



5-Lipoxygenase: underappreciated role of a pro-inflammatory enzyme in tumorigenesis

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Leukotrienes constitute a group of bioactive lipids generated by the 5-lipoxygenase (5-LO) pathway. An increasing body of evidence supports an acute role for 5-LO products already during the earliest stages of pancreatic, prostate, and colorectal carcinogenesis. Several pieces of experimental data form the basis for this hypothesis and suggest a correlation between 5-LO expression and tumor cell viability. First, several independent studies documented an overexpression of 5-LO in primary tumor cells as well as in established cancer cell lines. Second, addition of 5-LO products to cultured tumor cells also led to increased cell proliferation and activation of anti-apoptotic signaling pathways. 5-LO antisense technology approaches demonstrated impaired tumor cell growth due to reduction of 5-LO expression. Lastly, pharmacological inhibition of 5-LO potently suppressed tumor cell growth by inducing cell cycle arrest and triggering cell death via the intrinsic apoptotic pathway. However, the documented strong cytotoxic off-target effects of 5-LO inhibitors, in combination with the relatively high concentrations of 5-LO products needed to achieve mitogenic effects in cell culture assays, raise concern over the assignment of the cause, and question the relationship between 5-LO products and tumorigenesis.

Keywords: leukotriene, apoptosis, cell proliferation, mitogenic effects, cytotoxicity

INTRODUCTION

Cancer still ranks the second leading cause of death worldwide despite the emergence of a variety of novel therapeutic options over the past decade. Malignancy of cells reflects an up-regulation of various oncogenic signal cascades that elevate tumor cell proliferation, suppress apoptosis, trigger angiogenesis, and promote metastasis. Lipid mediators, such as leukotrienes (LTs) and prostaglandins constitute a recently discovered class of tumor promoters acting by increasing tumor cell viability and triggering metastasis inducing events. Pharmacological modulation of these biosynthetic pathways is steadily increasing in importance. The present article summarizes several of these experimental findings, which implicate an emerging role of 5-lipoxygenase (5-LO)-derived LTs in carcinogenesis and critically examines the potential shortcomings of previously conducted research. This can provide direction for future investigations on 5-LO in tumorigenesis.

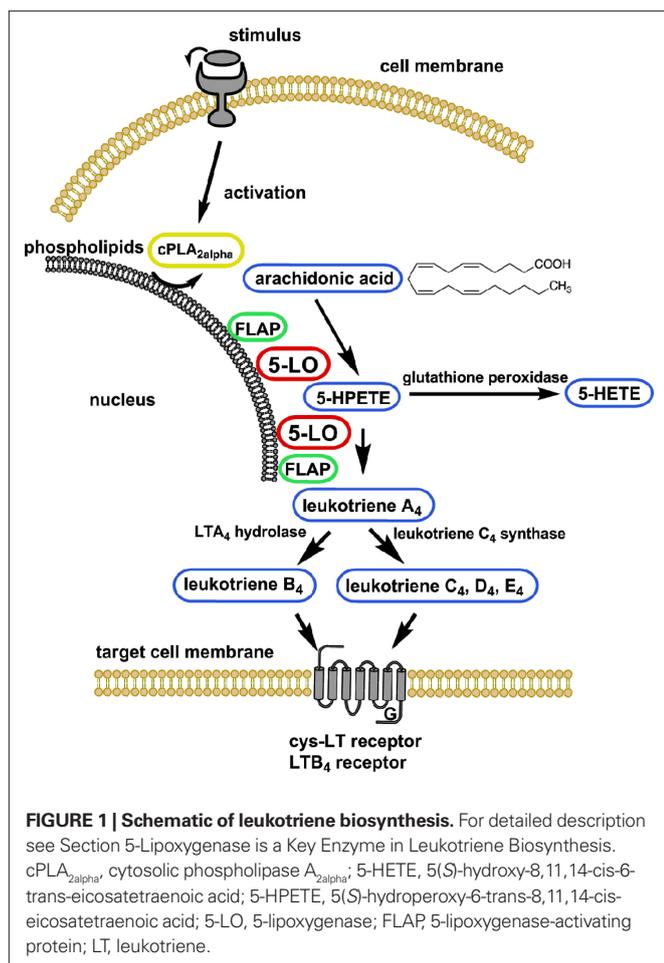
5-LIPOXYGENASE IS A KEY ENZYME IN LEUKOTRIENE BIOSYNTHESIS

Leukotrienes constitute a group of bioactive lipids that are generated by the 5-LO pathway. 5-LO expression is typically restricted to certain types of leukocytes, such as granulocytes (neutrophils,

eosinophils, and basophils) and monocytes/macrophages, which constitute the major source of LTs due to high 5-LO expression and enzymatic activity. 5-LO expression can also be detected in dendritic cells, mast cells, and B-lymphocytes. LTs primarily mediate inflammatory and allergic reactions (Funk, 2001; Tong et al., 2002; Ohd et al., 2003; Titos et al., 2003; Yoshimura et al., 2003; Zhi et al., 2003; Hayashi et al., 2006) by enhancing chemotaxis of migrating neutrophils and triggering vascular permeability and edema formation in inflamed tissues. Furthermore, LTs are potent bronchoconstrictory agents, crucially involved in asthma pathogenesis, and enhance plaque formation in foam cells of human atherosclerotic tissue. An increasing body of evidence also suggests a potential role of 5-LO products in early stage pancreatic, prostate, and colorectal carcinogenesis (Qiao et al., 1995; Bortuzzo et al., 1996; Avis et al., 2001; Ding et al., 2003; Tong et al., 2005).

5-Lipoxygenase catalyzes the first two steps in LT formation. Biosynthesis begins with dioxygenation of arachidonic acid (AA) released by cytosolic phospholipase A₂α (cPLA₂α) from cellular phospholipids, which results in two chemically unstable intermediates, 5(S)-hydroperoxy-6-trans-8,11,14-cis-eicosatetraenoic acid (5-HPETE) and LTA₄ (Figure 1). A non-heme coordinated iron atom, located in the catalytic site of 5-LO, is involved in both chemical reactions. Depending on the cellular enzymes present, LTA₄ can be either converted to LTB₄ by LTA₄-hydrolase or conjugated with glutathione by LTC₄-synthase to generate LTC₄. Further processing of LTC₄ produces LTD₄ and LTE₄. 5-HPETE can be reduced by glutathione peroxidases to form the corresponding 5(S)-hydroxy-6-trans-8,11,14-cis-eicosatetraenoic acid (5-HETE; Radmark et al., 2007).

Abbreviations: AA, arachidonic acid; cPLA₂α, cytosolic phospholipase A₂α; FLAP, 5-lipoxygenase-activating protein; 5-HETE, 5(S)-hydroxy-8,11,14-cis-6-trans-eicosatetraenoic acid; 5-HPETE, 5(S)-hydroperoxy-6-trans-8,11,14-cis-eicosatetraenoic acid; 5-LO, 5-lipoxygenase; LT, leukotriene; NDGA, nordihydroguaiaretic acid; PPAR, peroxisome proliferator-activated receptors; RTK, receptor tyrosine kinase; VEGF, vascular endothelial growth factor.



Notably, 5-LO is of importance not only for the biosynthesis of LTs but also of related bioactive eicosanoids, such as 5-*oxo*-ETE formed by the enzymatic oxidation of 5-HETE (Powell et al., 1992). Furthermore, the lipoxins are formed by the cooperative action of 5- and 15-LO on AA (Serhan et al., 1984) and are involved in programmed resolution of acute inflammation (Levy et al., 2001).

In resting cells, 5-LO resides in either the nucleus or the cytosol, depending on the cell type. Upon activation, 5-LO translocates to the nuclear membrane, where the 5-LO activating protein (FLAP) is thought to facilitate the transfer of phospholipid-derived AA to 5-LO and to enhance the efficiency of conversion of 5-HPETE to LTA₄, thereby triggering 5-LO product formation (Abramovitz et al., 1993; Mancini et al., 1993). The LTs produced then exert their biological effects by binding to specific G-protein-coupled transmembrane receptors at the cell surface denoted BLT1/2 for LTB₄ and CysLT1/2 activated by the cysteinyl LTs (Funk, 2001).

Several pharmacological strategies exist to suppress 5-LO product formation. Non-redox and redox type inhibitors, including CJ-13,610, Rev-5901, and AA-861 compete with fatty acids for binding to the active site cleft(s). Iron-ligand inhibitors such as zileuton and BWA4C suppress enzyme activity through chelation of the central iron atom and/or by stabilizing the ferrous oxidated state. FLAP inhibitors such as MK-886 act indirectly by interfering with the availability of AA (Ford-Hutchinson et al., 1994).

STUDIES THAT PROVIDE EVIDENCE FOR A ROLE OF 5-LO IN TUMOR CELL PROLIFERATION

Increasing evidence in literature implicates 5-LO in the growth of several tumor types, including pancreatic, colorectal, prostate, and breast cancer. Numerous studies demonstrated overexpression of 5-LO in tissue samples of primary tumor cells as well as in established cancer cell lines (Chen et al., 2006). Addition of 5-LO products to cultured tumor cells led to increased cell proliferation and activation of anti-apoptotic signaling pathways (Ding et al., 2003; Tong et al., 2005). 5-LO antisense technology approaches impaired tumor cell growth by reducing 5-LO expression (Sveinbjornsson et al., 2008). Finally, pharmacological inhibition of 5-LO has been shown to potently suppress tumor cell growth by inducing cell cycle arrest and triggering cell death via the intrinsic apoptotic pathway (Ghosh and Myers, 1998; Ding et al., 1999). Based on these findings, anti-LT drugs were considered a promising and novel pharmacological strategy for cancer prevention and therapy.

TUMOR-ASSOCIATED OVEREXPRESSION OF 5-LO, LT RECEPTORS AND OTHER ENZYMES INVOLVED IN LT BIOSYNTHESIS

The first evidence of a potential role for 5-LO in cancer growth was based on expression studies reported by Hong et al. (1999), who reported that 5-LO and FLAP were universally expressed in numerous epithelial cancer cell lines. These findings were in agreement with observations by Hennig et al. (2002), who demonstrated increased expression of 5-LO in a set of human pancreatic cancer cells and a decreased expression in normal pancreatic ductal cells. Subsequently, Gupta et al. (2001) demonstrated 5-LO overexpression in samples taken from prostate carcinoma patients, where the mean level of 5-LO mRNA was sixfold higher in malignant tissues compared to healthy tissues. Overexpression was further documented in malignant pleural mesothelial cells (Romano et al., 2001), in bladder carcinomas (Yoshimura et al., 2003), esophageal tumors (Zhi et al., 2003), and for breast cancer (Jiang et al., 2003).

In support of this hypothesis, an elevated expression of the LTB₄ receptor was detected in human pancreatic cancer tissue (Hennig et al., 2002). Assessing colorectal cancer samples from 84 patients, Ohd et al. (2003) were able to show a correlation between expression of CysLT receptors, 5-LO, an increased viability of the tumor cells and declined prognosis for patient survival. Notably, overexpression not only applies to 5-LO but also to other 5-LO binding enzymes involved in LT biosynthesis. Hong et al. (1999) found universal overexpression of FLAP in a series of epithelial cancer cell lines. Overexpression of FLAP associated with higher tumor aggressiveness and a poor prognosis for survival was demonstrated by analyzing breast cancer tissue samples of patients (Jiang et al., 2006). LTA₄ hydrolase overexpression and activity was found to be an early event in esophageal and oral adenocarcinogenesis (Chen et al., 2004; Sun et al., 2006), whereas a crucial role of LTC₄ synthase and cPLA_{2alpha} is evident particularly in pathogenesis of leukemia (Stenke et al., 1998; Sjolinder et al., 2000; Runarsson et al., 2007). Recently, simultaneous overexpression of various enzymes and receptors involved in LT biosynthesis and action, including, 5-LO, FLAP, LTC₄ synthase, LTA₄ hydrolase, BLT, and CysLT receptors, was detected in the majority of human primary neuroblastoma tumors as well as in respective cell lines. Whereas 5-LO is well-recognized as pro-carcinogenic, the related enzyme 15-LO-2 is down-regulated

in malignant tissues, considered to function as a tumor suppressor and to inhibit carcinogenesis (Shappell et al., 1999; Hsi et al., 2002). The role of 15-LO-1 is still discussed controversially in the literature (Pidgeon et al., 2007). Depending on the tumor cell type, overexpression of one enzyme of the LT synthesizing machinery in tumor cells does not inevitably account for an overexpression of the other enzymes. Thus, granulocytes from acute myeloid leukemia patients showed suppressed LTB₄ formation accompanied by elevated LTC₄ hydrolase expression and LTC₄ synthesis compared to leukocytes from healthy patients (Stenke et al., 1998).

CYTOTOXIC EFFECTS BY 5-LO INHIBITORS

Numerous studies have demonstrated cytotoxic and anti-proliferative effects of 5-LO inhibitors in cultured tumor cells as an important basis for the involvement of 5-LO in tumorigenesis. Tsukada et al. were amongst the first to describe the potent anti-proliferative effects of the 5-LO inhibitor AA-861 in a human leukemic cell line. This was accompanied by a sharp reduction in cellular DNA, RNA, and protein synthesis. It was concluded from this result that LTs potentially play an essential role in cancer cell viability (Tsukada et al., 1986). Similar growth-inhibitory effects were observed with the lipoxygenase inhibitor nordihydroguaiaretic acid (NDGA) in stomach cancer cells (Shimakura and Boland, 1992).

These data agree well with studies reporting that the 5-LO inhibitor AA-861 was capable of abolishing the AA stimulated increase of prostate cancer cell growth (Ghosh and Myers, 1997) and that inhibition of 5-LO by MK-886 (FLAP inhibitor) triggers severe apoptosis in human prostate cancer cells (Ghosh and Myers, 1998). Subsequent studies described that AA-861 is capable of suppressing the growth of esophageal cancer cells *in vitro* (Hoque et al., 2005) and the proliferation of MCF-7 breast cancer cells (Hammamieh et al., 2007). AA-861 also has been shown to have similar effects in colorectal cancer cells (Ihara et al., 2007). Strong cytotoxic effects in various cancer cell lines were also observed with the 5-LO inhibitor and LTD₄ receptor antagonist Rev-5901 (Ding et al., 1999; Tong et al., 2002; Titos et al., 2003; Hayashi et al., 2006; Melstrom et al., 2008; Sveinbjornsson et al., 2008). Recent findings with the LTB₄ receptor antagonist, LY293111,

which demonstrated potent anti-pancreatic cancer effects by inducing tumor cell apoptosis (Ding et al., 2005) and triggering S-phase cell cycle arrest (Tong et al., 2007), further supported the hypothesis that 5-LO and its downstream products, play crucial roles in tumorigenesis. Finally, a chemopreventive activity of Rev-5901 against colorectal adenocarcinoma xenografts was recently demonstrated in an animal tumor model (Melstrom et al., 2008). Taken together, the diverse set of 5-LO inhibitors was shown to suppress the growth of several types of tumor cells by cytotoxic mechanisms, primarily through activation of the intrinsic pathway of apoptosis. However, in addition to the pro-apoptotic activity of the drugs, it should be noted that additional anti-proliferative effects were observed, such as a decrease in DNA synthesis and induction of cell cycle arrest, which could harbor great significance. A summary of the described drug effects can be found in **Table 1**.

MITOGENIC EFFECTS OF 5-LO PRODUCTS

Several AA metabolites synthesized via the 5-LO pathways have been shown to promote tumor cell viability and to exert protective effects toward the 5-LO inhibitor induced cytotoxicity. The precise molecular mechanisms through which these molecules act on cancer cells remain incompletely understood. Direct proliferative and anti-apoptotic stimuli as well as an enhanced tumor angiogenesis may contribute. 5-HETE and LTB₄ increased cell proliferation and viability of pancreatic cancer cells by activating the mitogenic and anti-apoptotic MAPK and Akt kinase signaling pathways (Ding et al., 2003; Tong et al., 2005). A susceptibility toward 5-HETE also was described for several other tumor cell types, including breast cancer (Avis et al., 2001), and cancer of the lung (Avis et al., 1996). The effects of 5-HETE may, at least partially, derive from 5-*oxo*-ETE, which is formed by the cellular oxidation of 5-HETE (Powell et al., 1992). 5-*oxo*-ETE acts by binding to the G-protein-coupled OXE surface receptors (Grant et al., 2009). Some tumor cell types, including colorectal cancer cells, displayed specific mitogenic effects in response to LTB₄, but not to other 5-LO products (Qiao et al., 1995; Bortuzzo et al., 1996). A few additional studies report proliferative and anti-apoptotic effects for CysLTs. Accordingly, LTD₄

Table 1 | Cytotoxic and anti-proliferative effects by 5-LO inhibitors in cell culture assays.

Inhibitor	Concentration (μM)	Cell type	Effects	Literature
AA-861	20	Human leukemia cell lines	Potent anti-proliferative effects	Tsukada et al. (1986)
AA-861	60	Human prostate cancer cells	Inhibition of arachidonic acid stimulated cell growth	Ghosh et al. (1997)
MK-886	10	Human prostate cancer cells	Triggered cell death via activation of the apoptotic pathway	Ghosh et al. (1998)
Rev-5901	15	Human pancreatic cancer cell lines	Inhibition of cell proliferation, reversal by 5-HETE and 12-HETE	Ding et al. (1999)
LY293111	1	pancreatic cancer cells	Inhibition of pancreatic cancer growth, induction of tumor cell apoptosis	Ding et al. (2005)
AA-861	60	Esophageal cancer cells	Suppression of cell growth by induction of apoptosis	Hoque et al. (2005)
AA-861	30	Human bladder cancer cell lines	Strong growth suppression	Hayashi et al. (2006)
MK-591, MK-886	20	MCF-7 breast cancer cell line	Inhibition of cell proliferation	Hammamieh et al. (2007)

increased the proliferation and survival of intestinal epithelial cells (Paruchuri et al., 2005) and potentially enhanced tumor growth by up-regulating the transcriptional activity of the oncogenic protein beta-catenin (Mezhybovska et al., 2006). Romano et al. (2001) were able to show that 5(S)-HETE and LTA₄ but not LTB₄, potently up-regulated vascular endothelial growth factor (VEGF) transcription and expression in a human malignant mesothelioma model, which may contribute to the reported pro-angiogenic and anti-apoptotic effects of 5-LO products. Recent findings uncovered a novel function of LTB₄ in driving oncogenic ras-induced metastasis by acting on BLT-2 receptors (Kim et al., 2009). Furthermore, LTB₄-induced breast cancer cell survival was linked to BLT-2 mediated generation of reactive oxygen species. Collectively, the body of research supports a model in which 5-LO products modulate proliferative and anti-apoptotic events through multiple signaling pathways. **Table 2** provides a summary of the described mitogenic effects.

5-LO KNOCK-DOWN STUDIES IN CULTURED CELLS AND IN 5-LO KNOCK-OUT ANIMALS

Several studies found reduced cell proliferation rates due to down-regulation of 5-LO expression by antisense approaches. Using 5-LO antisense oligonucleotides, Romano et al. (2001) were amongst the first to provide direct evidence for 5-LO participation in the growth of malignant pleural mesothelial cells. Moreover, reduced expression of the 5-*oxo*-ETE receptor by siRNA approaches significantly impaired the viability of prostate cancer cells, suggesting a tumorigenic function of the 5-LO product 5-*oxo*-ETE (Sundaram and Ghosh, 2006). Finally, silencing of LT receptors was capable of suppressing growth of colorectal cancer cells (Ihara et al., 2007) and neuroblastoma cells (Sveinbjornsson et al., 2008). Comparatively few studies have investigated the role of 5-LO in mouse models. Chen et al. (2009) demonstrated that 5-LO-deficient knock-out (KO) mice show significantly impaired induction of chronic myeloid leukemia through specific suppression of leukemic stem cell proliferation. A summary of experimental findings accounting for a role of 5-LO in tumorigenesis can be found in **Figure 2**.

STUDIES THAT QUESTION THE ROLE OF 5-LO IN TUMORIGENESIS

CYTOTOXIC OFF-TARGET EFFECTS OF 5-LO INHIBITORS

The increasing number of publications that report a crucial role of 5-LO, and its products in tumorigenesis have been accompanied by additional studies that question the correlation between 5-LO and cancer. There is little disagreement that 5-LO inhibitors exert strong

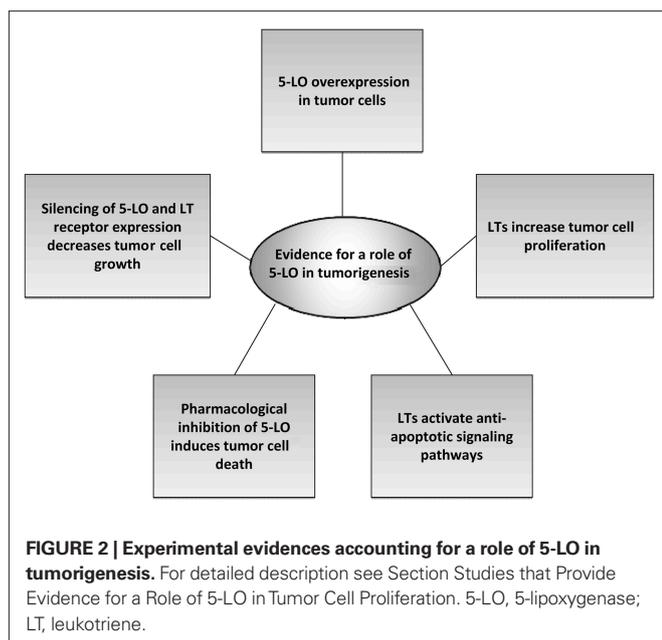
cytotoxic activities against 5-LO overexpressing tumor types and cultured tumor cells, which represents a significant basis for concluding that 5-LO products directly stimulate tumor cell proliferation. However, we recently demonstrated that the common 5-LO inhibitors AA-861, Rev-5901, BWA4C, and CJ-13,610 can reduce the viability of pancreatic cancer cells, cervix carcinoma cells, and leukemic cells independently of suppression of 5-LO product formation (Fischer et al., 2010). The hypothesis of 5-LO-independent cytotoxicity and anti-proliferation was substantiated using several experimental approaches. First, the various 5-LO inhibitors were shown to possess highly different abilities to reduce cell viability, to induce cytotoxic effects and to suppress the proliferation of cultured 5-LO-positive Capan-2 pancreas carcinoma cells. While the commonly used inhibitors AA-861, MK-886, and Rev-5901 produced strong cytotoxicity, other, more selective and more potent 5-LO inhibitors, including CJ-13,610, BWA4C, failed in this respect. Notably, zileuton, the only commercialized 5-LO inhibitor, failed to induce an anti-proliferative or cytotoxic response in all types of tumor cells employed. Additionally, the IC₅₀ values of cytotoxicity for AA-861, MK-886, and Rev-5901 exceeded the respective IC₅₀ values for inhibition of 5-LO enzyme activity by more than 20-fold (Rev-5901) and upto 5,000-fold (AA-861). Lastly, well-established 5-LO-negative tumor cell lines exhibited a higher susceptibility toward the 5-LO inhibitors than their morphologically related 5-LO-p counterparts. These observations are in line with a report by Datta et al. in which MK-886 induced severe apoptosis independently of FLAP (Datta et al., 1999; Fischer et al., 2010). Sabirsh et al. (2005) recently described 5-LO-independent effects of various LT synthesis inhibitors on Ca²⁺ signaling in 5-LO-deficient HeLa carcinoma cells. Also the apoptotic effects of licoferone, a dual COX/5-LO inhibitor, were found to occur independently of the ability of the drug to affect the AA cascade (Tavolari et al., 2008).

Recently, the 5-LO inhibitor zileuton was shown to suppress prostaglandin E₂ biosynthesis in macrophages with an IC₅₀ value of 1.94 μM (Rossi et al., 2010), a value which is close to the IC₅₀ of the drug for suppression of LT production in cell-based assays (Carter et al., 1991). Suppression of the tumor-promoting mediator PGE₂ by zileuton was also observed in human whole blood at clinically achievable concentrations and in rats at standard doses. This raises concerns over the relationship between the drugs chemopreventive effects and suppression of 5-LO.

In contrast to this, some non-tumor cell types showed obvious susceptibility toward 5-LO products, such as freshly isolated murine neuronal stem cells, which produced considerable basal amounts of LTB₄ (~7 ng per 10⁶ cells) and whose growth was suppressed

Table 2 | Mitogenic effects of 5-LO products in cell culture assays.

5-LO product	Cell type	Effect	Literature
LTB ₄	Colorectal cancer cells	Increase of cell proliferation	Qiao et al. (1995), Bortuzzo et al. (1996)
5-HETE, LTA ₄	Human malignant pleural mesothelial cells	Angiogenic and anti-apoptotic effects, in combination with potent up-regulation of vascular endothelial growth factor	Romano et al. (2001)
5-HETE, LTB ₄	Human pancreatic cancer cells	Stimulation of cell viability and proliferation via MAPK pathway	Ding et al. (2003), Tong et al. (2005)
LTD ₄	Intestinal epithelial cells	Increase of cell survival and cell proliferation possibly mediated via activation of wnt-signaling	Paruchuri et al. (2005), Mezhybovska et al. (2006)



by AA-861 at concentrations less than 1 μM (Wada et al., 2006). Furthermore, zileuton, the only drug devoid of anti-proliferative and cytotoxic off-target effects (Fischer et al., 2010), was capable of inhibiting the proliferation of RAW 264.7 macrophages below 1 μM . The macrophages considerably synthesized LTB_4 and addition of physiologically relevant concentrations of LTB_4 reversed the growth-inhibitory effects of zileuton (Nieves and Moreno, 2006). In sum, in many cases, the cytotoxic and chemopreventive effects 5-LO inhibitors in cell culture assays and in animal tumor models may derive from molecular mechanisms other than suppression of LT biosynthesis and warrant reassessment.

MITOGENIC EFFECTS OF 5-LO PRODUCTS

Several studies reported accelerated proliferation of tumor cells in the presence of exogenously added 5-LO products and attenuated anti-proliferative effects by 5-LO inhibitors (see Mitogenic Effects of 5-LO Products, page 3, 2nd paragraph). The extreme concentrations frequently required to observe these effects, however, represent one caveat to these experiments; the final concentrations of 5-LO products used often exceeded those present in the medium of untreated cells by up to 10,000-fold (Ghosh and Myers, 1998; Hoque et al., 2005; Sveinbjornsson et al., 2008). Experiments using physiologically relevant concentrations will be one step forward to test the mitogenicity and the pleiotropic effects of 5-LO products. Experiments are also needed to exclude that high concentrations of these 5-LO products induce non-specific anti-apoptotic responses that may counteract diverse stimuli or drugs inducing the intrinsic pathway of apoptosis.

LACKING EVIDENCE FOR A CLINICAL EFFICACY OF 5-LO INHIBITORS IN CANCER THERAPY

Few clinical trials have assessed the efficacy of anti-LT drugs in clinical cancer therapy. A randomized double-blind phase II study with the LTB_4 antagonist LY293111 in patients suffering from advanced

adenocarcinoma of the pancreas did not reveal any therapeutic benefit (Saif et al., 2009). This is a surprising result as a significant body of literature has demonstrated a remarkable susceptibility of pancreatic cancer cells toward anti-LT drugs in cell culture studies (see Cytotoxic Effects by 5-LO Inhibitors) and therefore suggests a certain lack of correlation between the effects of 5-LO inhibitors in cell culture assays and in patients. Notably, the mean C_{max} plasma concentrations of the drug achieved in patients after a dosage of 600 mg BID were found to be 4.4 μM (Schwartz et al., 2005) and should lead to almost complete suppression of LTB_4 signal transduction (Marder et al., 1995). Consequently, pleiotropic effects of the drug including modulation of PPAR (peroxisome proliferator-activated receptor) signal transduction are currently discussed (Adrian et al., 2008).

CONCLUSION AND FUTURE DIRECTION

A considerable number of studies has provided evidence for a role of 5-LO in tumorigenesis. The well-recognized overexpression of 5-LO in various types of malignant cells, the reduction of tumor cell viability by 5-LO gene silencing approaches, as well as experiments involving 5-LO KO mice, together constitute a substantial rationale for this hypothesis. However, considering that the cytotoxic activity of 5-LO inhibitors is substance-specific and may, in many cases, not derive from inhibition of 5-LO activity, the traditional hypothesis that 5-LO products are the exclusive players in 5-LO-triggered tumorigenesis may warrant reconsideration. Experiments on the role of 5-LO product formation in proliferation of cultured tumor cells using high concentrations (>1 μM) of certain 5-LO inhibitors may be misleading and the use of these agents as pharmacological tools should be critically considered. Also, possible indirect tumorigenic effects of 5-LO products (e.g., promotion of angiogenesis) with relevance toward 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 adaptor protein Grb-2, involved in receptor tyrosine kinase (RTK)-dependent growth factor signaling, have been reported for 5-LO (Lepley and Fitzpatrick, 1994). Because of the crucial role of oncogenic RTK signaling in cancer progression, a disrupted growth factor signaling may contribute to the reduction in tumor cell viability by 5-LO gene silencing approaches. Thus, experiments that assess Grb-2-dependent growth factor signaling after 5-LO gene silencing may be instructive. Taken together, a broad body of evidence from the literature suggests a crucial, albeit poorly defined, role of 5-LO in tumorigenesis of several cancer types. Elucidation of the molecular mechanisms underlying these effects may include direct proliferative actions of 5-LO products on tumor cells as well as indirect and so far neglected effects of 5-LO and thereby draw novel connections between pathways that are currently regarded as unrelated.

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