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Are Lipoxygenases Valid Targets of Cancer Prevention and Treatment?

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Abstract

Bioactive arachidonic acid (AA) metabolites produced by lipoxygenases (LOXs), and to a certain extent the LOX enzymes themselves, has been implicated in cancer development and progression. LOX enzymes and their AA metabolites have been shown to both promote and suppress cancer. *In vitro* and *in vivo* studies have shown that 5-LOX and *p*12-LOX promote tumor formation and progression, while 15-LOX-2 suppresses tumor progression. 15-LOX-1's role in cancer appears to be tissue dependent; inhibition of 15-LOX-1 suppresses tumors in some tissues while promoting tumors in other tissues. Further research on the roles of lipoxygenases in cancer is needed to develop new approaches to cancer prevention and treatment.

Introduction

After more than thirty years of research, there is a little doubt that a functional relationship exists between polyunsaturated fatty acids (PUFA), inflammation and cancer. When healthy, the arachidonic acids (AA) found in meat, egg, and dairy products are an essential part of our diet. These fatty acids are the precursors to important autocrine and paracrine hormones; sufficient quantities are needed to maintain normal physiological functions, including tissue regeneration and immune response [1]. However, excessive consumption of these fatty acids lead to accumulations of pro-inflammatory and proliferation-inducing lipid molecules in already chronically inflamed and premalignant tissues – further contributing to the conditions that promote abnormal growth and malignant transformation. Many studies have already connected dietary fat, more specifically ω-6 fats, to cancer incidence [2,3]. The expression of lipoxygenases (LOXs), one of the major AA metabolizing enzymes, is thought to be regulated by established oncogenes and tumor suppressors, and several lipoxygenase products have been shown to contribute to cancer incidence, progression, and invasion. Lipoxygenases are involved in carcinogenesis and are promising targets for cancer treatment.

Lipoxygenases form a complex family of non-heme iron containing enzymes that dioxygenate (1Z,4Z) -pentadiene groups [4], found in several PUFAs, into (1Z,3E) -1-R,5-R',5-peroxypentadienes via carbon radical intermediates. So far, at least eight distinctive human LOX isozymes, classified according to the regio-specificity of their AA dioxygenation, have been identified [5]. These enzymes synthesize more than a dozen biologically active metabolites, many of which from AA, that exert just as many, and often opposing, biological effects.

5-LOX

5-LOX stereo-specifically inserts O_2 into AA at C-5. The only known human 5-LOX isozyme, which is constitutively expressed in myeloid derived cells, B lymphocytes, and endothelial cells of the pulmonary artery, catalyzes the rate-limiting step of leukotriene (LT) and lipoxin (LX) synthesis [4]. After binding to 5-lipoxygenase activating proteins (FLAPs), 5-LOX primarily oxygenates AAs into 5*S*-hydroperoxyeicosatretraenoic acid (HpETE). After synthesis, 5*S*-HpETE spontaneously reduces to 5*S*-hydroxyeicosatetraenoic acid (HETE) and is further converted, by 5-LOX, into LTA_4, the precursor of pro-inflammatory LTB_4 and cysteinyl leukotrienes LTC_4, LTD_4, and LTE_4. Furthermore, 5-LOX converts eicosapentaenoic acid (EPA), an ω-3 acid, into anti-inflammatory series-5 leukotrienes.

In tissues that express 5-hydroxyeicosanoid dehydrogenase (HEDH), 5*S*-HETE is oxidized into 5-oxo-eicosatetraenoic acid (5-oxo-ETE). The pathophysiology of 5-oxo-ETE is not completely understood; however, evidence suggests that it acts as a chemo taxis agent and potently attract eosinophils and to a lesser extent, neutrophils. *In vivo* studies have shown that 5-oxo-ETEs also promote tumor survival; block 5-LOX inhibitor induced tumor apoptosis, and might be involved in asthma, allergies, and cardiovascular disease [6].

5-LOX also oxygenates 15*S*-HpETEs, formed by 12- and 15-LOXs, into (5*S*,6*S*) -epoxy-15*S*-HpETEs, which are in turn hydrolyzed into anti-inflammatory LXA_4, LXB_4, and cysteinyl lipoxins LXC_4, LXD_4, and LXE_4 during the resolution phases of inflammation. In the presence of aspirin, another AA metabolizing enzyme, cyclooxygenase-2 (COX-2), undergoes a biologically peculiar phenomena and initiates 15*R*-HpETE synthesis. Like 15*S*-HpETE, 5-LOX oxygenates 15*R*-HpETE into (5*S*,6*S*) -epoxy-15*R*-HpETE, the precursor to corresponding anti-inflammatory epi-lipoxins [7].

Because of the roles that 5-LOX metabolites play in inflammation and immune responses, several 5-LOX and leukotriene inhibitors are used clinically for the management of chronic asthma. The 5-LOX inhibitor, zileuton (ZYFLO CR), has been shown to also suppress cancer in the laboratory.

12-LOX

So far, there are four known human 12-LOX isozymes: platelet type 12-LOX (*p*12-LOX), leukocyte type 12/15-LOX (*l*12-LOX), epidermal type 12-LOX (*e*12-LOX), and 12*R*-LOX. *p*12-LOX is the first discovered mammalian lipoxygenase and oxygenates AAs exclusively into 12*S*-HpETEs. *l*12-LOX and *e*12-LOX are less regio- and substrate

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specific, both enzymes oxygenate AAs into 12*S*- and 15*S*-HpETEs, and linoleic acids (LAs) into13*S*-HODEs. 12*R*-LOX is the only LOX isozyme that generates products with *R* chirality and metabolizes AAs, predictably, into 12*R*-HpETEs [8].

Another LOX isozyme that exhibits hydroperoxide isomerase activity was discovered very recently. Epidermal lipoxygeanse-3 (*e*LOX-3) is not a 12-LOX, but is involved in the 12*R*-LOX pathway and known to play a role in the terminal differentiation of keratinocytes and adipocytes [5,9].

After synthesis, 12*S*-HpETE is either reduced to 12*S*-HETE or converted into hepoxilins (HX), which are speculated to mediate a wide array of physiological processes, including insulin secretion, neutrophil migration, pro-inflammatory responses in epidermal and mucosal tissues, and interestingly, anti-inflammatory responses in neutrophils [7]. Less is known about 12*R*-HETE, but evidence hints that it has pro-inflammatory effects and may play a role in the regulation of ocular transparency and tissue osmolarity [10]. If not reduced, 12*R*-HpETE enters the 12*R*-LOX/*e*LOX3 pathway.

Hydroperoxide isomerase cycling of 12*R*-HpETE is the preferred action of *e*LOX3, but purified *e*LOX3 has been observed to oxygenate AAs into a racemic mixture of 5-, 7-, and 9-HpETEs. Aside from 5*S*-HpETE, the biological effects of these AA metabolites have never been observed in humans and these reactions are not speculated to occur in nature [11].

15-LOX

Two 15-LOX isozymes have been identified in humans. 15-LOX-1 is the ortholog of murine *Leu*-12-LOX and is expressed in reticulocytes, eosinophils, macrophages, and airway epithelia in humans. 15-LOX-1 shares high homology with *l*12-LOX and also demonstrates little substrate specificity. 15-LOX-1 preferentially oxygenates LAs to 13*S*-HpODEs, but also oxygenates AAs into12*S*- and 15*S*-HpETEs [4,12,13].

15-LOX-2 is the ortholog of murine 8-LOX and is constitutively expressed in most healthy tissues, but most notably in the prostate, lung, and cornea. Unlike 15-LOX-1, 15-LOX-2 is substrate specific; 15-LOX-2 metabolizes LA poorly and AAs exclusively into 15*S*-HpETEs [14].

After synthesis, 15*S*-HpETEs are either converted to pro-inflammatory eoxins, anti-inflammatory lipoxins, or more often, reduced to 15*S*-HETEs that counteract the pro-inflammatory actions of LTs and prostaglandins, inhibit superoxide production, and neutrophil migration across cytokine-activated endothelium. The 13*S*-HpODE produced by 15-LOX-1 is reduced to anti-inflammatory 13*S*-HODE [7,15].

In addition, 15-LOX has also been reported metabolize ω-3 docosahexanenoic acids (DHA) into 17*S*-DHAs, the precursors to potent anti-inflammatory resolvins and protectins [15].

Lipoxygenase Involvement in Carcinogenesis

Speculation of LOX's involvement in cancer dates back to the 1970s. A study in 1977 probed for, and found, a previously unknown AA metabolite formed through the LOX pathway in rat basophilic leukemia cells [16]. During the 1980s, LOX inhibitors were shown to decrease the incidence of skin cancers in mouse models [17] and LOX metabolites were shown to potently attract circulating tumor cells [18] (Figure 1).

Our knowledge of LOX's role in cancer, however, remains incomplete. Evidence shows that this pathway's involvement is extraordinarily complex; not only because the LOX family of enzymes itself is complex, but also because the profile of LOX expression vary greatly from cancer to cancer, sometimes with conflicting effects. Nevertheless, 15-LOX-1 and 15-LOX-2, along with their murine orthologs, are preferentially expressed in healthy tissues and benign lesions and often absent from carcinomas; while 5-LOX and *p*12-LOX are absent in normal epithelia, unless induced by pro-inflammatory stimuli, and are constitutively expressed in carcinomas [5].

5-LOX and Cancer

A survey of malignant prostate tissues from 22 different patients showed that 5-LOX mRNA expression is, on average, 6 folds higher in malignant tissues than benign tissues from the same patient, and expression of 5-LOX protein and its primary metabolic product, 5-HETE, are more than 2 folds higher in malignant tissues than in benign tissues [19]. Other studies have confirmed up-regulated 5-LOX and FLAP expression in colon, lung, breast, pancreas, bone, and mesothelium cancers [4].

In vitro studies confirm that targeted inhibition of 5-LOX activity reduces malignant growth. Treatment with Rev-5901, a potent LTD_4 receptor antagonist and a less-potent 5-LOX inhibitor, down-regulates the expression of anti-apoptotic protein Bcl-2 and induces increased expression of the pro-apoptotic protein Bax in MiaPaCa-2 and AsPC-1 pancreatic cancer cells. Further, Rev-5901 treatment alone permeates the mitochondrial membrane and induces procasphase-3 cleavage into caspase-3, a protease involved in the execution phase of apoptosis, in both cell lines [20].

Animal models show that less than one half as many EGDA rats fed 1,000 ppm zileuton develops esophageal tumors as control EGDA rats over 40 weeks [21] and only $3/5^{th}$ as many hamsters treated with topical 6% zileuton develops oral carcinoma as untreated hamsters in DMBA tumor initiation models [22]. Inhibition of FLAP with 25 mg/kg MK-886 was also shown to both decreased the number of and reduce the average weight of lung tumors induced by NKK, a carcinogen found in cigarette smoke, by roughly 50% in 7 week old A/J mice [23].

The specific mechanism behind 5-LOX induced tumor survival is still not fully understood. Studies have generated evidence, using pharmacological interventions, suggesting that 5-HETE and LTB_4 simultaneously stimulate proliferation through activation of the MEK/ERK1/2 and the PI-3K/Akt kinase pathways. At the same time, up-regulations of 5-oxoETE, which is known to counteract the chemopreventative effects of 5-LOX inhibitors, have also been observed in many prostate cancers; up-regulations of CysLT1, a G-protein coupled receptor responsive to LTC_4, LTD_4, and LTE_4, have been observed in colorectal cancers; and genotoxic stress has been shown to selectively antagonize *p*53 induced apoptosis through increased 5-LOX expression. It's not clear if these mechanisms are somehow interconnected or which one is predominately responsible for the observable effects but the chemopreventitive phenomena of 5-LOX inactivation has been reproduced in prostate, renal, esophageal, gastric, pancreatic, and breast carcinomas [4-6].

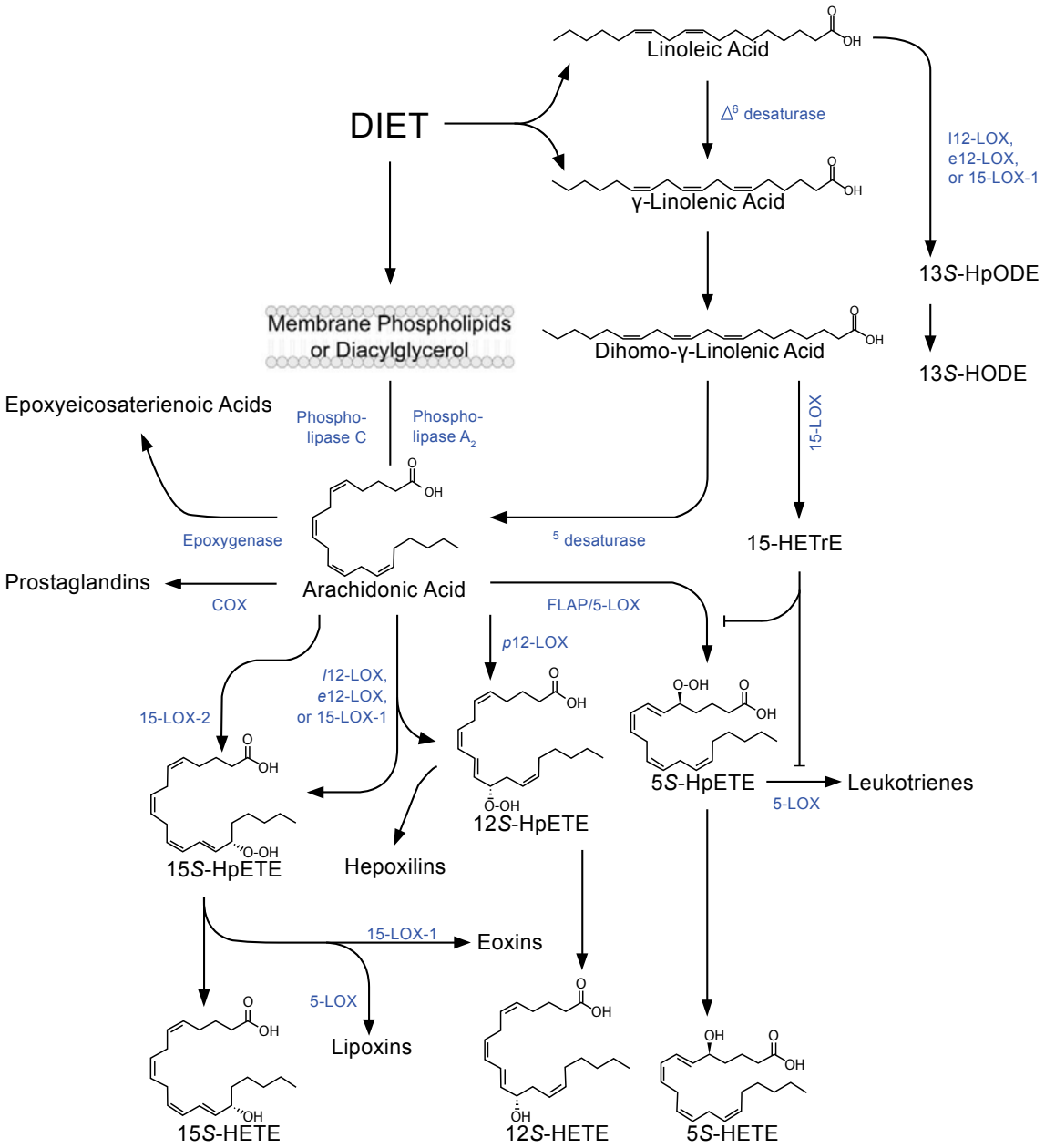

Figure 1: The lipoxygenase metabolic pathway. In humans, arachidonic acid is metabolized through three primary pathways: the epoxygenase pathway, cyclooxygenase pathway, and lipoxygenase pathway. Arachidonic acids that are metabolixed by lipoxygenases ultimately become leukotrienes, lipoxins, hepoxilins, and eoxins.

One interesting note is that this chemopreventitive property of 5-LOX inhibitors is compounded in the presence of COX inhibitors. When taken together, 5-LOX and COX inhibitors offered 2-4 times more protection against tumor incidence and growth than if taken alone at twice the dosage [21-23].

More recently, studies are beginning to show that 5-LOX also play a role in angiogenesis and metastasis. Changes in 5-LOX expression after exposure to inflammatory carcinogens or inhibitory antisense RNAs have been reported to coincide with corresponding rises and falls in MMP2 and VEGF levels [24-26] for some time now. No definitive evidence directly connects 5-LOX to VEGF yet; but one group, studying the destabilization of atherosclerotic plaque, directly implicated 5-LOX, and more importantly its metabolite LTB_4 in MMP2 production. Exposure to 4-hydroxynonenal (4-HNE), a major product of lipid peroxidation that is speculated to play roles in cell signal transduction, induces elevated MMP2 levels in 5-LOX positive vascular smooth muscle cells. Through pharmacological inhibition of LT receptors, this group further showed that BLT receptor antagonists, but not cysLT receptor antagonists, counteract this effect; indicating that 5-LOX mediates MMP2 production through the LTB_4-BLT receptor pathway [27].

12-LOX and Cancer

*p*12-LOX expression may not become dysregulated in all cancers.

A survey of prostate cancer specimens only detected *p*12-LOX mRNA in 38% of samples [28] and a survey of melanoma specimen showed that baseline *p*12-LOX expression in healthy epidermis varies greatly among patients and its expression is not necessarily uniform within individual malignant lesions [29]. Nevertheless, *p*12-LOX has been shown to exert similar tumor promoting properties as 5-LOX, and its expression is strongly correlated with tumor grade and associated with increased angiogenic and invasive potential.

Matched normal and cancerous epidermis tissue collected from 8 patients with melanoma showed a consistent correlation between *p*12-LOX expression and malignant transformation. Computed image analysis of IHC detection of *p*12-LOX revealed that *p*12-LOX protein levels are two-folds higher in melanoma tissues than in normal epidermis [29]. *In vitro* experiment showed that JB6 P+ murine epidermal cells that over express *p*12-LOX are significantly more sensitive to TPA induced neoplastic transformations than JB6 P- cells that do not over express *p*12-LOX. Inhibition of *p*12-LOX using either baicalein a selective but not especially potent *p*12-LOX inhibitor, or siRNA blocked this neoplastic transformation in JB6 P+ cells. Further, treatment of these cells with 12*S*-HETE enhances TPA actions and counteracts baicalein and siRNA effects [30].

Along a similar line, *p*12-LOX was demonstrated to promote tumor cell survival in two gastric cancer cell lines, AGS and MKN-28. Treatment with baicalein significantly inhibits the growth of both cell lines *in vitro*, and 12*S*-HETE, again, counteracts baicalein actions. This study further showed that both baicalein treatment and *p*12-LOX antisense RNA induces *p*53 independent, caspase mediated apoptosis in these cells [31].

In prostate cancers *p*12-LOX has also been shown to modulate tumor growth through increased vascularity. Like PGE_2, 12*S*-HETE induces the expression of VEGF and basic fibroblast growth factor (bFGF), another protein that modulates angiogenesis, and directly stimulates EC proliferation, migration, and tube formation. PC3 prostate carcinoma cells transfected with *p*12-LOX expression constructs only showed growth advantage *in vivo*. Both transfected and control cells doubled approximately every 36 hours *in vitro*, but compared to the control, subcutaneously implanted transfected cells showed significant growth acceleration after 15 days and a 7 fold increase in tumor volume after 40 days. The tumors formed by the transfected cells also showed significantly increased vascular density [32].

Further experiments confirmed that the growth advantage is secondary to increased angiogenic potential. Migration assays shows that 12*S*-HETE alone is capable of inducing EC migration at nanomolar concentrations, and treatment with N-benzyl-N-hydroxy-5-phenyl-pentanamid (BHPP), a compound that selectively inhibits 12*S*-HETE synthesis, significantly desensitizes ECs to bFGF and VEGF induced proliferation, blocks VEGF induced migrations, and inhibits EC tube-like structure formation in Matrigels. Conversely, *p*12-LOX overexpression in ECs induces migration and tube-like structure formation in Matrigels and *p*12-LOX over expression in PC3 cells produces dramatic, though never quantified, increases in vascular density and penetration in *in vivo* Matrigel plug assays [32,33].

*p*12-LOX expression is also positively correlated with metastatic potential in prostate cancer and melanoma cells; and its product, 12*S*-HETE, has been shown to regulate multiple steps of the metastatic process [4,5,34]. Two groups independently confirmed that *p*12-LOX expression in MCF-7 spheroids mediates tumor cell intravasation into lymphatic vessels. Both studies observed that MCF-7 spheroids that express *p*12-LOX induces the formation of circular chemorepellent-induced defects (CCID) in human lymphatic endothelial cells (LEC) monolayers, creating openings for tumor cells to metastasize into the lymphatic system. Further, the formation of CCIDs is accompanied by upregulations of mesenchymal markers and down regulation of VE-cadherin, a vascular endothelial adhesion protein. Baicalein treatment was found to counteract CCID formation and shRNA mediated knockdown of 15-LOX-1, MCF7 cells do not express *p*12-LOX and 12*S*-HpETE synthesis is instead catalyzed by 15-LOX-1, considerably reduced the size of the CCID openings. Knocking in of 12-LOX reestablished these cells' ability to form CCIDs [35,36].

12*S*-HETE may also, to some extent, regulate tumor cell adhesion. Several studies have suggested that 12*S*-HETE, but not 12*R*-HETE or 5*S*-HETE, exposure triggers immediate secretion of cathepsin B, a lysosomal cysteine enzyme involved in extracellular proteolysis, through the Protein Kinase C (PKC) pathway in highly malignant cells [37-39]. 12*S*-HETE has also been reported to increase Alpha-V/Beta-3 Integrin expression in endothelial cells, presumably increasing tumor cell adhesion to vascular endothelia [40].

*p*12-LOX's role in cancer is backed by more than 20 years of research; however, a handful of relatively recent studies have indicated that its effects are likely to be tissue dependent [4]. Studies have reported that inhibition of *p*12-LOX actually promotes survival in cortical neurons, and increased 12*S*-HETE concentration induces apoptosis in cortical neurons, fibroblasts, and neuroblastoma through distinct mechanisms [41-43].

15-LOX-1 and Cancer

15-LOX-1 has the most diverse set of substrates and products of the lipoxygenase enzymes; and as a consequence, appears to play conflicting roles in tumor initiation and progression. *In vivo* lymph metastasis model have confirmed that 15-LOX-1 knocked down MCF7 (MCF7/ALOX15^{kd}) cells have greatly reduced metastatic potential. While 60% of control MCF7 xenographs produced lymph node metastases in mice after 32 days, no MCF7/ALOX15^{kd} tumor xenograph produced metastasis; and while all control xenographs produced metastasis after 63 days and only 5% of MCF7/ALOX15^{kd} xenographs produced metastasis [35].

In addition to the role that 15-LOX-1 plays in MCF-7 cells, 15-LOX-1 expression is strongly associated with Gleason scores, a malignancy/prognosis indicator for prostate cancers, and its regulation is possibly lost due to mutations in p53 genes, a cell cycle regulator and tumor suppressor. IHC staining of prostate cancer specimens show that 15-LOX-1 and mutant p53 (mtp53) protein expressions are densest at the cancer foci [44].

In vitro experiments show that forced overexpression of 15-LOX-1 in PC-3 cells leads to significantly increased proliferation and diminished growth-stimulating effects of 13*S*-HODE that can be observed in parental cells. 15-LOX-1 over expressing cells also exhibited anchorage independent growth, not observed in parental or control cells, increased VEGF secretion, and generated more and larger tumors than the control in mice models [45].

Likewise, a survey of tissues extracted from LPB-Tag mice, transgenic mice that develop extensive high grade prostatic

intraepithelial neoplasia (HGPIN) and invasive and metastatic carcinoma, indicated that 12*S*-HETE expression is four folds higher in HGPIN than in healthy tissue, but interestingly secondary to *Leu*-12-LOX overexpression, not *p*12-LOX overexpression. This same study also confirmed that *Leu*-12-LOX is upregulated in invasive carcinomas, with detectable expression in about one half of metastatic nodes [46]. A later published, but less detailed study of Transgenic Adenocarcinoma of Mouse Prostate (TRAMP) mice also reported that prostate cancer progresses with corresponding increases in *Leu*-12-LOX activity, based on increased rates of 13*S*-HODE, 12*S*-HETE, and 15*S*-HETE synthesis [47].

In contrast, 15-LOX-1 expression is lost in colon, lung, and pancreatic cancers. Clinical specimens indicate that 15-LOX-1 expression is down regulated in 87% of colorectal adenocarcinomas. Loss of 15-LOX-1 functions also coincides with loss of apoptotic functions and terminal differentiation in colon cancer cells. Conversely, reestablishment of 15-LOX-1 expression alone is enough to restore apoptotic functions in Caco-2 colorectal carcinoma cells, and NSAID induced apoptosis are commonly associated with increases in 15-LOX-1 expression and 13*S*-HODE synthesis [4,48].

Similarly, 15-LOX-1 and its metabolites 15*S*-HETE and 13*S*-HODE are significantly reduced in malignant lung cancer tissues, and 15-LOX-1 expression is essentially undetectable via IHC staining of pancreatic intraepithelial neoplasias, primary tumor, and lymph node metastases. MiaPaCa2 and S2-O13 pancreatic adenocarcinoma cells stably transfected with 15-LOX-1 expression vectors show significantly decreased proliferation, compared to control groups, and treatment with 15*S*-HETE, but not 13*S*-HODE, have been shown to induce apoptosis. *In vivo* animal models confirm that the loss 15-LOX-1 is an early event in lung carcinogenesis. Mice treated with NKK showed declining 15*S*-HETE levels 26 weeks after initial exposure, followed by the emergence of lung tumors within six to eight weeks [49,50].

The specific mechanisms behind 15-LOX-1 modulated proliferation and apoptosis are not fully understood, but several studies have suggested that it is through the selective inhibition and activation of specific peroxisome proliferator-activated receptors (PPAR). In colorectal cancers, 13*S*-HODE, produced by endogenous 15-LOX-1, is reported to bind to and inactivate $PPAR_{\beta/\delta}$, a tumor promoter; and consequently up regulate $PPAR_{\gamma}$, a tumor suppressor, activity. In prostate cancers, both 15*S*-HETE and 13*S*-HODE are reported to bind directly to and activate $PPAR_{\gamma}$; however, 13*S*-HODE is also reported to more potently up regulate EGF-initiated MAP kinase activity that subsequently inactivates $PPAR_{\gamma}$, yielding net growth stimulation [5,51,52]. Other studies, however, have reported that 15*S*-HETE is the 15-LOX-1 product that exerts effects on tumor cell proliferation. *In vivo* lung tumor initiation models suggests that the emergence of tumors coincides more closely with diminishing 15*S*-HETE levels than with diminishing 13*S*-HODE levels [49,50].

15-LOX-2 and Cancer

Unlike 15-LOX-1, the effects of 15-LOX-2 are not, for the most part, tissue dependent. While alone study found elevated 5-LOX and 15-LOX-2 expressions in ovarian cancer specimens, 15-LOX-2 expression has consistently been reported to be down regulated and often lost in late stage esophageal, lung, breast, head and neck, and prostate cancers [53-59].

15-LOX-2 activity has never been reported to cause significant changes to tumor incidence, but *in vitro* studies strongly indicate that 15-LOX-2 is a negative cell cycle regulator and its expression is inversely correlated with malignant growth. Western blots of cultured prostate cell lines showed that, compared to non-malignant cells, 15-LOX-2 protein and mRNA are significantly reduced in all malignant lines and liquid chromatography/tandem mass spectroscopy indicated that the remaining enzymes have impaired catalytic activity [60]. Further *in vitro* studies have tied 15-LOX-2 activity to suppressed tumor growth. Compared to control groups, TE-1, TE-8, and TE-12 esophageal cancer cells transiently transfected with 15-LOX-2 expression vectors show a 33% decrease in tumor cell proliferation [56], and PC3 prostate cancer cells stably transfected with 15-LOX-2 expression vectors show as much as an 80% decrease in tumor cell proliferation. Further, these transfected PC3 cells showed an unspecified increase in the percentage of apoptotic cells in culture, dramatically decreased cell viability in the presence of 5 μM AA, and produced significantly smaller tumors when injected into nude mouse prostates [61,62].

Several distinct mechanisms been identified to account for this phenomena. Like 15-LOX-1, existing evidence suggests that 15-LOX-2 may exert its anti-proliferation effects through $PPAR_{\gamma}$. 15*S*-HETE has been confirmed to be an endogenous $PPAR_{\gamma}$ ligand, and $PPAR_{\gamma}$ agonists have been demonstrated to induce apoptosis in cancer cells [63]. Other studies have shown that 15*S*-HETE and 8*S*-HETE also inhibit DNA synthesis and induce cell cycle arrest through the activation of p38 mitogen-activated protein kinases [64,65]. However, an alternatively spliced 15-LOX-2 variant, 15-LOX-2sv-b, that has no AA metabolizing ability also appears to inhibit tumor growth [61], suggesting that a third, 15*S*-HETE independent mechanism exist.

Studies also indicated that 15-LOX-2 also suppresses tumor progression. IHC staining of matched-pairs of prostate cancer specimens showed that 15-LOX-2 expression is almost universally reduced in higher grade tumors [53] and a survey of 160 clinical lung cancer specimens showed that 15-LOX-2 expression is correlated with better differentiation in NSCLCs [58]. *In vitro*, 15-LOX-2 expression has also been correlated with larger, flatter phenotype in normal human prostate (NHP) cells and NHP cells have been observed to autonomously upregulated 15-LOX-2 mRNA and protein expression shortly before they become senescent. Treatment of cultured NHP and prostate carcinoma cells with 25 μM 15*S*-HETE induces an enlarged, flattened, and immotile morphology within 72 hours in both cell types; and transgenic expression of 15-LOX-2 in mouse prostates induced early expression of senescence associated β-galactosidase [66,67].

Studies have also reported that 15-LOX-2 expression promotes less invasive phenotypes. Western blot analysis of nine prostate cancer cell lines clearly showed a correlation between the loss of wild type 15-LOX-2 and emergence of truncated variants of E-cadherin in all 9 malignant lines and complete loss of wild type E-cadherin in three malignant lines [67]. Treatment with either 30 μM AA or 20 μM 15*S*-HETE significantly and almost immediately increased MDA-MB-435 breast cancer cells adhesion to type IV collagen, likely through the MAP kinase pathway [65]. DU145 and PC3 cells transfected with 15-LOX-2 expression constructs also show significant reductions in VEGF-A levels [62].

The loss of 15-LOX-2 expression in cancer cells still, for the most part, remains to be defined. Analysis of the ALOX15B promoter regions have suggested that the transcription factors Sp1 positively and Sp3 negatively regulate 15-LOX-2 expression [68]. However, analysis of prostate cancers showed that Sp1 expression is surprisingly higher in cancer cells than normal cells. Further experiments also ruled out

genomic mutations, DNA hypermethylation, and deficient KLF6 expression [67], a Sp1 family protein that is down regulated in prostate cancers. Two studies did indicate that 15-LOX-2 expression could be down regulated by glucocorticoid receptor activity [69] or by the creation of a negative feedback mechanism responsive to 15*S*-HETE levels and $PPAR_{\gamma}$ activation [70].

Targeting Lipoxygenases for Cancer Treatment

The efficacy of lipoxygenase inhibitors and lipoxygenase metabolite inhibitors as chemotherapeutics is not well studied and has not been established yet. As of now, there are no published clinical trials involving lipoxygenase inhibitors and there are no indications that patients who take zileuton or the CysLT receptor 1 ($CysLT_1$) antagonist montelukast (Singulair) exhibit lower instances of cancer.

A leukotriene B_4 receptor antagonist and selective 5-LOX inhibitor, LY293111, did complete phase I trials and all indications suggest that it can be safely administered to patients with advanced stage tumors. *In vitro* and animal models have consistently produced promising results, especially when used in combination with existing chemotherapeutics to treat pancreatic and colorectal cancers. Unfortunately, LY293111 could not reproduce any beneficial effects for patients with metastatic pancreatic adenocarcinoma during phase II trials [71-77].

Conclusion and Perspective

Despite the progress that has already been made, many unknowns still lie with the LOX enzymes. It is still not clear how the expressions and activities of enzymes become dysregulated in cancers or why some isozymes, such as *p*12-LOX and 15-LOX-1, exert radically different effects in different tissues. Many studies have reported interactions between LOX products and PPAR receptors, while others have indicated that these enzymes may exert their effects independent of these receptors or even independent of their products. There is no holistic explanation and a fuller understanding of these mechanisms is needed before treatments that target these pathways can be implemented successfully.

Furthermore, the LOX isozymes oxygenate a diverse set of PUFAs, not just AA. Metabolites generated from other substrates often exert effects that oppose those of the AA metabolites. Studies have found correlations between dietary ω-6 fatty acids and cancer [3], and at least one study has reported that dietary ω-3 fatty acid supplements enhance the efficacy of therapies that target VEGF and AA metabolizing enzymes [78]. Research into this field can have profound impact on our understanding of nutrition and identify diets that supplement and enhance existing treatments.

The expression of LOX enzymes become dysregulated in cancers and all research indicates that this pathway is involved in carcinogenesis and tumor progression. Studies using animal models have demonstrated that these enzymes are promising targets for intervention; *in vivo* models have shown that many LOX inhibitors reduce tumor incidence, growth, and acquisition of invasive phenotypes. Unfortunately, these results have not yet been translated into the clinic successfully. The development of new and the repurposing of existing inhibitors of LOX, LOX metabolites, or even the downstream receptors can provide novel and effective therapeutic options. Considering the evidence that is presented above, treatments that target the LOX pathway, whether used alone or in combination with existing treatments, can have profound impacts on cancer patients - including pancreatic cancer patients, since modulation of the LOX pathway has produced promising anti-cancer results in pancreatic cancer cell lines. Because these LOX enzymes have also been shown to be involved in many other disorders, including asthma, allergies, arthritis, and various other cardiovascular and immune disorders, the development of these LOX based treatments can be especially cost-efficient, since the treatments can be repurposed quickly and used to treat various other conditions.

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