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Authors

Wang, Weicang
Zhu, Julia
Lyu, Fei
et al.

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ω -3 polyunsaturated fatty acids-derived lipid metabolites on angiogenesis, inflammation and cancer

Weicang Wang¹, Julia Zhu¹, Fei Lyu¹, Dipak Panigrahy², Katherine W. Ferrara³, Bruce Hammock^{4,*}, and Guodong Zhang^{1,*}

¹Department of Food Science, University of Massachusetts-Amherst, Amherst, MA 01003

²Center for Vascular Biology Research and Department of Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02115

³Department of Biomedical Engineering, University of California, Davis, CA 95616

⁴Department of Entomology and Comprehensive Cancer Center, University of California, Davis, CA 95616

Abstract

Epidemiological and pre-clinical studies support the anti-tumor effects of ω -3 PUFAs; however, the results from human trials are mixed, making it difficult to provide dietary guidelines or recommendations of ω -3 PUFAs for disease prevention or treatment. Understanding the molecular mechanisms by which ω -3 PUFAs inhibit cancer could lead to better nutritional paradigms and human trials to clarify their health effects. The ω -3 PUFAs exert their biological activities mainly through the formation of bioactive lipid metabolites. Here we discuss the biology of cyclooxygenase, lipoxygenase and cytochrome P450 enzymes-derived ω -3-series lipid metabolites on angiogenesis, inflammation and cancer.

Keywords

ω -3 polyunsaturated fatty acids; cyclooxygenase; lipoxygenase; cytochrome P450

Introduction

In the U.S., there are ~1,665,540 new cases and ~585,720 deaths from cancers expected in 2014. It is estimated that 30% of cancer in developed countries are diet-related¹. Human studies support that dietary ω -3 polyunsaturated fatty acids (PUFAs), in particular eicosapentaenoic acid (EPA, 20:5 ω -3) and docosahexaenoic acid (DHA, 22:6 ω -3), may reduce cancer risks^{2–10}; while ω -6 PUFAs, such as linoleic acid (18:2 ω -6) and arachidonic

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*To whom correspondence should be addressed. Guodong Zhang, Tel: (413) 545-1014, guodongzhang@umass.edu, and Bruce Hammock, Tel: (530) 752-7519, bdhammock@ucdavis.edu.

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acid (ARA, 20:4 ω -6), could promote tumor progression^{11–16}. For example, in the VITamins And Lifestyle (VITAL) Cohort, current use of fish oil, but not other dietary supplements, was associated with reduced risks of breast cancer¹⁰. The breast cancer patients with high tissue levels of DHA also respond better to chemotherapeutic drugs¹⁷. In contrary to the effects of ω -3 PUFAs, studies carried out in Mexico, Sweden, Singapore and China showed that dietary intake of ω -6 PUFAs was associated with increased breast cancer risks^{11, 14, 16, 18}. This is important because current western diet contains 20–30 times more ω -6 than ω -3 PUFAs¹⁹. Validation of the anti-tumor effects of ω -3 PUFAs will have significant impact on public health. However, there are inconsistent results from human studies, which showed that ω -3 PUFAs had no effects^{20, 21} or detrimental effects on cancers²², making it difficult provide dietary guidelines or recommendations of ω -3 PUFAs for cancer prevention or treatment.

The inconsistent results could be due to many reasons. In previous ω -3 human trials, different types, doses and treatment durations of ω -3 PUFAs were used, making it difficult to compare and analyze the results of different ω -3 studies. Besides the heterogeneity of experimental designs, the mixed results could be, in part, due to inter-individual variations to metabolize ω -3 PUFAs to generate bioactive ω -3 lipid mediators (LMs)^{23–26}. The ω -3 PUFAs act in part via formation of certain LMs such as cyclooxygenase (COX)-derived prostaglandin E₃ (PGE₃)²⁷, lipoxygenase (LOX)-derived 4-hydroxy-docosahexaenoic acid (4-HDHA)²⁸, cytochrome P450 (CYP)-derived epoxydocosapentaenoic acids (EDPs)²⁹, as well as unique ω -3 lipid autacoids such as resolvins and protectins^{30, 31}. These ω -3 LMs serve as autocrine and/or paracrine mediators to regulate inflammation and homeostasis³²; many of these mediators are short-lived, locally produced and locally acting in response to cellular stimuli, followed with degradation or metabolism to maintain homeostasis³³. The polymorphisms in the genes encoding ω -3 metabolism enzymes could affect the ω -3 metabolism, leading to different levels of ω -3/ ω -6 LMs in tissues and varied biological responses to ω -3 supplementation. Indeed, recent research showed that there is a high degree of inter-individual variability in metabolizing ω -3 PUFAs to generate LMs upon dietary intake of ω -3 PUFAs²³. Clearly, it is important to elucidate the specific lipid metabolizing enzymes and metabolites required for the anti-tumor effects of ω -3 PUFAs. The identified enzymes and metabolites could serve as biomarkers to screen the sub-populations which are most likely to respond to ω -3 PUFAs, or develop personalized doses for ω -3 supplementation. In addition, the bioactive ω -3 LMs could serve as biotemplates to design more potent and safer therapeutic drugs³².

The enzymatic metabolism of ω -6 ARA leads to formation of predominately though not exclusively pro-inflammatory and pro-tumorigenic LMs, which have been shown to play a central role in tumor progression^{34–36}. Compared with the ω -6-series LMs, the roles of ω -3-series LMs in angiogenesis, inflammation and cancer are less known. The ω -3 LMs were thought to be less-active mediators, while emerging evidences support that certain classes of ω -3 LMs have potent effects to modulate inflammation, angiogenesis and tumorigenesis. In this review we will discuss the biology of COX, LOX and CYP-derived ω -3 LMs on angiogenesis, inflammation and cancer. The ω -3 PUFAs also act as precursors for biosynthesis of unique lipid autacoids such as resolvins and protectins, which also regulate

multiple cellular processes including inflammation, angiogenesis and cancer³¹. These ω -3 autacoids have been discussed in several recent reviews^{30–32} and will not be discussed here.

ω -3 and ω -6 PUFAs

The ω -3 and ω -6 PUFAs are polyunsaturated fatty acids which have a double bond at the third and the sixth carbon atom from the end of the carbon chain respectively. Linoleic acid (LA, 18:2 ω -6), which is an essential fatty acid and is highly abundant in common vegetable oils, is the major source of dietary ω -6 PUFA in the western diet. The average adult intake of LA in the U.S. ranges from 12–17 g/day for men and 9–11 g/day for women. LA can be further converted to arachidonic acid (ARA, 20:4 ω -6), which is an important PUFA involved in cell signaling by generation of ω -6-series LMs (termed eicosanoids). Most research of ω -3 PUFAs have focused on EPA and DHA. Food sources of EPA and DHA include fish and fish oil supplements. Fish oil is among the most popular dietary supplements in United States. It is the most popular nonvitamin/nonmineral supplements in adults and the second most popular in children. In addition, major food companies are increasingly adding ω -3 PUFAs to various foods as value-added ingredients. FDA has approved Lovaza[®], a mixture of EPA and DHA ethyl ester, as a prescription drug to treat hypertriglyceridemia (high levels of triglycerides). Another drug Vascepa[®] that is a pure EPA ethyl ester is currently seeking approval from FDA targeting hypertriglyceridemia. Recent technology development, using transgenic yeast, algae or supercritical carbon dioxide separation, allows the industry to prepare large-scale of highly purified EPA or DHA. Diets with a ω -6-to- ω -3 PUFA ratio of 1 are recommended by nutritionists, however, current western diets have a ratio of 20–30 due to too much consumption of LA and too low consumption of ω -3 PUFAs¹⁹. Due to the high dietary intake of ω -6 PUFAs, ARA is among the most abundant PUFAs in most tissues. The ω -3 PUFAs are highly enriched in retina and brain tissues, mainly in the form of DHA, the tissue levels of EPA are usually low³⁷.

Molecular mechanisms of ω -3 PUFAs on cancer

Recent research shows that the lipid signaling is deregulated in tumor tissues, leading to increased production of pro-inflammatory and pro-tumorigenic eicosanoids from ARA in the tumor microenvironment. For example, the expressions of delta-6-desaturase which is a rate-limiting enzyme to convert linoleic acid to ARA, as well as phospholipases which release ARA from membrane phospholipids to initiate the biosynthesis of eicosanoids, are significantly up-regulated in tumor tissues^{38, 39}. The expressions of pro-tumorigenic lipid metabolizing enzymes, such as COX-2, 5-LOX and CYP epoxygenases, have been reported to be up-regulated in tumors^{34, 36}; while the anti-tumorigenic enzymes such as 15-LOX-1 are down-regulated³⁴. Together, these changes lead to a supportive microenvironment to support tumor progression.

An important mechanism for the health-promoting effects of ω -3 PUFAs is that they suppress the metabolism of ARA to generate eicosanoids⁴⁰. Upon dietary consumption, ω -3 PUFAs, including EPA and DHA, are incorporated into the membrane phospholipids at the expense of ω -6 ARA. Upon cellular stimulation, the incorporated ω -3 and ω -6 PUFAs are enzymatically released to generate intracellular free fatty acids (FFAs), which are rapidly

metabolized by COX, LOX and CYP enzymes to generate ω -3-series and ω -6-series LMs. EPA and DHA inhibit the formation of ARA-derived ω -6-series LMs via multiple mechanisms, including reduced release of ARA from membrane phospholipids, inhibition of the enzymatic activities of the metabolizing enzymes, and direct competition with ARA for the enzymatic conversions. Besides inhibition of enzymatic metabolism of ARA, EPA and DHA also serve as alternative substrates of the lipid metabolism enzymes, leading to increased formation of ω -3-series LMs. Some of these mediators have potent effects to inhibit inflammation, angiogenesis and cancer^{28, 29}, and will be discussed below.

COX-derived ω -3 LMs in angiogenesis, inflammation and cancer

The COX-2 pathway plays a critical role in angiogenesis, inflammation and cancer (Figure 1)³⁴. The COX-2 metabolite of ARA, prostaglandin E₂ (PGE₂), is widely known to promote inflammation, neovascularization, primary tumor growth and metastasis. Increased expression of COX-2 has been observed in many tumor tissues³⁴. COX-2 inhibitors, including non-steroidal anti-inflammatory drugs (NSAIDs) and cyclooxygenase (COX)-2 selective inhibitors (coxibs), have been shown to reduce cancer risks^{41, 42}. However, the life-threatening cardiovascular risks as well as other adverse effects induced by long-term and high-dose use of these drugs have jeopardized their therapeutic applications^{43, 44}.

The ω -3 PUFAs reduce cancer risks via COX-2-dependent mechanisms. Human studies support that polymorphisms in the genes encoding COX-2 modulate the anti-tumor effects of ω -3 PUFAs^{45, 46}. EPA and DHA reduced the formation of COX-2-derived PGE₂, which contributes to the beneficial effects of ω -3 PUFAs⁴⁰. DHA is widely believed not to be a substrate of COX enzymes, although it has been reported that DHA is converted by COX-2 to form hydroxyl DHA, which is further metabolized to generate electrophilic LMs with anti-inflammatory actions⁴⁷. EPA has been shown to be an alternative substrate of COX-2, which converts it to the ω -3-series of prostaglandin termed prostaglandin E₃ (PGE₃) and other LMs. Compared with ARA, EPA is a poor substrate for COX enzymes^{48, 49}. Previous studies have shown that PGE₃ has less detrimental or even beneficial effects on cancer, the biology of PGE₃ was discussed in a recent review²⁷. PGE₃ has been shown to have less pro-inflammatory and pro-angiogenic effects than PGE₂. In NIH 3T3 fibroblasts, PGE₂ induced cell proliferation while PGE₃ had no effect in the same dose range. Both PGE₂ and PGE₃ induced COX-2 transcription in NIH 3T3 cells and IL-6 production in RAW 264.7 cells, but PGE₃ had a significantly reduced pro-inflammatory effect⁵⁰. In human endothelial cells, PGE₂ further increased Ang2 expression induced by a combination of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), while PGE₃ had no such effect⁵¹. PGE₃ has also been shown to inhibit cancer cell proliferation and invasion. At a dose of 1 μ M, PGE₂ had no effect, while PGE₃ inhibited proliferation of lung cancer A549 cells⁵². PGE₃ also inhibited proliferation and induced apoptosis in B16F10 melanoma cells via mechanisms involving increased expression of PTEN⁵³. In a Matrigel-based Boyden chamber assay, PGE₃ inhibited cell invasion in the highly aggressive brain-metastatic melanoma 70W cell line⁵⁴. The cellular receptors of PGE₃ have not been confirmed; some studies have shown that PGE₃ binds to the same receptors as PGE₂ with reduced affinity and potency^{27, 55}. Further, a recent study showed that ¹²-prostaglandin J₃ (¹²-PGJ₃), which is

a novel COX-derived metabolite of EPA, potently inhibited progression of leukemia in animal models⁵⁶.

LOX-derived ω -3 LMs in angiogenesis, inflammation and cancer

The metabolism of PUFAs by LOX enzymes leads to the formation of leukotrienes and hydroxyl fatty acids (Figure 2)³³. The LOX pathway in cancer is more complicated as there are multiple isoforms of LOX enzymes. It is generally believed that 5-LOX and 12-LOX and their metabolites promote cancer, while 15-LOX-1 and 15-LOX-2 have anti-tumor effects^{34, 57, 58}. 5-HETE, a 5-LOX metabolite of ARA, has been shown to induce angiogenesis, inflammation, and tumor progression. Pharmacological inhibitors of the 5-LOX enzyme have been shown to suppress tumor progression in animal models³⁴. Since both COX-2 and 5-LOX are up-regulated in tumor tissues, dual inhibition of COX-2 and 5-LOX has been shown to cause enhanced anti-tumor effect^{59, 60}.

The 5-LOX metabolites of ARA are generally believed to promote inflammation and angiogenesis³⁴. Surprisingly, 5-LOX was recently shown to play a central role in the anti-angiogenic effect of DHA via formation of an anti-angiogenic metabolite 4-hydroxydocosahexaenoic acid (4-HDHA)²⁸. Dietary supplementation of DHA has been shown to suppress retinal angiogenesis in an oxygen-induced retinopathy model⁶¹. Transgenic deletion of 5-LOX greatly reduced the anti-angiogenic effect of DHA, while deletion of COX-1/2 or 12/15-LOX had little effect, suggesting a central role of 5-LOX in the anti-angiogenic effect of DHA²⁸. The 5-LOX enzyme mediates the anti-angiogenic effect of DHA via formation of 4-HDHA, which inhibited angiogenesis through a PPAR- γ -dependent mechanism²⁸. Considering the importance of angiogenesis in tumor progression, it would be important to study the effect of the 4-HDHA pathway in tumor angiogenesis and associated tumor progression and metastasis.

EPA and DHA are also substrates of 15-LOX, which convert them to 15-hydroxyeicosapentaenoic acid (15-HEPE) and 17-hydroxy-docosahexaenoic acid (17-HDHA) respectively^{62, 63}. Both 15-HEPE and 17-HDHA have been shown to inhibit the enzymatic activity of 5-LOX (a major enzyme to generate pro-inflammatory LMs), suggesting their potential anti-inflammatory effects^{62, 64}. Further animal studies demonstrate the potent anti-inflammatory effects of 17-HDHA in colitis models. In a dextran sulfate sodium (DSS)-or 2,4,6-trinitrobenzene sulfonic acid-induced colitis model, treatment with 0.1–1 μ g/animal/day 17-HDHA significantly reduced the disease activity index, body weight loss, colonic damage and polymorphonuclear infiltration in both colitis models. 17-HDHA also reduced levels of pro-inflammatory cytokines such as TNF- α , IL-1 β , MIP-2, and CXCL1/KC and mRNA expression of NF- κ B and adhesion molecules in colon tissue⁶⁵. In another study, 17-HDHA also suppressed DSS-induced colitis in mice. In murine macrophage RAW264.7 cells, 17-HDHA increased phagocytosis in macrophages and promoted polarization towards an anti-inflammatory M2 phenotype⁶⁶. These studies demonstrate the potent anti-inflammatory effect of 17-HDHA, indicating a critical role of the 15-LOX enzyme in the biological activities of DHA. 17-HDHA is a precursor for the biosynthesis of resolvins, also shown to have potent anti-inflammatory effects in recent reviews^{25, 26}.

The 15-LOX metabolites of EPA and DHA have also been shown to directly inhibit cancer cell proliferation. EPA-derived 15-HEPE inhibited the formation of PGE₂ and 5-HETE, as well as cancer cell proliferation in PC-3 and LNCaP cells⁶⁷. DHA-derived 17-HDHA inhibited the proliferation of prostate cancer cells (PC-3, LNCaP and DU145) at doses much lower than the corresponding metabolite of ARA (15-HETE) and DHA⁶⁸. The other 15-LOX metabolites of DHA, including 17-hydroperoxy-, 10,17-dihydroxy- and 7,17-dihydroxy-DHA, also inhibited cell proliferation via mechanisms involving activation of PPAR- γ and syndecan-1 signaling in prostate cancer cells⁶⁸. The 15-LOX-mediated metabolism is required for the effect of DHA to induce syndecan-1 signaling and apoptosis in prostate cancer cells⁶⁹. In another study, 17-hydroperoxy-DHA inhibited cell proliferation in neuroblastoma cells with an IC₅₀ of 3–6 μ M, compared with 12–15 μ M for DHA⁷⁰.

Human studies support a critical role of LOX in the effects of ω -3 PUFAs. Carriers of variant 5-LOX genotypes have been shown to have increased risks for inflammation and atherosclerosis compared with the carriers of common allele. Dietary intake of ω -6 PUFAs increased, while intake of ω -3 PUFAs decreased, the risk of atherosclerosis only in the carriers of variant alleles but not in the common alleles⁷¹. This study suggests that 5-LOX may be a biomarker to distinguish people who respond to the anti-atherosclerotic benefits of ω -3 PUFAs from non-responders⁷¹. In terms of cancer, Wang et al. showed that there is a significant interaction of the polymorphism in the genes encoding 5-LOX-activating protein (ALOX5AP) and dietary intake of ω -6 PUFA LA in terms of breast cancer risks in a population-based case-control study in San Francisco bay area¹². Among the women with high dietary intake of LA, carrying the ALOX5AP -4900 AA genotype was associated with higher risk of breast cancer compared with other genotypes. No such correlations were observed in women consuming low levels of LA¹². It would be interesting to test whether dietary intervention with ω -3 PUFAs would inhibit breast cancer progression in this high-risk sub-population. These two human studies demonstrate a strong diet-gene interaction, supporting the critical importance of PUFA metabolism enzyme in the biological activity of ω -3 and ω -6 PUFAs.

CYP-derived ω -3 LMs in angiogenesis, inflammation and cancer

The CYP pathway has two branches, converting ARA to epoxyeicosatrienoic acids (EETs) by CYP epoxygenases (largely CYP2C and CYP2J) and 20-hydroxyeicosatetraenoic acid (20-HETE) by CYP ω -hydroxylase (largely CYP4A and CYP4F)⁷². EETs have been shown to have an array of beneficial actions, including anti-inflammatory, vasodilatory, anti-hypertensive, renal-protective, cardio-protective, and tissue regenerative actions. Pharmacological inhibitors of soluble epoxide hydrolase (sEH, the major enzyme to degrade EETs) which stabilize and increase EETs, are being developed to treat many human disorders⁷². 20-HETE has been shown to have predominately detrimental effects, inducing inflammation, vasoconstriction, hypertension, and cardiovascular problems. Pharmacological inhibitors of 20-HETE biosynthesis have been shown to alleviate many disease states in animal models⁷³.

The roles of EETs and 20-HETE in cancer have not been well studied. EETs are mildly pro-angiogenic, stimulating endothelial cell proliferation, migration, invasion and tube

formation⁷², as well as tissue regeneration⁷⁴. Due to the pro-angiogenic actions, increased levels of EETs stimulate primary tumor growth and metastasis in implantable and spontaneous transgenic murine tumor models⁷⁵. On the other hand, EETs are anti-inflammatory, where increased level of EETs inhibited colon cancer progression and tumor-associated inflammation in inflammation-driven colon cancer models^{76, 77}. 20-HETE has been shown to increase primary tumor growth in murine tumor models, in part via induction of tumor angiogenesis and inflammation. Pharmacological inhibitors targeting 20-HETE biosynthesis have been shown to inhibit tumor progression in animal models³⁵.

EPA and DHA have been shown to be highly efficient alternative substrates of CYP epoxygenases, leading to the formation of epoxyoxygenated ω -3 PUFAs termed epoxyeicosatetraenoic acids (EEQs) and epoxydocosapentaenoic acids (EDPs) respectively^{72, 78}. CYP epoxygenases selectively catalyze the epoxidation of the terminal double bond of ω -3 PUFAs, leading to the predominate formation of 17,18-EEQ from EPA and 19,20-EDP from DHA^{78–81}. Compared with other DHA epoxide regioisomers, 19,20-EDP is the poorest substrate of sEH, which increases the relative proportion of 19,20-EDP in tissues⁸². Many studies have shown that ω -3 supplementation significantly increased levels of EEQs and EDPs in animal and human plasma and tissues^{78, 83–87}.

EEQs and EDPs have similar or more potent effects for vasodilation, anti-inflammation and analgesia than EETs. EPA-derived 17,18-EEQ, as well as ARA-derived 14–15-EET, inhibited TNF- α -induced inflammation in human bronchi via NF- κ B- and PPAR- γ -related mechanisms^{88, 89}. In a carrageenan-induced inflammatory pain model in rats, all epoxyoxygenated PUFAs (EETs, EEQs and EDPs) inhibited inflammatory pain, while the effects of EEQs were less potent than those of EETs and EDPs⁸². Also, a 12-LOX-derived metabolite of 17,18-EEQ, 12-hydroxy-17,18-epoxyeicosatetraenoic acid (12-OH-17,18-EEQ), inhibited LTB₄-induced neutrophil chemotaxis and polarization *in vitro* at a low nM range⁹⁰. In terms of vasodilation, EDPs are among the most potent vasodilators ever discovered (dilation EC₅₀ = 0.5–24 pM)⁹¹. Direct treatment of EDPs suppressed Angiotensin II-induced hypertension in mice⁹². The CYP-mediated formation of EDPs has been hypothesized to contribute to the anti-hypertensive effects of DHA, as shown by transgenic deletion of CYP1A1 (a CYP epoxygenase enzyme) which attenuated the anti-hypertensive effects of DHA⁹³.

Opposite to the pro-angiogenic effects of EETs, the ω -3-series fatty acid epoxides (EEQs and EDPs) have been shown to inhibit angiogenesis. 17,18-EEQ, but not other EEQ regioisomers, inhibited cell proliferation in the immortalized endothelial cell line bEND.3 at a dose of 10 μ M, while EETs at the same dose range showed opposite effects to increase cell proliferation in bEND.3 cells⁹⁴. This study suggests a potential anti-angiogenic effect of EEQs, however, more studies are needed to characterize their effects on angiogenesis, in particular in animal models of neovascularization. Our recent study showed that EDPs potently inhibited angiogenesis, primary tumor growth and metastasis²⁹. In a Matrigel plug assay in mice, all EDP regioisomers (except 4,5-EDP which is chemically unstable) inhibited VEGF-induced angiogenesis. 19,20-EDP, which is a major EDP isomer in tissues, inhibited VEGF-induced angiogenesis with an EC₅₀ value of 0.3 μ g/animal, suggesting its potent anti-angiogenic effect. 19,20-EDP also suppressed basic fibroblast growth factor

(bFGF)-induced angiogenesis in mice, suggesting a potential broad-spectrum anti-angiogenic effect. In human endothelial cells, 19,20-EDP inhibited endothelial tube formation, migration, and production of matrix metalloproteinases, via a mechanism involving VEGF receptor 2 (VEGFR2)-dependent signaling. Given that tumor metastasis causes 90% of human cancer deaths, anti-metastatic agents are very important therapeutic agents⁹⁵. We demonstrated that two EDP regioisomers (i.e., 16,17-EDP and 19,20-EDP, dose = 0.05 mg/kg/day), when stabilized in circulation by co-administration of a selective sEH inhibitor, suppressed ~70% of tumor metastasis in mice²⁹. In fact, EDPs are the first fatty acid metabolites to be shown to have anti-metastatic activities. Moreover, the stabilized EDP also inhibited Met-1 breast tumor growth (a highly aggressive triple-negative breast cancer model) in mice by ~70%²⁹. Our findings demonstrate potent effects of EDPs on tumor angiogenesis, however, two recent studies showed that EDPs did not impact angiogenesis in retinal angiogenesis models^{96, 97}. More studies are needed to characterize the effects and mechanisms of ω -3-series epoxides and diols on angiogenesis in different disease models as it is likely that the effects of these LMs may be disease- and tissue-specific.

Future Directions

The ω -3 PUFAs are among the most intensively studied nutritional compounds, as demonstrated by epidemiological and pre-clinical studies. However, after decades of ω -3 PUFA research, many of the health claims of ω -3 PUFAs remain controversial and have therefore had limited impact in disease prevention and treatment. The mixed results obtained with the use of ω -3 PUFAs in human trials may result in part from failing to recognize the importance of ω -3 PUFA metabolism. As we have discussed in this review, the enzymatic metabolism of ω -3 PUFAs generates ω -3-series LMs, which have potent actions to regulate inflammation, angiogenesis and tumor progression. The ω -3 LMs, rather than the parent ω -3 PUFAs (EPA or DHA), are more likely to be the ultimate bioactive species interacting with cellular targets to exert the biological effects of ω -3 PUFA supplementation. However, the vast majority of previous ω -3 PUFA research has focused on tissue levels of ω -3 PUFAs, instead of ω -3 LMs, as biomarkers to establish the nutritional or therapeutic effects of ω -3 PUFAs.

As discussed above, it is critical to elucidate the lipid metabolizing enzymes and metabolites which are required for the biological effects of ω -3 PUFAs. We expect that increased dietary intake of ω -3 PUFAs is associated with reduced cancer risks among those with genetic variant that result in increased activity of the required ω -3 metabolizing enzymes. Recently, the development of transgenic animal models, LC-MS/MS-based lipidomics and standards for LMs has greatly facilitated the study of LMs. In the past decade, transgenic animal models with deletion or over-expression of lipid metabolism enzymes (COX-2, COX-1, 5-LOX, 12/15-LOX, CYP epoxygenase, CYP ω -hydroxylase, sEH, etc) have been developed and many of them are commercially available. Multiple laboratories in the U.S. have developed LC-MS/MS-based lipidomics methods, which can systematically analyze >100 LMs derived from ω -3/ ω -6 PUFAs using minimal plasma or tissues^{98, 99}. Many ω -3/ ω -6 LM have been chemically synthesized and some of these LMs are commercially available, allowing cell culture and animal experiments to directly study their effects and mechanisms.

These resources will greatly help to elucidate the roles of specific lipid metabolism pathway(s) and metabolite(s) in the effects of ω -3 and ω -6 PUFAs in different disease states. The knowledge obtained in pre-clinical models must be further verified in human trials. The identified lipid metabolism enzymes or metabolites could be used as biomarkers to distinguish " ω -3 responders" from "non-responders", leading to targeted human trials. Such knowledge could also help to provide personalized dietary recommendations. For example, sub-populations carrying certain genotypes of 5-LOX or 5-LOXAP could be educated to optimize their diet^{12, 71}. An in-depth understanding of the molecular mechanisms of PUFAs, together with utilization of nutrigenomic and metabolomic approaches, could lead to targeted nutritional paradigms to better understand the metabolic individuality and nutrition effects of ω -3 PUFAs on human health¹⁰⁰.

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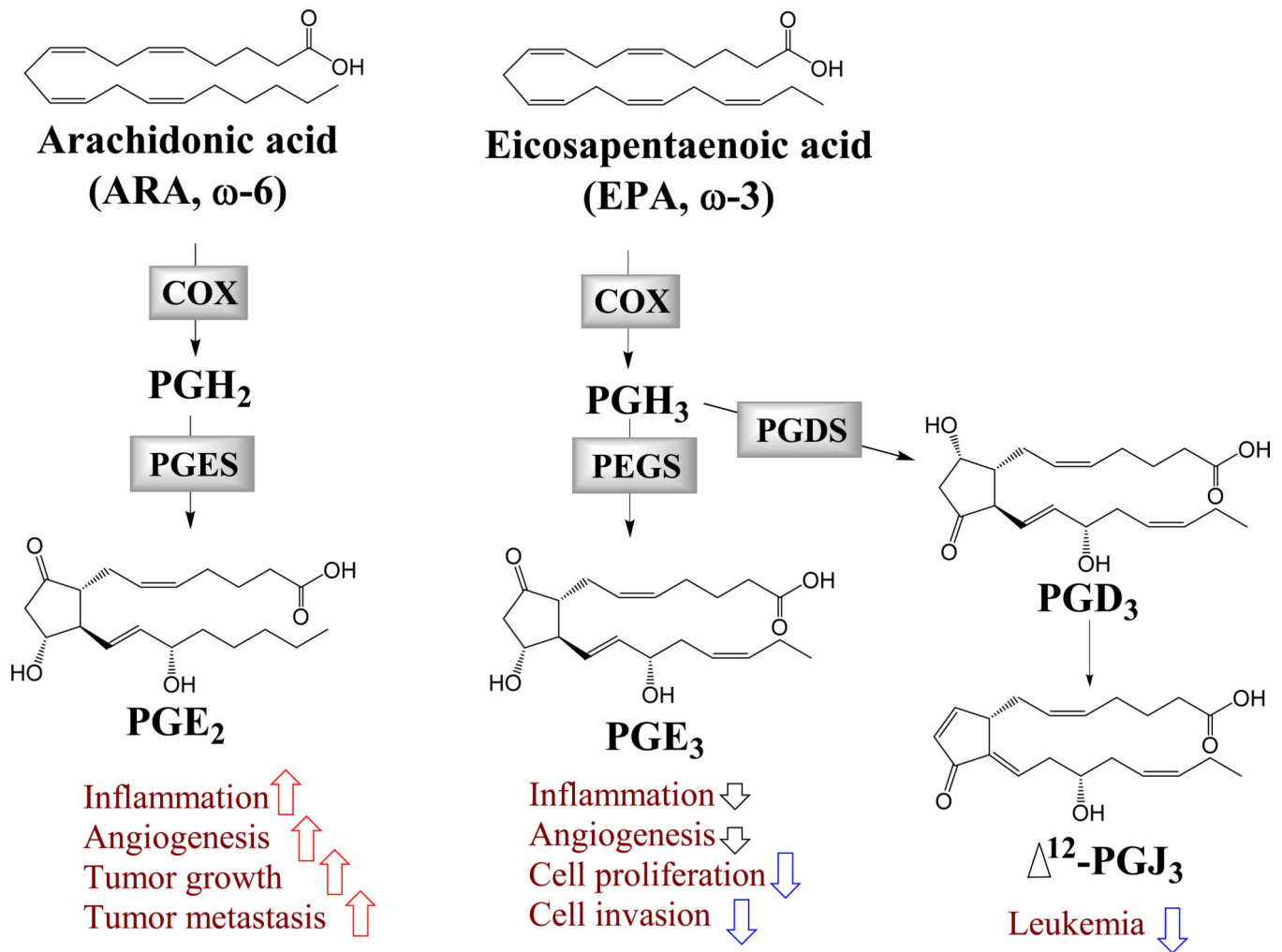


Figure 1.

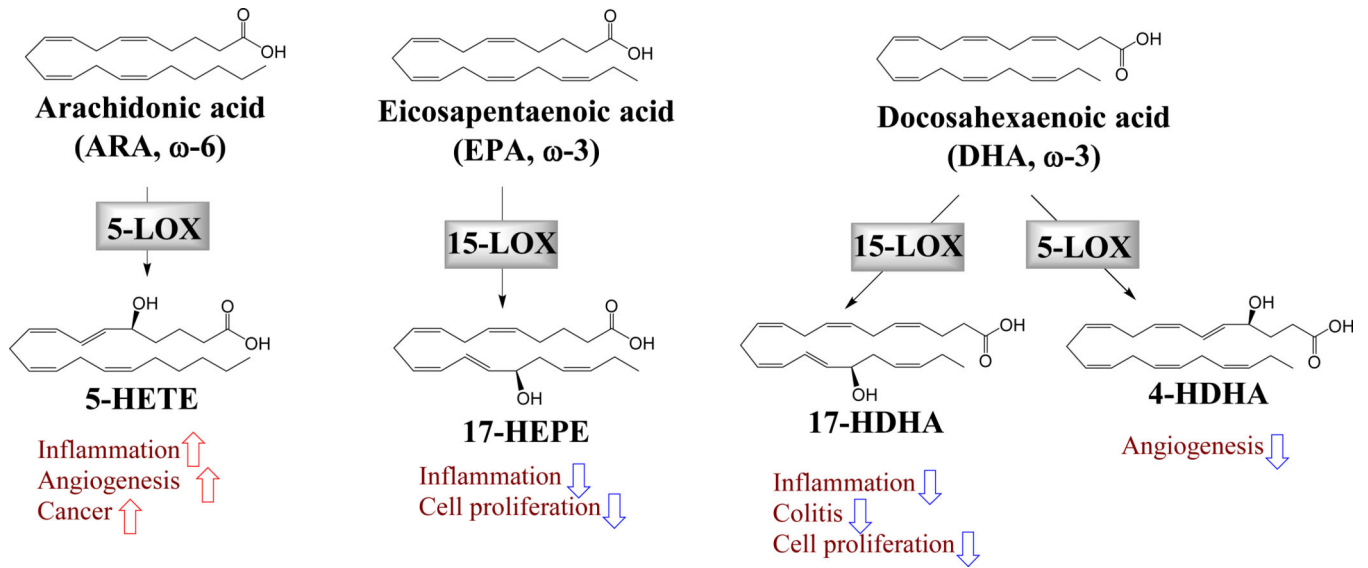


Figure 2.

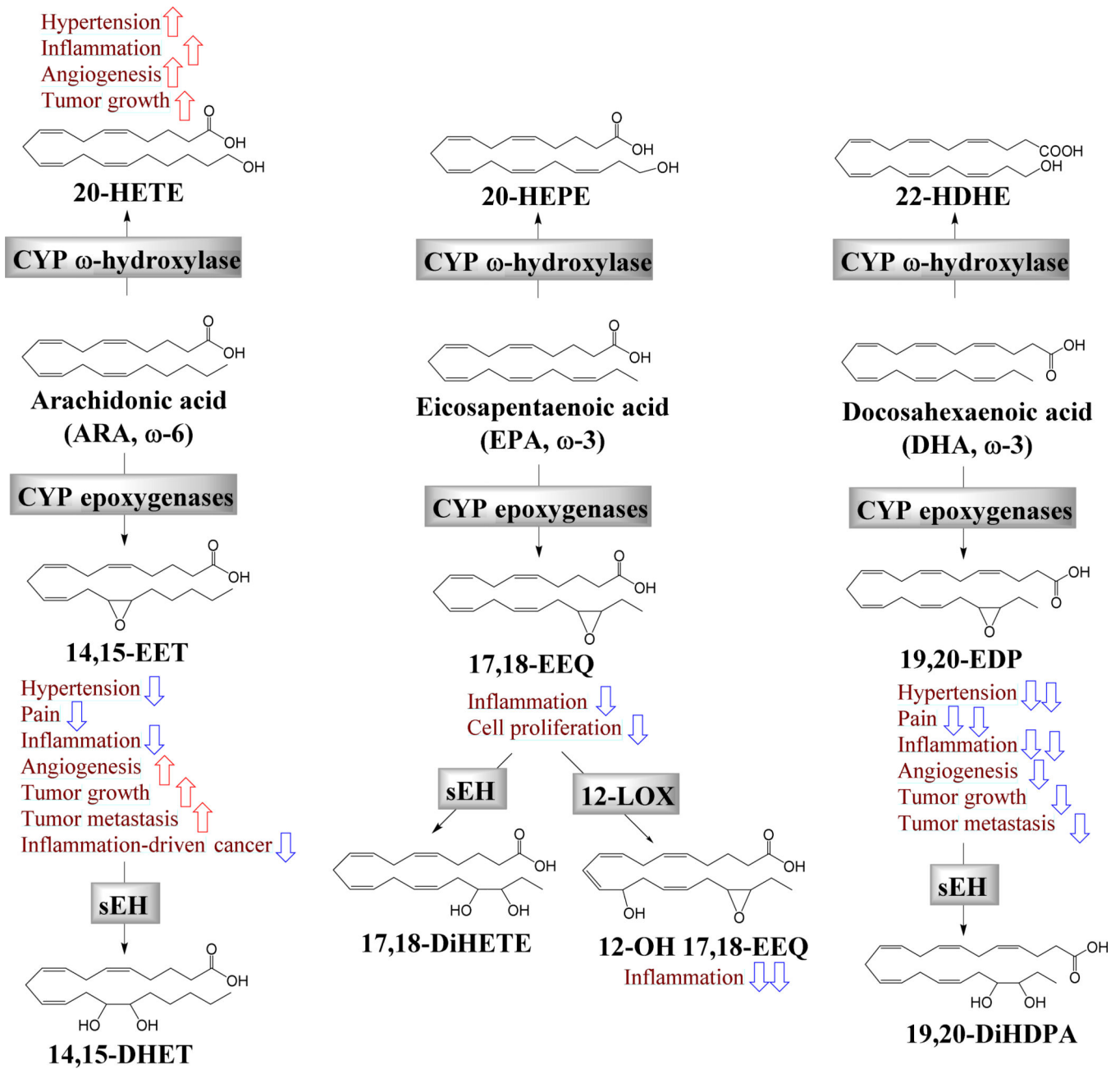


Figure 3.