

Review

5-Lipoxygenase Antagonist therapy: a new approach towards targeted cancer chemotherapy

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Leukotrienes are the bioactive group of fatty acids and major constituents of arachidonic acid metabolism molded by the catalytic activity of 5-lipoxygenase (5-LOX). Evidence is accumulating in support of the direct involvement of 5-LOX in the progression of different types of cancer including prostate, lung, colon, and colorectal cancers. Several independent studies now support the correlation between the 5-LOX expression and cancer cell viability, proliferation, cell migration, invasion through extracellular matrix destruction, metastasis, and activation of anti-apoptotic signaling cascades. The involvement of epidermal growth factor receptor and 5-oxo-ETE receptor (OXER1) is the major talking point in the downstream of the 5-LOX pathway, which relates the cancer cells to the proliferative pathways. Antisense technology approaches and use of different kinds of blocker targeted to 5-LOX, FLAP (5-LOX-activating protein), and OXER1 have shown a greater efficiency in combating different cancer cell types. Lastly, suppression of 5-LOX activity that reduces the cell proliferation activity also induces intrinsic mitochondrial apoptotic pathway in either p53-dependent or independent manner. Pharmacological agents that specifically inhibit the LOX-mediated signaling pathways have been used during last few years to treat inflammatory diseases such as asthma and arthritis. Studies of these well-characterized agents are therefore warranted for their use as possible candidates for chemotherapeutic studies against the killer disease cancer.

Keywords leukotriene; 5-LOX; receptor; proliferation; apoptosis

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Introduction

Cancer is a dreadful disease primarily caused by abnormal and uncontrolled cell proliferation. The disease is triggered by various extrinsic and intrinsic factors. Some agents are

considered initiators and some as promoters of cancer. While some of the dietary components can act as antagonists of cancer [1], some are known to contribute to the cause of cancer progression [2]. One such example is that of high-fat diet that has been linked to the progression of cancer.

Diet is then a critical determinant of cancer risk. The risk has been attributed both to dietary chemical constituents and to overall energy consumption. As much as 14%–20% of cancer deaths have been attributed to overweight and obesity. Overweight and obesity, as defined by the ratio of weight to height known as body mass index [3], are on the rise in the developing countries. Traditionally overweight and obesity have been associated with elevated risk of cancers of the colon, breast, endometrium, kidney, prostate, lung, and esophagus [1]. The metabolites of fatty acid intend to form different bioactive molecules like eicosanoids. These derived substances implicated in the pathogenesis of variety of human diseases, including cancer, are now believed to play greater role in tumor progression, metastasis, angiogenesis, etc. [4]. Two main enzymes namely cyclooxygenase (COX) and lipoxygenase (LOX) are responsible for production of eicosanoids while metabolizing fats [5]. Inhibition of these two enzymes delays tumorigenesis in animals and humans by many non-steroidal anti-inflammatory drugs, which in turn can obstruct tumor progression in various tissues [6]. In the last decade, agents that specifically inhibit the LOX metabolic pathway have been developed to treat inflammatory diseases, such as asthma and arthritis [7]. In recent days these compounds, showing inhibitory action on LOX pathway, are also showing promising block against cell proliferation. This profound activity of LOX as proliferation-blocker may play a significant role as a key factor in the treatment of the killer disease cancer to a certain extent.

Thus treating cancer as a disease of cells brings up the basic question: what is the cause of abnormal proliferation of cancer cells? Inappropriate number of chromosomes with their incorrect structure or the faulty metabolism which produces mitogen like substances has often been reported when studying cancer cells.

5-LOX: Key Enzyme in Leukotriene Biosynthesis

Human 5-LOX is a non-heme iron containing dioxygenase [8]. Its gene spans >82 kb and consists of 14 exons [9]. This enzyme is also known as arachidonate:oxygen 5-oxido-reductase that catalyzes the formation of leukotriene (LT) or eicosatetraenoic acid from arachidonic acid [10]. Arachidonic acid (5,8,11,14-eicosatetraenoic acid), a common member of the omega-6 poly-unsaturated fatty acids in high-fat product, is a strong stimulating agent for different types of cancer [11]. The progression of carcinogenicity is associated with the formation of bioactive arachidonic acid metabolites like eicosanoids, which act as mitogen [12].

5-LOX catalyzes the first two steps in LT formation [4], and the reaction starts with the intracellular release of arachidonic acid. 5-LOX in the presence of FLAP (5-LOX-activating protein) catalyzes the oxidation of arachidonic acid into 5(*S*)-hydroxy-6-*trans*-8,11,14-*cis*-eicosatetraenoic acid (5-HETE) [10], followed by a second reaction in which 5-HETE is dehydrated to form the epoxide LTA₄ [13]. Once formed, LTA₄ is further metabolized to either LTB₄ via stereo-selective hydration by LTA₄ hydrolase or to LTC₄ through glutathione conjugation catalyzed by LTC₄ synthase [14]. Sequential metabolic reactions, catalyzed by γ -glutamyltransferase and a specific membrane-bound dipeptidase, convert LTC₄ into LTD₄ and LTE₄, respectively [15]. Now in resting cells, 5-LOX resides in either the nucleus or the cytosol, in several tissues and cells, including epithelial cells, and vascular smooth muscle cells [16]. Upon activation, 5-LOX translocates to the nuclear membrane, where the FLAP is thought to facilitate the transfer of phospholipid-derived arachidonic acid to 5-LOX and to enhance the efficiency of conversion of 5-HETE to LTA₄, thereby triggering 5-LOX product formation [4]. The oxidized forms of 5-HETE, LTs, increased cell proliferation, and viability of cancer cells [17] by binding to the G-protein-coupled trans-membrane receptor OXER1 [18] (Fig. 1).

Involvement of 5-LOX Metabolites in Tumor Cell Proliferation

Several metabolites of arachidonic acid synthesized by the 5-LOX-mediated pathway are known to have the potential to promote the cell proliferation, increase the cellular viability, and protect the cells from different chemo-preventive measures [19], but the proper molecular mechanism of action of these metabolites still remains in the dark though these metabolites play a critical role in the tissue repair and lipid homeostasis [20]. Tumor cells, like growing tissues or embryonic cells, emit signals that initiate the formation of new

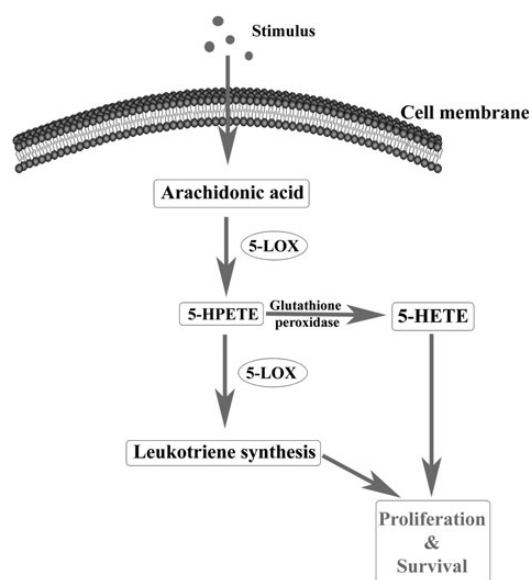


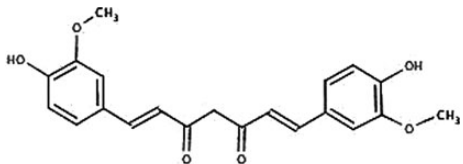
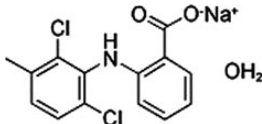
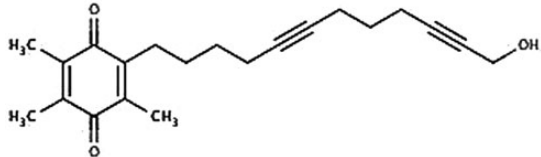
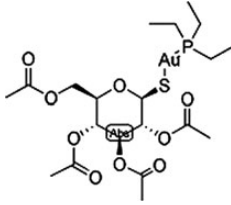
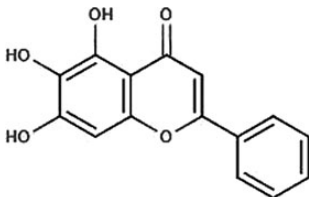
Figure 1 Schematic of LT biosynthesis

blood vessels. This adoptive process, termed angiogenesis, is a general feature of every tissue, mainly activated during wound repair process, and is a pre-requisite for tumor expansion beyond a limiting size [21]. Direct proliferative and anti-apoptotic stimuli are an enhanced tumor angiogenesis which can contribute to the tumor metastasis in the later stages. Recent studies demonstrated the involvement of growth factors, such as epidermal growth factor (EGF) and neurotensin in the 5-LOX-mediated tumor progression in prostate cancer [22,23]. Recent studies with 5-LOX siRNA [10] and specific blocker of 5-LOX [24] revealed the relation of this gene with the tumor cell proliferation.

Involvement of 5-LOX Metabolites in Angiogenesis

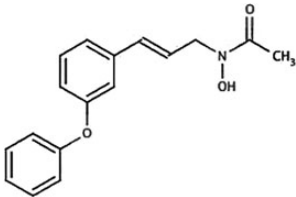
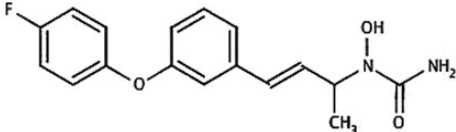
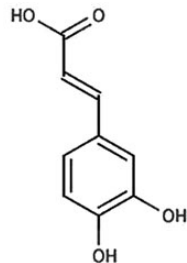
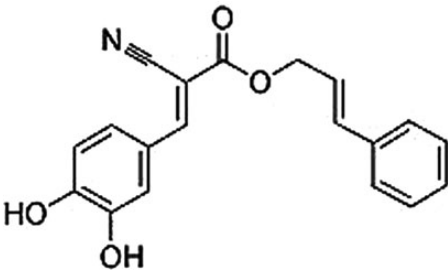
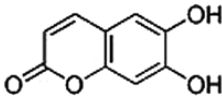
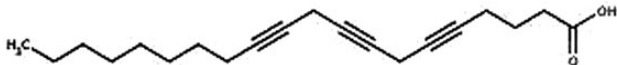
Growth factors are the key regulators of angiogenic genes. Requirements of nutrients and oxygen in the growing tissue make them emit signals that initiate the formation of new blood vessels. Formation of new blood vessels is a general feature in the wound healing process and is a pre-requisite for tumor expansion beyond a limiting size of 2–3 mm³. Vascular endothelial growth factor (VEGF) is the most potent tumor angiogenic factor identified and a critical initiator for vessel formation. Recently, 5-HETE, a metabolite of 5-LOX, was found to stimulate angiogenesis by inducing the expression of VEGF in case of colon cancer [25–27]. The involvement of AKT (also known as Protein Kinase B) and ERK (extracellular-signal-regulated kinases) in the downstream of 5-LOX activation also could play a crucial role in the formation of vasculogenesis or angiogenesis in the tumor cells [28].

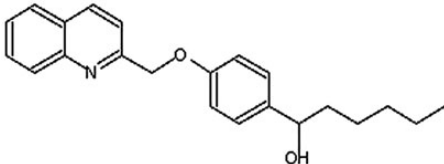
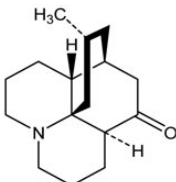
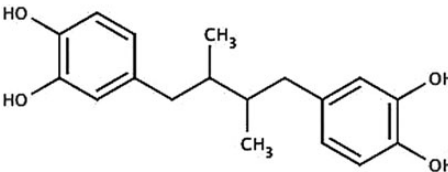
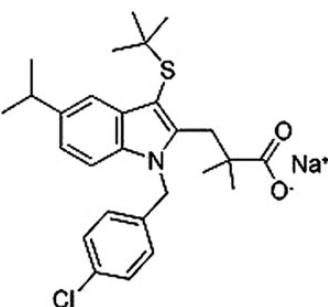
Table 1 Names and structures of 5-LOX inhibitors, their targets of action, and types of cancer tested along with respective references

Name and structure	Target	Status ^a	Types of cancer tested and IC ₅₀ /EC ₅₀ values	References
Curcumin 	5-LOX	Pancreatic neoplasms: phase II	Inhibit 5-LOX activity in RAW264.7 cells (IC ₅₀ = 0.3 μM), inhibits 5-LOX (IC ₅₀ = 8 μM), Cox-2 (IC ₅₀ = 52 μM) in colorectal cancer	[36]
Meclofenamate sodium 	5-LOX and COX		Inhibit human recombinant hCox-1 (IC ₅₀ = 1.5 μM) and hCox-2 (IC ₅₀ = 9.7 μM)	[37–39]
AA-861 	5-LOX	Discontinued phase II (asthma, allergy) Phase III (rheumatoid arthritis)	Human leukemia cells (IC ₅₀ = 6–20 mM), lung cancer cells (IC ₅₀ = 5–10 mM), guinea pig peritoneal PMNL 5-LOX (IC ₅₀ = 0.8 mM), mouse epidermal 12-LO (IC ₅₀ = 1.9 mM), murine bladder cancer cell line MBT-2 (IC ₅₀ = 8.2 μM), Capan-2 (IC ₅₀ = 57 μM), Panc-1 (IC ₅₀ = 27 μM), THP-1 (IC ₅₀ = 40 μM), U937 (IC ₅₀ = 12 μM), Capan-Z (IC ₅₀ = 25 μM)	[36,40–45]
Auranofin 	5-LOX/LTA synthase and disrupt MMP	Phase II (chronic lymphocytic leukemia, stage IV non-small cell lung cancer)	Human lung macrophages, human neutrophils stimulated with either fMLP (IC ₅₀ = 10 μM), human neutrophils (IC ₅₀ = 17.4 μM), MCF-7 human breast cancer cells (IC ₅₀ = 11.4 μM)	[46–50]
Baicalein 	5- and 12-LOX		Gastric cancer cell lines, murine bladder cancer cell line MBT-2 (IC ₅₀ = 0.43 μM), rat heart endothelial cells (IC ₅₀ = 20 μM), rat platelet (IC ₅₀ = 0.12 μM), rat PMN (IC ₅₀ = 9.5 μM)	[51–57]

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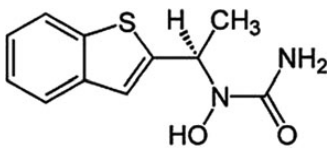
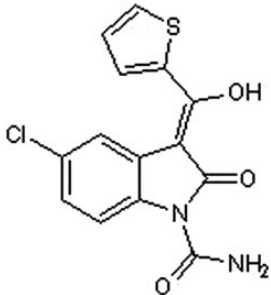
Table 1 Continued

Name and structure	Target	Status ^a	Types of cancer tested and IC ₅₀ /EC ₅₀ values	References
BW A4C 	5-LOX		Murine adenocarcinomas: MAC16 (IC ₅₀ = 5 μM), MAC13 (IC ₅₀ = 2 μM), MAC26 (IC ₅₀ = 4 μM), U937 (IC ₅₀ = 29 μM), Capan-Z (IC ₅₀ = 13 μM)	[15,58–62]
BW B70C 	Arachidonic acid, 5-LOX		Murine adenocarcinomas: MAC16 (IC ₅₀ = 5 μM), MAC13 (IC ₅₀ = 2 μM), MAC26 (IC ₅₀ = 4 μM)	[61]
Caffeic acid 	5-LOX	Discontinued phase III (common cold)	HepG2 (IC ₅₀ = 100 μg/ml), RAW 264.7 macrophages, DPE inhibited platelet (IC ₅₀ = 4.2 μM), DPE inhibited PMNL (IC ₅₀ = 13 μM), intact platelets (IC ₅₀ = 50 μM), intact PMNL (IC ₅₀ = 26 μM), colorectal cancer cells (IC ₅₀ = 0.8 μM), human lung macrophages, human neutrophils stimulated with either fMLP (IC ₅₀ = 10 μM), human neutrophils (IC ₅₀ = 17.4 μM), MCF-7 human breast cancer cells (IC ₅₀ = 11.4 μM)	[63–67]
CDC 	12-LOX inhibitor also inhibits 5-LOX and 15-LOX	Phase I (adenocarcinoma) Phase II (renal cell cancer, kidney cancer)	Inhibit 5-LOX at 1.89 μM, growth inhibition at 10 μM, induce apoptosis at 24.2 μM in epithelial cancer cell lines, prostate cancer cells PC3, breast cancer cells MCF-7, Bladder cancer cells (BOY, T24, HT1376, SCaBeR, RT4) IC ₅₀ ≥ 50 μM, lung pulmonary arteries (IC ₅₀ = 10 μM)	[68–71]
Esculetin 	12-LOX and LT synthesis		Inhibits 5-LOX at 0.1–1.0 μM, mastocytoma 12-LOX (IC ₅₀ = 2.5 μM)	[72,73]
ETI 	5-LOX and biosynthesis of LT C4	Phase I (healthy volunteers)	Cox (IC ₅₀ = 14 μM), human 12-LO (IC ₅₀ = 0.46 μM), 5-LO (IC ₅₀ = 25 μM), effective against mammary cancer	[74,75]

L-655,238	FLAP	Platelets ($IC_{50} = 10 \mu M$), cerebellar granule neurons ($IC_{50} = 25 \mu M$), 5(<i>S</i>)-HETE production in RBL-2H3 cells was inhibited by L-655,238 with an IC_{50} of 135.2 ± 11.5 nM, bronchial epithelial cells ($IC_{50} = 1 \mu M$)	[45,75–77]	
				
Lycopodine	5-LOX	Inhibit prostate cancer PC3 ($IC_{50} = 121 \mu M$), LnCap ($IC_{50} = 101 \mu M$)	[78]	
				
NDGA	5-LOX	Discontinued phase I (prostate cancer), completed phase II (prostate cancer, brain tumor, CNS tumors, cervical intraepithelial neoplasia)	[79,80]	
				
MK-886 sodium salt	FLAP	Discontinued phase I (asthma)	Human and rat neutrophil LT biosynthesis ($IC_{50} = 3–5$ nM), human PMNs ($IC_{50} = 2.5$ nM), DNA synthesis inhibition in acute myelogenous leukemia cells at 100 nM, growth inhibition of chronic myelogenous leukemia (10–20 mM), lung cancer cells (5–10 mM), Capan-1 ($IC_{50} = 37 \mu M$), THP-1 ($IC_{50} = 32 \mu M$), U937 ($IC_{50} = 10 \mu M$), Capan-2 ($IC_{50} = 15 \mu M$), Capan-Z ($IC_{50} = 24 \mu M$)	[76,81,82]
				

Continued

Table 1 Continued

Name and structure	Target	Status ^a	Types of cancer tested and IC ₅₀ /EC ₅₀ values	References
Zileuton 	5-LOX, LTB ₄ synthesis	Phase II (lung cancer, head and neck cancer) Completed phase II (head and neck cancer) FDA approved 12/96, launched in the USA January 1997 (asthma)	FDA approved 12/96, launched in the USA January 1997 (asthma), RBL-1 (IC ₅₀ = 0.5 mM) and rat PMNL (IC ₅₀ = 0.3 mM) 5-HETE synthesis, human whole blood (IC ₅₀ = 0.9 mM) and PMNL (IC ₅₀ = 0.4 mM) LTB ₄ , murine colon adenocarcinoma cells (IC ₅₀ = 43–58 mM), murine adenocarcinomas: MAC16 (IC ₅₀ = 58 μM), MAC13 (IC ₅₀ = 43 μM), MAC26 (IC ₅₀ = 58 μM)	[82,83]
Tenidap 	5-LOX, COX-1		Plasma-free leucocyte suspensions (IC ₅₀ = 10 μM), inhibition of IL-1 production (IC ₅₀ = 3 μM) <i>in vitro</i>	[84–86]

5-LOX inhibitors can block the metabolism of arachidonic acid, which in turn produces LTs at the downstream of this pathway. These LTs directly help the cancer cells to proliferate via up-regulating the EGFR.

^aData were obtained from the site: ClinicalTrials.gov (A service of the US National Institutes of Health).

DPE, 2-(3,4-dihydroxyphenyl)ethanol; FDA, Food And Drug Administration; IL-1, interleukin-1; fMLP, formylmethionyleucylphenylalanine; PMNL, endometrial epithelial cell line.

Involvement of 5-LOX in the Regulation of Migration and Invasion

There are also evidences for the role of 5-LOX in cell migration and invasion through extracellular matrix (ECM) destruction. Matrix metalloproteinases (MMPs) are involved in the degradation of matrix components and they play an important role in the invasion of tumor cells through basement membrane barriers [27]. Distinct changes in ECM homeostasis, which in some respects imitate those that occur in fibrotic diseases, play a crucial role in tumor development. They occur due to destruction of the balance between ECM synthesis and secretion, and owing to alterations in the normal levels of matrix-remodeling enzymes such as LOX [28] and MMPs. Elevated LOX expression is ominously connected with metastasis and is known to reduce survival in cancer patients and mouse models of cancer [29]. LOX has been validated as a predictive marker in patients with head and neck cancer [30,31]. Augmented LOX activity results in increased ECM stiffness [32], and has been shown to increase the invasiveness of many cancer cell types [33,34]. Thus, in addition to proliferation and angiogenesis, 5-LOX and MMP are now linked to the tumor migration and invasion. So, 5-LOX can be believed as a potent cancer-causing agent, which helps in tumor progression, angiogenesis, migration, and lastly in invasion.

5-LOX Inhibitors in Combating Cancer

Barbey *et al.* [35] used 3-[[3-fluoro-5-(tetrahydro-4-methoxy-2H-pyran-4-yl)phenoxy]methyl]-1-[4-(methylsulfonyl)phenyl]-5-phenyl-1H-pyrazole to inhibit growth of prostate cancer cell lines, and surprisingly it brought a marked reduction in the growth of PC3 and LnCaP cell lines, showing a greater efficiency on the androgen-dependent cell lines. The growth was reduced to 50% at 83 μ M of the drug used (see **Table 1** for other details). Meclofenamate sodium (MS) is known for its anti-inflammatory activity, and apart from this, Boctor *et al.* [37] reported that it caused reduction in the formation of 5-HETE in human leucocytes when used. MS can thus be considered a dual inhibitor of 5-LOX and COX pathways of arachidonic acid cascade. Further investigation with this substance revealed that it could interfere with the LT receptors in the lung carcinoma [38]. In a recent study, a group of scientists have shown the effect of MS on prostate cancer cells both *in vitro* and *in vivo* [39], and their result suggests a profound reduction in the tumor growth and cancer metastasis.

Studies have shown that 2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadienyl)-1,4-benzoquinone (AA861) can inhibit 5-LOX of guinea pig peritoneal polymorphonuclear leucocytes [40] and also can suppress the formation of 5-HETE

and LTD₄ [41]. Zou *et al.* [42] reported that AA-861 reduced gastric cancer metastasis and induced apoptosis, and could act as a potent, selective, and orally active 5-LOX inhibitor. Inhibition of 5-LOX has shown to accelerate and enhance fracture healing and increase Ca²⁺ in MDCK cells (also known as NBL-2, Canis familiaris kidney disease) by releasing Ca²⁺ from multiple internal stores. Hoque *et al.* [36] studied the role of AA-861 on the esophageal cancer, and found that increased expression of 5-LOX prevented the cells from apoptosis and by using this inhibitor, a reduced growth of cancer cell lines was noted. Hayashi *et al.* [43] studied the effect of AA-861 on bladder cancer cells and suggested that the inhibition of 5-LOX pathway suppressed the growth of bladder cancer cells. Goto *et al.* [44] showed that AA-861 could bring apoptosis on estrogen-responsive mouse Leydig cell tumor cells by inhibiting LT production. Uz *et al.* [45] also showed that AA-861 could reduce proliferation in immature cerebellar granule neurons *in vitro*.

Auranofin, a gold(I)-phosphine thiolate small molecule, is reported to completely block all LOX products through inhibition of 5-LOX and to induce mitochondrial permeability transition [46–49]. It can bring up apoptosis on cisplatin-resistant human ovarian cancer cells [50].

Baicalein is a cell-permeable flavone, originally isolated from the roots of *Scutellaria baicalensis*. Baicalein has been shown to inhibit platelet 5-LOX and 12-LOX. Baicalein is a potent anti-inflammatory and anti-tumor agent. Hsu *et al.* [51] reported about this active flavone and also reported it to have anti-proliferative effect on several cell types; it could bring apoptosis to them by arresting the cell cycle mechanism [52]. This compound has an anti-inflammatory activity on leucocytes [53,54]. For the first time, Deschamps *et al.* [55] stated about the LOX inhibitory nature of this active principle. Baicalein can induce apoptosis in human gastric cancer cells and also on murine bladder cancer cell line by inhibiting LOX pathway [56,57].

BW A4C is a selective 5-LOX inhibitor and can induce apoptosis on lymphoma [58,59]. This compound shows its inhibitory effect on MAC26 and MAC16 tumors and at dose levels in between 5 and 25 mg/kg [60]. Similar inhibition occurs in murine colon adenocarcinoma cell lines MAC16, MAC13, and MAC26 treated with BWA4C at micro-molar concentrations and it has also been reported to have inhibitory growth effect on colorectal cancer by modulating 5-LOX pathway [61]. BWA4C is the most effective inhibitor, significantly decreasing both growth rate and tumor volume after 8–13 days of treatment [15]. Fischer *et al.* [62] showed the effect of BW A4C on various kinds of tumor cell including pancreatic cancer cell line (Capan-2).

Caffeic acid is an endogenous phenolic phytochemical compound that exists in plants and many foods [63]. A major metabolite product upon hydrolization of chlorogenic acid, caffeic acid inhibits a number of LOXs such as 5-LOX in a

non-competitive manner [64] in various kinds of cancer like colorectal cancer [65], liver cancer [66], and gastric cancer [67].

Esculetin, a phenolic compound, acts as a 5-LOX inhibitor. Additionally, esculetin also inhibits the activity of 12-LOX, and decreases LT biosynthesis during 5-LOX inhibition [72]. This compound acts as a potent therapeutic agent for leukemia, and induces apoptosis through Bcl-2-mediated pathway [73].

Other compounds that can also inhibit 5-LOX and 12-LOX selectively are also used as an anti-cancer agent. ETI, 5,8,11-eicosatriynoic acid, demonstrates the ability to inhibit 5-LOX activity and halt biosynthesis of LT C4 by mastocytoma cells [71,74]. L-655,238 is a potent and selective inhibitor of FLAP (5-LO-activating protein) [45,75–77]. NDGA (nordihydroguaiaretic acid) [79,80], MK-886 sodium salt [76,81,82], zileuton [82,83], tenidap [84–86], CDC (cinamyl-3,4-dihydroxy- α -cyanocinnamat, amebolite of ciprofloxacin) [68–71], BW B70C [61], curcumin [36], and lycopodine [78] can alter LOX metabolism by inhibiting 5-LOX, 12-LOX, and 15-LOX; otherwise they can also inhibit FLAP receptor and other proteins of related pathways. These effects, on the other hand, induce apoptosis of different kinds of cancer *in vitro* and *in vivo*.

Off-target Effect of 5-LOX Inhibitors

The increasing numbers of publications that report a crucial role of 5-LOX, and its products in tumorigenesis have been accompanied by additional studies that question the correlation between 5-LOX and cancer. There is little disagreement that 5-LOX inhibitors exert strong cytotoxic activities against 5-LOX over-expressing tumor types and cultured tumor cells, which represents significant basis for concluding that 5-LOX products directly stimulate tumor cell proliferation. These metabolites might induce cancer progression, but not carry sole responsiveness. Other factors related to fat deposition also play a crucial role in inducing neoplasia. LOX pathway also carries out a metabolism related to normal physiological growth. Blocking of this pathway on the other hand hinders normal physiological conditions related to metabolism. So, it is most important to carry out study onside/off-target effects of these blocker substances. Cytotoxic natures of these substances generally harm the normal body cells.

However, it was recently demonstrated that the common 5-LO inhibitors could reduce the viability of pancreatic cancer cells, cervix carcinoma cells, and leukemic cells independently of the suppression of 5-LO product formation [62]. The hypothesis of 5-LO-independent cytotoxicity and anti-proliferation was substantiated using several experimental approaches. While the commonly used inhibitors produced strong cytotoxicity, notably, zileuton, the only commercialized 5-LOX inhibitor, failed to induce an anti-proliferative or cytotoxic response in all other types of

tumor cells where 5-LOX was in inactive state (e.g. HeLa cells); however, where 5-LOX was in active state, zileuton could effectively inhibit progression, as in case of prostate cancer.

In fine, the cytotoxic and chemo-preventive effects of 5-LOX inhibitors in cell culture assays and in animal tumor models may derive from molecular mechanisms other than suppression of LT biosynthesis and warrant re-assessment in some cases.

Apoptosis Induction

The blocking of 5-LOX enzyme can induce apoptosis in several cancer cells. Initiation of cells' programmed-killing process is generally triggered by depolarization of mitochondrial membrane potential. Recently in our lab, we have shown the activation of mitochondrial-mediated apoptosis without the involvement of p53 gene. Lycopodine, a plant-derived active compound, could activate caspase 3 protein in PC3 and LnCaP prostate cancer cells [78]. In another study, Rev-5901, a 5-LOX inhibitor, showed its efficiency to reduce cancer growth in lung tissue, and activated cytochrome c-mediated caspase-dependent pathway to induce apoptosis [87]. Again a group of researchers have shown licoferone, a dual COX/5-LOX inhibitor, could depolarize mitochondrial membrane potential to induce apoptosis; on the other hand, it also affected the arachidonic pathway by inhibiting 5-LOX enzyme activity in HCA-7 colon cancer cells [88]. MK-591 in prostate cancer cells could induce protein kinase C-epsilon at the downstream of 5-LOX inhibition [89]. From all these cumulative data, it is clear that, the LOX inhibitor acts upon mitochondria to depolarize its potential and releases out the cytochrome c to activate caspase cascade. The involvement of proteins like p53 [78] and AKT [89] is always not needed for caspase cascade initiation. Proteins like p21 and PKC could play here a greater role in the initiation of apoptosis through mitochondrial membrane potential imbalance (Fig. 2).

Implications and Future Directions

This review aims at presenting a conceptual framework for integrative signaling and proposes 5-LOX as a cancer-

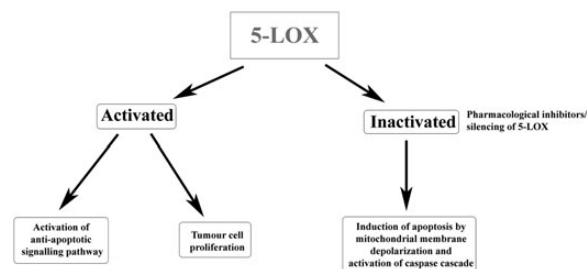


Figure 2 Effect of 5-LOX inhibition

causing element. A casual connection between arachidonic acid cascade and cell proliferation and cancer has been proposed for many years and many events on COX pathway have been well documented in recent years, but the implication of LOX pathway for the metabolism of arachidonic acid and its relation with the cancer growth is not yet well understood. As 5-HETE and LTs synthesis have been associated with several malignancies involving epithelial cells, these arachidonic acid metabolites might be the missing link between arachidonic acid metabolites and cancer progression.

The well-recognized over-expression of 5-LOX in various types of malignant cells, the reduction of tumor cell viability by 5-LOX gene silencing approaches, as well as experiments involving 5-LOX knockout mice, together constitute a substantial rationale for the relation between 5-LOX gene and malignancy. However, considering that the cytotoxic activity of 5-LOX inhibitors is substance-specific and may, in many cases, have not derived from inhibition of 5-LOX activity, the traditional hypothesis that 5-LOX products are the exclusive players in 5-LOX-triggered tumorigenesis may warrant re-consideration. Experiments on the role of 5-LOX product formation in proliferation of cultured tumor cells using high concentrations ($>1 \mu\text{M}$) of certain 5-LOX inhibitors may be misleading and the use of these agents as pharmacological tools should be critically considered. Also, possible indirect tumorigenic effects of 5-LOX products (e.g. promotion of angiogenesis) with relevance towards the observed situation *in vivo* but not for cell culture assays should be taken into account. Notably, non-enzymatic functions, including an interaction with cytoskeleton proteins, or with the EGF receptor (EGFR) molecule, or with mitogen-activated protein kinases (MAPKs) have been reported for 5-LOX. Because of the crucial role of oncogenic EGFR and MAPK signaling in cancer progression, a disrupted growth factor signaling may contribute to the reduction in tumor cell viability by 5-LOX gene silencing approaches. Taken together, a broad body of evidence from the literature suggests a crucial, albeit poorly defined, role of 5-LOX in tumorigenesis of several cancer types. Elucidation of the molecular mechanisms underlying these effects may include direct proliferative actions of 5-LOX products on tumor cells as well as indirect and so far neglected effects of 5-LOX, and thereby tempt one to draw novel connections between pathways that are currently regarded as unrelated.

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