PATHOLOGY ONCOLOGY RESEARCH
Vol 3, No 2, 1997

ARÁNYI LAJOS
Foundation

REVIEW

12-Lipoxygenase in Human Tumor Cells

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Tumor cell proliferation and metastasis proceed via a network of interdependent molecular events with a vast array of molecular players and signal transduction mechanisms differing in various types of human tumors. In the sequence of events necessary for carcinogenesis, arachidonate metabolites have been documented to play a significant role at several steps. Arachidonate metabolism in human cells occurs via several enzymatic pathways, including enzymes such as cyclo-oxygenases and lipoxygenases. This review pays particular attention to one member of the lipoxygenase family of enzymes, namely 12-lipoxygenase, since an arachidonate metabolite generated via 12-lipoxygenase action, 12(S)-HETE, has been shown to elicit various prometastatic effects of tumor cells in vivo and in vitro. We focus especially on mechanisms of activation and modulation of 12-lipoxygenase expression in human tumor cells, since various tumor cells express 12-lipoxygenase or are responsive to metabolites derived from 12-lipoxygenase action, thus offering a potential for successful therapeutic intervention against such tumors (Pathology Oncology Research Vol 3, No 2, 83–88, 1997)

Keywords: epidermal growth factor, 12-lipoxygenase, metastasis, nuclear translocation, tumor cells

Introduction

Human 12-lipoxygenase in platelets was the first lipoxygenase discovered in the animal kingdom.[1] The lipoxygenases constitute a group of non-heme iron-containing dioxygenases present in plants and animals which stereospecifically insert molecular oxygen into *cis, cis*-1,4-pentadiene-containing polyunsaturated fatty acids. So far, several types of lipoxygenases have been cloned, namely 5-, 12-, and 15-lipoxygenase, which stereospecifically integrate oxygen at carbon atom 5, 12, or 15 of the substrate fatty acid, respectively,[2-7] thus generating *S* configuration fatty acid hydroperoxides. More recently, an 8*R*-lipoxygenase was cloned from a coral source.[8] Another 8-lipoxygenase has been reported,[9] but the cDNA coding for such an 8-lipoxygenase protein has not been cloned yet. This review summarizes the current knowledge on 12-lipoxygenase in human cells and tissues, particularly in human tumor cells, in view of the suggested role of 12-lipoxygenase in the sequence of events during human tumor cell metastasis.[10]

Received: April 22, 1997 *accepted*: May 18, 1997

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Abbreviations: AA, arachidonate; DAG, diacylglycerol; EGF, epidermal growth factor; EGFR, EGF receptor; Gp, G-protein; IP_3, inositoltriphosphate; HEL, human erythroleukemia; 12(S)-HETE, 12(S)-hydroxyeicosatetraenoate; 13(S)-HODE, 13(S)-hydroxyoctadecadienoate; 12(S)-HPETE, 12(S)-hydroperoxyeicosatetraenoate; 12-LOX, 12-lipoxygenase; PKC, protein kinase C; PLC, phospholipase C; PI, phosphatidylinositol; PTK, protein tyrosine kinase; RT-PCR, reverse transcription polymerase chain reaction; TPA, tetradecanoyl phorbolacetate

Gene Structure of 12-Lipoxygenase

Mammalian lipoxygenase genes including those of 5-, 12-, and 15-lipoxygenase are organized at the genomic level in a 14 exon format with exon/intron boundaries in exactly corresponding positions.[11-16] In contrast to the introns of 12-lipoxygenase genes, the sizes of the exons are highly conserved among different species. Among the known 12-lipoxygenase enzymes, several cell type-specific isoforms have been characterized from different species, and have been termed "leukocyte-type", "platelet-type", and "epidermal" 12-lipoxygenase. Accordingly, porcine leukocyte 12-lipoxygenase and epidermal 12-

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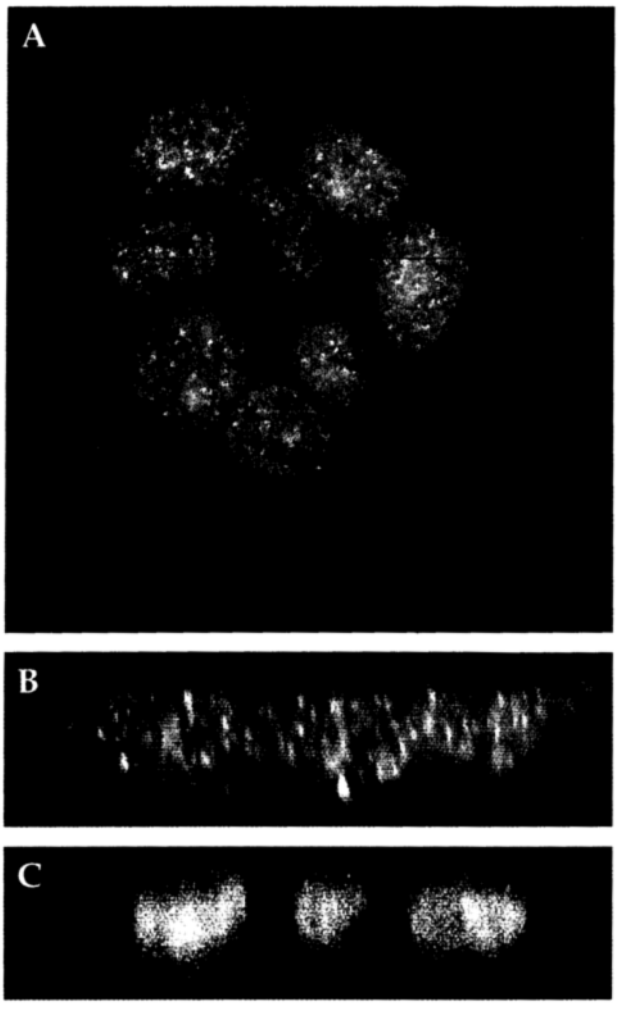

Figure 1. Intracellular including nuclear localization of 12-LOX in A431 cells. A: Confocal laserscan microscopy (LSM) picture of unstimulated A431 cells showing immunofluorescent staining specific for 12-LOX (red), nuclear staining with DAPI (green), and colocalization of both stains in yellow. B,C: LSM-generated optical sections perpendicular to the substratum (position of section at horizonal line indicated in panel A) showing immunofluorescent staining for 12-LOX (panel B) and nuclear staining with DAPI (panel C).

lipoxygenase detected in murine tissue are genetically closer to human and rabbit reticulocyte 15-lipoxygenase than to the human platelet-type 12-lipoxygenase.[15,16] In human cells, the expression of a leukocyte-type 12-lipoxygenase was demonstrated in adrenal glomerulosa cells,[17] the platelet-type 12-lipoxygenase in platelets,[1] epithelial cells,[18] and in several tumor cells.[19-21] The concomitant expression of both platelet-type and leukocyte-type 12-lipoxygenase has not been observed in human cells. The gene of the human platelet 12-lipoxygenase comprises 15-17 kb,[12,13] is localized on subband p13.1 of chromosome 17,[13] and encodes a protein of 663 amino acids with a molecular mass of approximatively 75 kDa. In addition, a putative pseudogene for platelet-type 12-lipoxygenase has been cloned from human genomic libraries.[12] The putative promoter sequence in the 5' flanking region of the human 12-lipoxygenase gene[12] contains multiple GC boxes, a "TATA-like" box, but no CCAAT boxes,[12] suggesting that the 12-lipoxygenase is a product of a housekeeping gene; however, the expression of 12-lipoxygenase on both mRNA and protein level can vary in a bidirectional manner (see below). Various consensus sequences for transcriptional regulatory factors were found in the 12-lipoxygenase promoter including GC boxes as potential Sp1-binding sites, AP-2 and NFkB sites, and a core sequence of a glucocorticoid-responsive element.[12,13] Primer extension analyses determined the transcriptional start site in the human 12-lipoxygenase gene at bp -306[13] or at bp -62 and bp -88, respectively.[12]

Intracellular Distribution of 12-Lipoxygenase Protein and of 12-Lipoxygenase Activity

In human tumor cells and tissues studied so far, expression of the mRNA for 12-lipoxygenase has been demonstrated by RT-PCR and Northern blot analysis for erythroleukemia (HEL)[19] and megakaryoblastic leukemia (DAMI) cells,[22] epidermoid carcinoma (A431) cells,[23] and colon carcinoma (Clone A) cells.[24] However, the presence of 12-lipoxygenase protein and of 12-lipoxygenase activity in Clone A cells could not be demonstrated yet with certainty which points to a possible post-transcriptional regulation of 12-lipoxygenase, at least in these cells. Where present in human tumor cells, the intracellular localization of 12-lipoxygenase protein in an unstimulated cell is predominantly cytosolic, as in platelets.[21,25] This localization has been demonstrated by quantitation of Western blots from subcellular fractions and by immunofluorescence in intact tumor cells.[21,25] Interestingly, the intracellular distribution of 12-lipoxygenase protein in tumor cells does not necessarily parallel the subcellular localization of 12-lipoxygenase activity. In murine 3LL tumor cells, both the 12-lipoxygenase protein[26] and its activity[26,27] are predominantly cytosolic. In human A431 and HEL cells[21,25] and in murine W256 sarcoma and B16a melanoma cells,[24] however, the 12-lipoxygenase protein is localized mainly in the cytosol, but is enzymatically far less active in this subcellular compartment as compared to the corresponding membrane-associated fraction of 12-lipoxygenase protein.

More recently, an association of the 12-lipoxygenase with nuclear membranes[21] and also a distinct intranuclear localization of 12-lipoxygenase was observed in A431 cells *(Fig.1)*. While most of the immunologically detectable 12-lipoxygenase resides in the cytosol, staining of nuclei with DAPI concomitant with immunologic

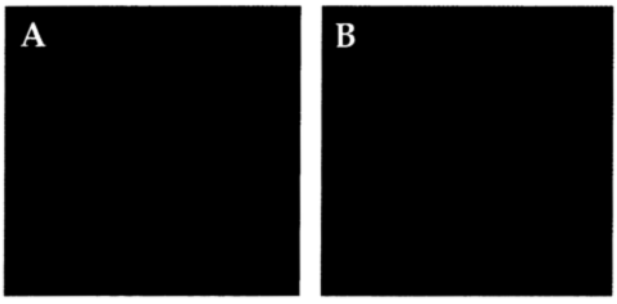

Figure 2. EGF-induced up-regulation and translocation of 12-LOX. A431 cells were starved for 24 h in medium containing 0.5% (v/v) FBS (panel A) before treatment with EGF (50 ng/ml) for 18 h (panel B). Panels depict immunofluorescence detection of 12-LOX using 12-LOX-specific primary and immunofluorescent (Cy3-labeled) secondary antibody. Modified from ref. 21.

detection of 12-lipoxygenase clearly indicates the additional intranuclear presence of the enzyme protein in unstimulated A431 cells *(Fig.1)*. Further, confocal laser scan microscopy-generated optical sections through A431 cells perpendicular to the substratum demonstrate that the observed intranuclear 12-lipoxygenase is not due to cytosolic contamination on the nuclear membrane surface *(Fig.1)*. This intranuclear fraction of 12-lipoxygenase is enzymatically active. The activity of intranuclear 12-lipoxygenase was determined[21] in isolated purified nuclei,[28] and amounts to approximately 14% of total cellular 12-lipoxygenase activity in these cells. This novel aspect of subcellular localization of 12-lipoxygenase suggests a possible role of mediators generated by intranuclear 12-lipoxygenase activity in the modulation and regulation of 12-lipoxygenase-dependent actions.

Modulation of Expression and Activity of 12-Lipoxygenase

The activity of 12-lipoxygenase in a given cell can be modulated by extra- and intracellular factors which influence its expression at the mRNA and protein level, by the subcellular localization of the 12-lipoxygenase protein, and by the redox status of the compartment in which it is contained. Interestingly, this expression of 12-lipoxygenase is modulated bidirectionally, i.e. it can be up- and down-regulated within a wide range.[21] Extracellular factors modulating 12-lipoxygenase include glucose, angiotensin II, tumor promoting phorbol ester TPA, epidermal growth factor (EGF), glucocorticoids, autocrine motility factor, and components of extracellular matrix and of serum. Whereas expression of 12-lipoxygenase is upregulated by glucose, angiotensin II,[29] EGF,[20,23] autocrine motility factor[30] and serum,[21] varying results have been reported for TPA,[19,25,31] and down-regulation of 12-lipoxygenase was observed under the influence of dexamethasone[32] and fibronectin.[22] Most interestingly, according to pharmacologic evidence 12-lipoxygenase expression is controlled also by exogenously added 12-HETE[10] or by other factors acting downstream of 12-lipoxygenase-dependent metabolism of arachidonate.[33] Under physiologic conditions, 12-HETE may be regarded as an essentially intracellular regulatory metabolite, since – except for platelets – endogenously generated 12-HETE is not secreted to a major extent from the intracellular compartment in most cell types studied. Intracellularly, receptor-mediated signal transduction elicited e.g. by EGF and leading towards 12-lipoxygenase activation[21] involves protein tyrosine kinase of the EGF receptor.[33] Downstream of 12-lipoxygenase, the major arachidonate metabolite generated following 12-lipoxygenase activity, namely 12(*S*)-HETE, is able to act as a second messenger in various pathways including angiotensin II-induced aldosterone secretion,[34] neurotransmitter peptide-induced hyperpolarization of neuronal cells[35], and epithelial DNA synthesis and epidermal proliferation.[36,37] Most, if not all, molecular mechanisms involving 12(*S*)-HETE actions in tumor cells and endothelial cells are probably exerted via intracellular activation of protein kinase C.[10] Furthermore, 12(*S*)-HETE apparently activates selective isoforms of PKC in different cell types.[38] It is not clear, however, how this activation of PKC is triggered by 12(*S*)-HETE, since direct activation in PKC activity assays could not be demonstrated.[10] Another link between 12-lipoxygenase and PKC appears to exist in the dependency of EGF-induced increase in 12-lipoxygenase expression on functional PKC activity.[39] The exact events in this pathway downstream of PKC activation are still unknown, but an involvement of protein tyrosine kinase-dependent formation of focal adhesions and tyrosine phosphorylation of focal adhesion kinase (pp125FAK) has recently been demonstrated.[40]

Similar to 5-lipoxygenase,[28,41,42] the intracellular 12-lipoxygenase can also be activated by translocation from one subcellular compartment to the other. Such an intracellular shift of 12-lipoxygenase protein and 12-lipoxygenase activity is elicited upon Ca^{2+}-mediated cell stimulation,[25,43] with tumor promoting phorbol ester TPA,[25] or with EGF.[21] In the latter case, EGF causes both an up-regulation of 12-lipoxygenase expression[20,23] and a translocation from cytosol to membranes including nuclear membrane[21] *(Fig.2)*. It remains to be demonstrated whether an EGF-induced intracellular translocation of 12-lipoxygenase proceeds would lead to increased intranuclear presence of 12-lipoxygenase.

As reported for other lipoxygenases,[44] the redox conditions surrounding the 12-lipoxygenase enzyme in a given subcellular compartment strongly affect its activity. In platelets[45] and tumor cells,[25] 12-lipoxygenase activity is

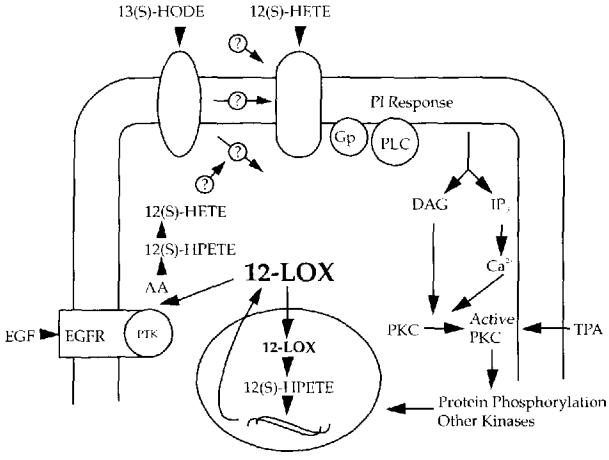

Figure 3. Activation and modulation of expression of 12-LOX. Details are given in the text. AA, arachidonate; DAG, diacylglycerol; EGF, epidermal growth factor; EGFR, EGF receptor; Gp, G-protein; IP_3, inositoltriphosphate; 12-LOX, 12-lipoxygenase; 12(S)-HETE, 12(S)-hydroxyeicosatetraenoate; 13(S)-HODE, 13(S)-hydroxyoctadecadienoate; 12(S)-HPETE, 12(S)-hydroperoxyeicosatetraenoate; PKC, protein kinase C; PLC, phospholipase C; PI, phosphatidylinositol; PTK, protein tyrosine kinase; TPA, tetradecanoyl phorbolacetate.

sensitive to reducing agents such as glutathione present in physiologic concentrations. On the other hand, in tumor cells where the predominant amount of 12-lipoxygenase resides in the cytosol in a largely inactive status its activity can be directly restored in the presence of hydroperoxides as shown for 13-HPODE[25] and H_2O_2.[39]

Regulation of 12-lipoxygenase expression at the mRNA level represents another physiologic means to control 12-lipoxygenase activity within cells. Such transcriptional control of 12-lipoxygenase expression has been demonstrated for the transcription factor NFkB.[46] In a heterodimer form as NFkB/Rel, it down-regulates 12-lipoxygenase expression. Also, glucocorticoids suppress 12-lipoxygenase expression, probably via glucocorticoid receptor activation[32] and interaction on the GRE site of the 12-lipoxygenase promoter.[12,13] Recently, a positive transcriptional regulatory function was found to exist as an EGF-responsive element in the 12-lipoxygenase promoter (WC Chang, personal communication).

Further, the expression of 12-lipoxygenase mRNA and subsequently of 12-lipoxygenase protein appears to depend on functional 12-lipoxygenase activity, which points to the possible role of 12-lipoxygenase-derived arachidonate metabolites such as 12(*S*)-HPETE and/or 12(*S*)-HETE as direct or indirect transcriptional activators of 12-lipoxygenase expression. Such a positive transcriptional regulatory function of 12(*S*)-HETE on 12-lipoxygenase expression has been reported recently for human tumor cells.[33]

12-LOX-derived 12(S)-HETE and tumor cell metastasis

A vast body of evidence has accumulated supporting the functional link of 12-lipoxygenase-derived 12(*S*)-HETE and the metastatic behaviour of tumor cells in experimental animals *in vivo* and *in vitro*.[24] In short, 12(*S*)-HETE at submicromolar concentrations was shown to increase tumor cell adhesion to vascular endothelium and to upregulate integrin expression,[47] elicit retraction of vascular endothelial cells,[48] stimulate tumor cell motility[49] and invasiveness[38] via activation of protein kinase C,[38] as well as enhanced release of cathepsin B.[50,51] On the other hand, some tumor cells including human tumor cells express the platelet-type isoform of 12-lipoxygenase.[5,21,23] The existing evidence does not rule out, however, the possible contribution of other enzymes including 15-lipoxygenase to 12(*S*)-HETE production in some cases where the presence of 12-lipoxygenase or 15-lipoxygenase protein, respectively, has not as yet been demonstrated with certainty. In addition, the lack of a truly specific inhibitor for platelet-type 12-lipoxygenase in the studies published to date argues for cautious interpretation of data which solely rely on the use of pharmacologic inhibitors.

Tumor cell proliferation and 12-LOX

Growth factors including EGF can stimulate cellular proliferation, and EGF-dependent actions on responsive cells such as epithelial cells are mediated via the EGF receptor (EGF-R).[52] Strong evidence suggests that the EGF-R plays a role in malignant tumor growth, and its overexpression can result in a neoplastic phenotype in transgenic animals and cells.[53,54] Earlier studies had shown an activation and induction of lipoxygenase activity by EGF[21,23,55] or, reversely, a suppression of expression of 12-lipoxygenase by inhibition of protein tyrosine kinase activity.[21] Most recently, a functional link between growth factor-dependent activation of growth-factor receptor, phosphorylation of its protein tyrosine kinase, activation of 12-lipoxygenase, and finally, the dependency of 12-lipoxygenase mRNA expression on the presence of active lipoxygenase enzyme *(Fig.3)* was established.[33] Thus, in future chemotherapy, the combined use of selective inhibitors of EGF-R tyrosine kinase and of 12-lipoxygenase may offer an attractive therapeutic potential against appropriate 12-lipoxygenase-expressing and/or 12-HETE-responsive human tumor cells. The further development of inhibitors of 12-lipoxygenase with higher specificity than the currently available compounds would certainly help to substantiate this suggestion. Other potential sites of pharmacologic interference include the "unidentified" cellular receptor(s) for 12(*S*)-HPETE or 12(*S*)-HETE, which mediate their stereospecific actions in responsive cells *(Fig.3)*. It remains to be established

whether such intracellular actions of these eicosanoid mediators include modulations of the 12-lipoxygenase promoter activity in human tumor cells.

Acknowledgements

The author thanks Prof. E. Spiess and A.-R. Strohmaier for the confocal laserscan imaging, S. Borgers for excellent technical assistance, and Prof. D. Keppler for continuing support.

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