Histol Histopathol (2005) 20: 969-975

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

New aspects on the role of lipoxygenases in cancer progression

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Summary. The Lipoxygenases (LOXs) are a class of enzymes that convert arachidonic, linoleic, and other polyunsaturated fatty acid into biologically active metabolites involved in the inflammatory and immune responses. Recent evidences indicate that LOXs and the signaling pathways that are involved in their activation are also important for carcinogenesis and tumor progression. LOXs should therefore receive as much attention from cancer researchers as it has already from immunologists. In this article, we will review some evidence that the LOXs pathways affect several aspects of lung, pancreatic and prostate cancer progression. Moreover, we discuss how this new perspective on the roles of LOXs and their metabolites can have important implications to cancer therapy.

Key words: Lipoxygenase, Lung, Pancreas, Prostate, Tumor progression

Introduction: lipoxygenases

Polyunsaturated fatty acids, such as arachidonic and linoleic acids, are metabolized by three classes of enzymes (Funk, 2001). These are (a) Cyclooxygenases (COXs), which initiate the synthesis of prostaglandin (PGs); (b) lipoxygenases (LOXs), which forms hydroxy-eicosatetraenoic acids (HETEs) and leukotrienes (LTs); and (c) cytochrome P450s, which catalyze the formation of epoxyeicosatrienoic acids (EETs) or the formation of HETEs. These enzymes generate products of differing biological activity by inserting oxygen at different positions in the substrate. In particular, mammalian LOXs have two principal functions (Brash, 1999). One is to modify membranes by peroxidation reactions; 12/15-LOX, in homo 15-LOX type 1, is typically connected with this function. The other is to produce signaling lipid mediators which exert effects via G protein-coupled plasma membrane-bound receptors; maybe the best example is 5-LOX and the LTs. Classes of major human LOXs and their metabolites are listed in Figure 1. The 5-LOX pathway leads to the formation of 5(S)-HETE and LTs. The 12- and 15-LOXs can form 12(S)- and 15(S)-HETE from arachidonic acid. Functionally distinct isoforms of 12-LOX have been cloned, including platelet, leukocyte, and epidermal 12-LOXs (Kuhn and Thiele, 1999). Human and rabbit 15-LOXs and the leukocyte 12-LOX have high homology and are classified as 12/15-LOXs because they can form both 12(S)-HETE and 15(S)-HETE from arachidonic acid via their hydroperoxy precursors and mainly hydroperoxyocatadecadienoic acids (13(S)-and 9(S)-HPODE) and hydroxyocatadecadienoic acids from linoleic acid (Yamamoto, 1992). The production of LOXs metabolites has been shown in several vascular tissues and cells, such as vascular smooth muscle cells (VSMC), endothelial cells, monocytes, and leukocytes.

The generation of LOX-derived metabolites is under a complex set of controls; maybe the best example is that of 5-LOX. Firstly, their synthesis from arachidonic acid is initiated by cytosolic phospholipase A2 (cPLA2), which is specific for phospholipids that contain arachidonic acid in the SN2 position (Clark et al., 1991). cPLA2 is calcium-dependent and is translocated to the nuclear membrane in several cells stimulated with IgE or calcium ionophore A23187 (Glover et al., 1995). This translocation is required for cellular activity and is mediated by a phospholipid-binding domain (Nalefski et al., 1994). Moreover, all LOXs require membrane translocation to exert activity. When leukocytes are activated to produce LTs, 5-LOX moves from the cytosol, or from a soluble locus inside the nucleus, to the perinuclear membrane (Peters-Golden and Brock, 2001). Another critical aspect of LOXs control is its capability to bind to proteins. The clustering of cPLA2, 5-LOX, and permanently membrane-bound FLAP (5LO activating protein, transfers arachidonate to 5LOX) is reasonable because membrane phospholipid is the source of arachidonic acid. Some LOXs are also regulated by post-translational modification. Indeed, 5-LOX is

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phosphorylated concomitantly with translocation to the nucleus and nuclear envelope (Lepley et al., 1996). Phosphorylation via MAPK-activated protein kinase-2 is critical in controlling targeting and activation of this enzyme (Werz et al., 2000). Therefore, the activation of LOXs is generally rapid and transient, a property assumed to be well adapted to their role in infection or injury, which need urgent and time-limited expression of proinflammatory molecules. Although LOXs pathways have been most intensely studied for their involvement in immunity and inflammation, a constitutive LOX activity has been shown to be involved in the pathogenesis of several diseases, including asthma (Drazen et al., 1994), ulcerative colitis (Cole et al., 1996), psoriasis (Iversen et al., 1997), atherosclerosis (Natarajan and Nadler, 2004), and cancer (Shureiqi and Lippman, 2001). This constitutive LOX activity was shown to influence the proliferative rate of the cells, their apoptosis resistance/sensitivity or their senescence response (Catalano et al., 2004a,b; Soberman and Christmas, 2003). In this review, we attempt to provide an overview into the differing roles of the known LOXs and their metabolic products in the context of cancer progression.

A role for LOXs in tumorigenesis

According to Hanahan and Weinberg, tumor development requires six essential alterations to normal cell physiology: self-sufficiency in growth signals; insensitivity to growth inhibition; evasion of apoptosis; immortalization; sustained angiogenesis; and tissue invasion and metastasis (Hanahan and Weinberg, 2000). The LOXs are constitutively expressed in some types of cancer cell (Boado et al., 1992; Hong et al., 1999; Avis et al., 2001; Romano et al., 2001; Hennig et al., 2004) and their activity has been associated with several aspects of tumorigenesis, including promoting cancer-cell proliferation and genotoxicity, preventing apoptosis, and increasing tumor angiogenic and metastatic potential (Fig. 2).

Stimulating cell proliferation

Several LOXs form different metabolites within arachidonic acid pathways that appear to enhance cancer cell growth. HETEs can activate certain isoforms of protein kinase C directly or indirectly by incorporating into membrane phospholipids, which then generate HETE-containing diacylglycerol species to activate protein kinase C (PKC) (Natarajan and Nadler, 2004). They can also activate several mitogen activated protein kinases (MAPKs) and thereby activate key transcription factors that stimulate DNA synthesis and proliferation of cancer cells (Ding et al., 2003). Reactive oxygen species (ROS) generated during LOX pathway activation (Roy et al., 1994) may mediate some growth effects. In several model systems, the 5-LOX cascade contributes to the generation of ROS that activate the transcription factors NF-κB (Lepley and Fitzpatrick, 1998) and p53 (Catalano et al., 2004a). The potential for 5-LOX to generate sufficient ROS to affect transcription factors activity provides a direct and feasible link between LOX-generated redox changes and transcription. In addition, some LOXs metabolites have also been shown to directly stimulate the transcription of target genes apparently via activation of the nuclear receptors, such as peroxisome-proliferator-activated receptors (PPARs) (Michalik et al., 2004). The best characterized at the moment are: 1) LTB4 and 8(S)-HETE, which preferentially activate PPARß-δ 2) 15-deoxy-prostaglandin J2 (15-dPGJ2) and 15-HETE, which are PPAR-γ-selective ligands. PPARγ is also activated by 9-HODE (hydroxyoctadecadienoic acid) and 13-HODE, either derived from linoleic acid or as components of oxidized low-density lipoprotein (oxLDL); 3) the prostaglandin I2 (PGI2, also called prostacyclin), which

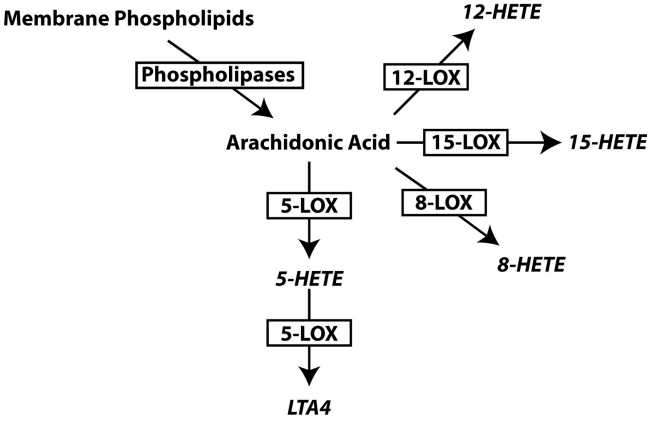

Fig. 1. Polyunsaturated fatty acid metabolic pathways of arachidonic acid through LOXs. Note that certain LOXs, including 12- and 15-LOX, also react with other fatty acid substrates, such as linoleic acid, to yield additional products.

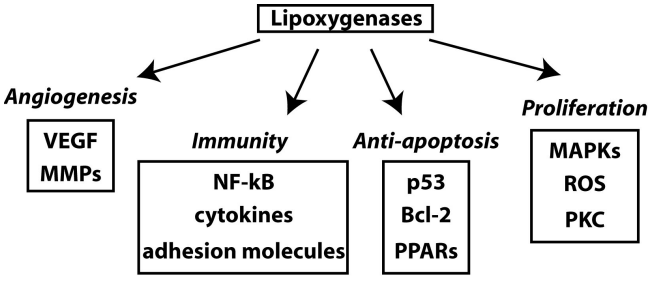

Fig. 2. LOXs contribute to the induction of four classes of molecules. The factors that are induced in the response to lipoxygenases activation can be divided into four functional classes: molecules that are involved in angiogenesis control; molecules that serve various immunoregulatory functions; molecules that control programmed cell death; and molecules that promote cell proliferation.

is probably a PPARß/δ natural ligand. There is strong evidence that PPAR·-dependent DNA induction drives the proliferation of hepatocytes during hepatocarcinogenesis (Rusyn et al., 2000; Yeldandi et al., 2000).

Genotoxicity

The 5-LOX product LTA4 has the potential to form adducts with DNA bases, suggesting that it may potentially serve as a modulator of transcription or as a mutagen (Reiber and Murphy, 2000). In the case of terminally differentiated myeloid cells, the adduction of LTA4 may function as a modulator of gene expression similar to methylation. Moreover, studies have shown that 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a tobacco carcinogen, is metabolized in several steps by enzymes of the cytochrome P450, COX or LOX families to yield products that bind to DNA and form methylated and pyridyloxobutylated DNA adducts (Rioux and Castonguay, 1999). Of these products, O6-methylguanine causes a GC-AT transitional mispairing. O^6-methylguanine formation in mice and rats has also been associated with an activating point mutation in the *K-ras* gene in NNK-induced pulmonary adenocarcinomas (Belinsky et al., 1989, 1990). Studies in mice have implicated O6-methylguanine as a crucial determinant of NNK-induced lung carcino-genesis, whereas experiments in rats provided evidence for an important role of DNA pyridyloxobutylation (Peterson and Hecht, 1991; Staretz et al., 1997). As well as controlling of the cell cycle, the production of ROS in response to LOX activation might damage DNA and promote mutation in target genes. However, the implication of LOX in this DNA damage effect remains to be proven. Nevertheless, recent evidence suggests that the activation of LOX is required and causal to the adverse effects of oxidative stress in primary cells (Catalano et al., 2004b). The underlying mechanisms probably include perturbation of the cell cycle and the production of ROS.

Inhibition of apoptosis

Several LOXs are also inhibitors of programmed cell death (Maccarrone et al., 2001; Shureiqi and Lippman, 2001). The LOXs products activate the transcription of several target genes, such as members of the bcl-2 family (Tang et al., 1996; Pidgeon et al., 2002), that are known to block the induction of apoptosis. 5-LOX metabolism of arachidonic acid can also attenuate the apoptotic response to genotoxic anticancer drugs and ionizing radiation (Catalano et al., 2004a). Tumor cells in which 5-LOX is constitutively active are highly resistant to anticancer drugs or ionizing radiation, and inhibition of 5-LOX activity in these cells greatly increases their sensitivity to such treatments (Catalano et al., 2004a). In addition to conferring resistance to cancer therapies, the anti-apoptotic activity of some LOXs can also have an important role in the emergence of neoplasms, by preventing the death of cells that have undergone chromosomal rearrangements or other types of DNA damage. Such cells are normally eliminated by means of checkpoint controls, such as the p53 pathway (Appella and Anderson, 2001). In fact, there is evidence for transcriptional antagonism between 5-LOX-derived metabolites and p53 (Catalano et al., 2004a). Regardless of mechanism, prevention of apoptosis increases the pool of genetically altered cells, which will eventually give rise to transformed progeny.

Increased metastasis and angiogenesis

12-LOX and 12(S)-HETE can modulate several parameters related to the metastatic potential of tumor cells, such as motility (Timar et al., 1993), secretion of lysosomal proteinases cathepsin B and L (Ulbricht et al., 1996), expression of integrin receptor αIIb,3 (Timar et al., 1995), tumor cell adhesion to endothelium and spreading on subendothelial matrix (Honn et al., 1989), and lung colonizing ability *in vivo* (Liu et al., 1994).

Another important component of tumor growth is angiogenesis, a process that requires both migratory and invasive capabilities of vascular endothelial cells. Recently, 5-LOX activation was found to stimulate angiogenesis, possibly by inducing expression of vascular endothelial growth factor (VEGF) and matrix metalloproteinase-2 (MMP-2), proteolytic enzymes that promote tumor invasion of surrounding tissue (Romano et al., 2001; Ye et al., 2004).

LOXs and carcinomas

It is known that several LOX metabolites have mitogenic activity and promote the proliferation of normal human cells of different types. For example, the 5-LOX products LTB4, LTC4 and LTD4 stimulate DNA synthesis in cultured human epidermal keratinocytes (Luo et al., 2003). Therefore, it is not all that surprising that some LOXs and their metabolites are implicated in the growth control of specific cancer cell-types. Notably, LOXs have been shown to be involved in the development of a number of carcinomas, cancers of epithelial origin. Numerous studies have documented elevated or constitutive LOXs activity in colon, lung, pleural, breast, prostate, pancreas, bone, and brain cancer cells (Boado et al., 1992; Hong et al., 1999; Avis et al., 2001; Romano et al., 2001; Hennig et al., 2004). Human cancer tissues also display an enhanced expression of 5-LOX and 12-LOX. At least for human prostate, lung and pancreatic cancer, very low or undetectable 5-LOX as well as 12-LOX expression is observed in normal cells or tissues, whereas high 5-LOX as well as 12-LOX expression and activity are detected in the corresponding transformed tissues (Nie et al., 2001; Schuller, 2002; Hennig et al., 2004). However, although the role of LOXs in promoting prostate, lung, and pancreatic carcinogenesis is strongly supported by experimental data, LOXs may not promote carcinogenesis in all

epithelial-derived tumors.

LOXs in lung and pancreatic adenocarcinomas

Adenocarcinoma of the lungs and pancreas are among the most common and most deadly smoking-associated cancers. The enzymes COX-2 as well as 5-, 12- and 15-LOX have been shown to be overexpressed by pulmonary and pancreatic adenocarcinoma cells (Nie et al., 2001; Schuller, 2002; Hennig et al., 2004). Cigarette smoke contains various toxic chemicals, such as nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit COX enzymes, and LOX inhibitors have been shown to significantly reduce the formation of NNK-induced lung adenomas in mice (Rioux and Castonguay, 1997, 1999). Therefore, both COXs- and LOXs-derived metabolites seem to contribute to NNK-induced lung carcinogenesis. In support of this theory, high systemic levels of the PGE2 and LTB4 have been identified in the lungs of NNK-treated mice (Castonguay et al., 1998). One of the most well-known features of NNK is the ability of its metabolites to bind to DNA and induce activating point mutations in the *ras* gene. However, NNK is also a ß-adrenergic-receptor agonist that stimulates arachidonic acid release from cell-membrane phospholipids and DNA synthesis, resulting in proliferation of human pulmonary adenocarcinoma cells (Schuller et al., 1999). Treatment of adenocarcinoma cells with the ß-adrenergic-receptor antagonists, with the COX inhibitor aspirin, or with the 5-LOX inhibitor MK886, reduces the mitogenic response to NNK (Schuller et al., 1999).

NNK seems to have the same effects on pancreatic cancer cells that it has on pulmonary cancers. Recent investigations in cell lines that are derived from human pancreatic adenocarcinomas showed that NNK, and also the ß-adrenergic-receptor agonist isoproterenol, stimulate the release of arachidonic acid, leading to DNA synthesis and cell proliferation (Weddle et al., 2001).This response to NNK was inhibited by the general ß-adrenergic antagonist propranolol or the $ß_2$-adrenergic antagonist ICI118,551. Aspirin and the 5-LOX inhibitor MK886 also significantly reduced NNK-induced DNA synthesis and cell proliferation (Weddle et al., 2001). Collectively, these findings indicate that the growth of pancreatic, like pulmonary, adenocarcinomas is controlled by NNK activation of ß2-adrenergic receptor signaling, the release of arachidonic acid and the formation of mitogenic metabolites derived from both COXs and LOXs pathways. In support of this hypothesis, aspirin and the 5-LOX inhibitor MK886 significantly reduced the incidence of pancreatic adenocarcinomas induced in hamsters by prenatal exposure to NNK and ethanol (Schuller et al., 2002).

LOXs and prostate cancer

Prostate cancer is another epithelial-derived tumor extremely common with more than 220,000 new cases diagnosed annually in the United States alone (Jemal et al., 2004). 5-LOX overexpression recently has been documented in human prostate cancer tissue (Gupta et al., 2001), and 5-S-HETE formation and inhibition respectively promote and inhibit the growth of prostate cancer cells (Ghosh and Myers, 1997). 5-S-HETE but not other HETE products (LTB4, 12-, or 15-HETE) can also inhibit apoptosis induction by MK-886 in prostate cancer cell lines (Ghosh and Myers, 1998). Similarly, Gao et al. (1995) investigated the expression pattern of 12-LOX in prostate cancer and found an elevation of 12-LOX mRNA expression in advanced stage, high-grade prostate cancer. The sequence of RT-PCR 12-LOX products was found identical to platelet-type 12-LOX (Hagmann et al., 1995). The relation of 12-S-LOX to tumor metastatic potential of prostate cancer is additionally supported by the finding that 12-S-LOX expression levels were higher in metastatic prostate cancer cells (DU-145) than in nonmetastatic prostate cancer cells (PC-3) that were transplanted into severe combined immunodeficient mice (Timar et al., 2000). Besides being higher, the 12-S-LOX expression was also more localized in the cytoskeleton in DU-145 (metastatic) cells than in PC-3 (nonmetastatic) cells, and 12-S-LOX inhibition markedly reduced the metastatic potential of the DU-145 cells (Timar et al., 2000). Baicalein, which inhibits LOXs activity, impairs the proliferation of androgen-independent PC-3 and DU-145 prostate cancer cell lines, and induces cell-cycle arrest at G0-G1 and apoptosis at concentrations achieved in humans. Administration of the 12-S-HETE rescued baicalein-treated cells, indicating that the ability of baicalein to inhibit 12-LOX was responsible for its antineoplastic activities (Pidgeon et al., 2002). Similar effects on cell cycle and cell proliferation were shown in androgen-sensitive LNCaP cells with an additional finding that, at clinically achievable concentrations, baicalein markedly suppressed the expression of the androgen receptor (Chen et al., 2001). Preclinical data for the efficacy of the LOX inhibitor baicalein in prostate cancer models is limited to a single report in which DU-145 prostate cancer cells were pretreated with baicalein before the injection of tumor cells into tail veins of SCID mice. The number of lung metastases was decreased of about 20% by baicalein pretreatment (Timar et al., 2000).

Implications and future directions

A causal connection between inflammation and cancer has been suspected for many years. However, the mechanistic link between inflammation and tumorigenesis is not well understood. Because some LOXs become activated in response to inflammatory stimuli and their constitutive activation has been associated with several epithelial-derived malignancies, LOXs might be the missing link between these two processes. By virtue of the antiapoptotic activity, the

persistent activation of 12- or 5-LOX that occurs during chronic inflammation or infection might prevent the elimination of genetically altered, precancerous cells. In addition, by stimulating the transcription of VEGF and other growth factors, constitutively active 12- or 5-LOX might cause enhanced cell proliferation and tumor angiogenesis. Until recently, LOX cancer chemoprevention research focused exclusively on the tumor-promoting effects of LOXs and on inhibiting LOX, in general, and 5- and 12-LOX in particular (Rioux and Castonguay, 1999; Steele et al., 1999). Now we know that some LOXs (i.e. 15-LOX-1 and -2) can also have a tumor-suppressor function. Antagonistic interactions may occur between 5- and 15-LOX and between 12- and 15-LOX in various experimental models, which involved direct effects of the 15-LOX-1 and -2 products (13-S-HODE and 15-HETE, respectively) on 5- and 12-LOX (Vanderhoek et al., 1980; Takata et al., 1994). Therefore, a novel approach for cancer chemoprevention would involve LOX modulators (i.e., agents that can induce the anticarcinogenic and/or inhibit the procarcinogenic LOXs) thereby shifting the balance of LOX activities from procarcinogenic (by 12- and 5-LOX activity) to anticarcinogenic (by 15-LOXs) metabolism of polyunsaturated fatty acids (Shureiqi and Lippman, 2001).

To further investigate the role of LOXs in cancer development, we also need to analyze a large collection of genetically altered mouse strains that carry deletions or other genetic alterations in genes that encode for LOXs. In general, single LOX gene knockout experiments in mice indicate no obvious problems in development or life expectance (Chen et al., 1994). However, the susceptibility of such mouse strains to a variety of cancers and cancer treatments needs to be examined. The identification of LOXs target genes in different types of normal cell and their transformed derivatives is another important area for future research. As we begin to understand which genes are activated by LOXs under different conditions, and which transcription factors and signaling pathways are involved in LOXs activation, we should be able to design new therapeutic strategies that will allow the blocking of certain LOXs target genes and not others.

While more studies are needed to resolve many pitfalls and unanswered issues on the role of LOXs in cancer progression, several lines of evidence suggest that the up-regulation of specific LOX in different type of adenocarcinomas is related to the increased tumor growth and progression. Therefore, inhibition of these enzymes may represent a promising approach to halt or reverse the progression of epithelial-derived malignancies.

Acknowledgements. Research in the authors' laboratory is supported by grants from AIRC, FIRC, MIUR and Ministero della Sanità. A. Catalano is a regular member in the American Society for Biochemistry and Molecular Biology, and is supported by a fellowship from FIRC.

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Accepted March 14, 2005