer patient survival [131, 132]. Some polymorphisms in human CYP2C8 have been identified and shown to decrease the metabolism of paclitaxel and arachidonic acid [133]. Recent studies have implicated that genetic polymorphisms in CYP2C8 and CYP2C9 influence disease-free survival in breast cancer patients [132]. Furthermore, a genetic polymorphism of CYP2C19 is associated with increased susceptibility to biliary tract cancer [134].

11 Outlook

Several types of drugs, originally designed to target inflammation and cardiovascular diseases, have now been discovered to play an important role in cancer biology. This appears to be the case with the cytochrome P450-derived metabolites of arachidonic acid, EETs and HETEs. These lipid autacoids may be involved in cancer in a few different ways: either in cell-autonomous tumor survival and growth or in modulating stromal processes, such as angiogenesis and inflammation that can support tumor progression. Recently, classical mediators of inflammation, such as

prostaglandins, have received new attention as potential targets in cancer treatment. The EET and HETE pathways should be evaluated as potential targets in cancer therapy, directed both against tumor cells and their surrounding stroma. Today, the role of cytochrome P450 metabolites in cancer is still poorly characterized, in part because of their biochemical complexity. The increasing availability of research tools, such as novel synthetic agonists, antagonists, and enzyme inhibitors, now offer a reasonable platform for dissecting the role of EETs and HETEs family autacoids in cancer.

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