Minireview

15-Lipooxygenases in cancer: A double-edged sword?

Adi J. Kil-Drori a, Amiram Ariel b, a

a Department of Hematology and Bone Marrow Transplantation, Rambam Health Care Campus, P.O. Box 9602, Haifa 31096, Israel
b Department of Biology, Faculty of Natural Sciences, University of Haifa, Haifa 31905, Israel

A R T I C L E   I N F O
Article history:
Received 26 February 2013
Received in revised form 11 July 2013
Accepted 30 July 2013
Available online 8 August 2013

Keywords:
Lipoxygenase
Neoplastic growth
Tumor biology
Arachidonic acid
Docosahexaenoic acid

A B S T R A C T
Among the lipoxygenases, a diverse family of fatty acid dioxygenases with varying tissue-specific expression, 15-lipooxygenase (15-LOX) was found to be involved in many aspects of human cancer, such as angiogenesis, chronic inflammation, metastasis formation, and direct and indirect tumor suppression. Herein, evidence for the expression and action of 15-LOX and its orthologs in various neoplasms, including solid tumors and hematologic malignancies, is reviewed. The debate surrounding the impact of 15-LOX as either a tumor-promoting or a tumor-suppressing enzyme is highlighted and discussed in the context of its role in other biological systems.

© 2013 Elsevier Inc. All rights reserved.

Contents
1. Introduction ................................................................. 16
2. Prostate cancer ............................................................ 18
3. Renal cell carcinoma ......................................................... 18
4. Lung cancer ............................................................... 18
5. Colorectal carcinoma ....................................................... 19
6. Breast cancer ............................................................. 19
7. Hematologic malignancies ................................................. 20
8. Discussion ................................................................. 21
   Disclosure statements ....................................................... 21
   Conflict of interest statement ............................................ 21
   Acknowledgements ......................................................... 21
   References ................................................................. 21

1. Introduction

The lipoxygenase (LOX) superfamily is abundant in plants, fungi, and animals, and catalyzes the formation of a single specific hydroperoxide derivative from polyunsaturated fatty acids [1]. The nomenclature of LOXs is based on the substrate carbon where oxygenation is catalyzed, and largely the chain length of common substrates determines the specificity of the enzyme. Whereas in plants 18-carbon fatty acids (such as linoleate and linolenate) are the dominant LOX substrates, in animals arachidonate (20-carbon fatty acid) is more common, corresponding to plant 13-LOX and animal 15-LOXs, respectively. Mammalian LOXs oxygenate arachidonic acid to hydroperoxycicosatetraenoic acids (HPETEs) that are subsequently reduced to their corresponding hydroxyicosatetraenoic acids (HETEs).

Fig. 1 summarizes the oxidative metabolism of arachidonic acid by lipoxygenases cyclooxygenases and cytochrome P450.

Lipoxygenases in humans are expressed in a tissue-specific fashion: 5-LOX is mainly expressed in leukocytes, 12-LOX in platelets, and 15-LOX-1 in reticulocytes, eosinophils, and macrophages. In mice, the analogous enzyme to the human 15-LOXs is 12/15-LOX, an enzyme biased toward the production of oxygenated products with a hydroxyl group on carbon 12 in arachidonic acid and carbon 14 in DHA, rather than carbon 15 and 17, respectively. In the 1990s, an additional 15-LOX isozyme, 15-LOX-2, was reported. Hence, publications through 1997 preceded the

* Corresponding author. Tel.: +972 4 8288771; fax: +972 4 8288763.
E-mail address: amiram@research.haifa.ac.il (A. Ariel).

1098-823/5 – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.prostaglandins.2013.07.006
Fig. 1. Arachidonic acid metabolism. Arachidonic acid (AA) is oxidized by lipoxygenases (LOX), cyclooxygenases (COX) and cytochrome P450 (CYP 450) to produce either pro-inflammatory (red border) or anti-inflammatory and pro-resolving (green border) metabolites. Dashed arrow represents incomplete description of reaction sequence. 20-HETE, 20-hydroxyeicosatetraenoic acid; EET, epoxyeicosatrienoic acid; H(p)ETE, hydroperoxyeicosatetraenoic acid; LX, lipoxin; LT, leukotriene; PG, prostaglandin; PLA, phospholipase A; TX, thromboxane. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
15-Lipoxygenase isozymes in humans. From Gene – National Center for Biotechnology Information.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Gene/location</th>
<th>Abbreviation</th>
<th>Tissue/cellular distribution</th>
<th>Substrates</th>
<th>Metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-</td>
<td>Arachidonate</td>
<td>ALOX15</td>
<td>Reticulocytes, eicosinophils, macrophages</td>
<td>Arachidonic acid</td>
<td>15S-H(p)ETE</td>
</tr>
<tr>
<td>LOX-</td>
<td>15-</td>
<td></td>
<td>skin, cornea, prostate, lung, esophagus</td>
<td>Linoleic acid</td>
<td>15S-H(p)ODE</td>
</tr>
<tr>
<td>1</td>
<td>lipoxygenase/17p13.3</td>
<td>ALOX15B</td>
<td></td>
<td>DHA</td>
<td>17S-H(p)DHA</td>
</tr>
<tr>
<td>15-LOX-2</td>
<td>Arachidonate</td>
<td>ALOX15B</td>
<td></td>
<td>Arachidonic acid</td>
<td>15S-H(p)ETE</td>
</tr>
</tbody>
</table>


distinction between the two 15-LOXs and results from these studies are limited in their interpretation. 15-LOX-2 is expressed in skin, cornea, prostate, lung, and esophagus [2,3]. It shares a 35% identity in amino acids with 15-LOX-1, and is more restricted to 15-carbon oxygenation and to arachidonic acid as substrate than 15-LOX-1 [4]. The latter can also metabolize linoleic acid, thus forming 13-hydroxyoctadecadienoic acid (13-HODE). Fig. 2 summarizes the oxidative metabolism of linoleic acid and other polyunsaturated acids (PUFA) by lipoxygenases. In addition, the main differences between the two 15-LOXs are briefly reviewed in Table 1. The metabolic products of LOXs are diverse. 5-LOX oxygenates arachidonic acid to 5-HETE, which is further metabolized by 5-LOX to the unstable leukotriene (LT) A4. This LT is transformed in part to the proinflammatory leukotrienes LTxB, LTCa, LTDb, and LTE4.

Fig. 2. Polyunsaturated fatty acids (PUFA) metabolism. Linoleic acid is a substrate for 15-lipoxygenase-1 (15-LOX-1) metabolism by which 13-hydroperoxyicosatetraenoic acid [13-H(p)ODE] is produced. Eicosapentaenoic acid (EPA) is oxidized by acetylated cyclooxygenase II (Ac COX-II) and subsequently by 5-lipoxygenase (5-LOX) to the resolvins of the E-series (RvEs). Docosahexaenoic acid (DHA) is oxidized by either Ac COX-II or 15-LOX-1. Green border indicates pro-resolving metabolites. See text for further information. AT-RvD, aspirin-triggered resolvin of the D-series; H(p)ETE, hydroperoxyoctadecadienoic acid; RvD, D-series resolvin. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
5-HPETE may also undergo reduction to 5-HETE, and both 5-HETE and LTA₄ have been reported to recruit and activate inflammatory cells, as well as to increase vascular permeability, both key steps in tumorigenesis [5]. LTA₄ released from leukocytes may also be transformed by platelet 12-LOX or mucosal 15-LOX to lipoxin (LX) A₄ and B₄. Lipoxins counter-regulate the main aspects of inflammation as well as halt the recruitment of inflammatory cells [6].

Opposing actions were reported for both 15-LOX-1 [7] and 15-LOX-2 [5] in carcinogenesis. This dispute extends to both solid tumors and hematological malignancies. The following sections will review evidence that supports either a pro-carcinogenic role or a tumor-suppressor effect for metabolites of the different 15-LOXs in various types of neoplasms.

2. Prostate cancer

The interest in 15-LOX in prostate cancer (PCa) has spanned more than a decade and a half. Spindler et al. [8] first suggested a carcinogenic role for 15-LOX-1 metabolites by detecting high levels of 13-HODE, a linoleic acid derivative, in both human PC specimens and PCa cell lines. Concomitantly, the presence of indeterminate 15-LOX was documented in these cell lines. When athymic nude mice were injected with PCa cell lines overexpressing 15-LOX-1, much larger prostatic tumors were generated as compared to those injected with PC cell lines with normal expression of 15-LOX-1 [9].

Following the discovery of 15-LOX-2 and its expression in prostate tissue, the development of a specific antibody directed against this isozyme allowed its detection in prostate hyperplasia, whereas in PCa cells its levels were reduced [10]. This paved the way for the tumor-suppressor paradigm for 15-LOX-2 in PCa, later substantiated by evidence that its metabolite, 15-HETr, activates peroxisome proliferator-activated receptor gamma (PPARγ) and inhibits the proliferation of PCa cell lines [11].

The contrasting roles of 15-LOX-1 as an oncogene and 15-LOX-2 as a tumor-suppressor were supported by opposing effects of their products on mitogen-activated protein kinase (MAPK) signaling: 13-HODE up-regulates MAPK activity, thus enhancing PPARγ phosphorylation and subsequently decreasing PPARγ transcriptional activity, whereas 15-HETr down-regulates MAPK activity with a stimulatory effect on PPARγ activity [12]. The Akt kinase is another signaling molecule that is affected by 13-HODE and 15-HETr in opposing manners, although to a lesser degree than MAPK. Hence, the effect of synthetic 15-LOX metabolites on MAPK signaling was demonstrated at lower, more physiologically relevant concentrations. Activation of the insulin growth factor-1 receptor (IGF-1R) promoter and IGF-1 binding were also promoted by 13-HODE [13]. IGF-1-stimulated MAPK and Akt activation was also increased by 15-LOX-1 overexpression, and blocking of 15-LOX-1 activity inhibited the proliferation of PCa cells, thus supporting a pro-tumorigenic role for 15-LOX-1 metabolites [13].

The tumor-suppressor function of 15-LOX-2 in normal prostate epithelial cells may be explained by the induction of replicative senescence [14,15]. Thus, 15-LOX-2 is overexpressed in age-dependent prostatic hyperplasia, but cell senescence may hinder progression to malignant transformation. Notwithstanding arachidonic acid, additional exogenous compounds found in foodstuffs may invoke 15-LOX-2 mediated growth arrest in PCa cells; for instance, docosahexaenoic acid (DHA) has been shown to be metabolized by 15-LOX-2 to different 17-hydroxy DHA derivatives, which induce this effect via a PPARγ-dependent pathway [16]. Hence, the opposing roles of the 15-LOX isozymes in PCa are in wide agreement. The necessity for this duality is still under scrutiny.

3. Renal cell carcinoma

Lipoxygenase involvement in renal cell carcinoma (RCC) development has been scarcely probed. There is one report on macrophages in the renal tumor microenvironment which show an upregulated expression of 15-LOX-2, but not 15-LOX-1, and enhanced production of 15-HETE. Pharmacologic inhibition of LOX promoted the production of CCL2 and IL-10 by these tumor-associated macrophages, suggesting 15-LOX-2 supports immune evasion by tumor cells [17]. Notably, tumor cells were obtained from newly diagnosed patients. On the other hand, a different report indicated enhanced expression of 15-LOX-1 in early RCC samples [18]. This expression was decreased in progressive tumor biopsies. The differences reported in 15-LOX-1 levels in RCC may stem from different methodologies: in the former study polymerase chain reaction (PCR) and Western blotting were used, whereas in the latter, immunohistochemistry was the method of choice. In sum, the scarcity of data regarding 15-LOX expression in RCC does not allow a definitive conclusion as to its role in this malignancy.

4. Lung cancer

A lung carcinoma cell line was demonstrated by Brinckmann and Kuhn [19] to express an indeterminate 15-LOX after culturing with interleukin 4 (IL-4), a type 2 cytokine that exerts a similar effect on human monocytes/macrophages [20]. Detectable mRNA levels of both indeterminate 15-LOX in non-small lung carcinoma (NSCLC) cell lines were also reported by Moody et al. in the absence of IL-4 [21]. Along these lines, nordihydroguaiaretic acid (NDGA), a nonspecific LOX inhibitor, inhibited NSCLC cell growth and induced its apoptosis [21]. This effect, however, cannot be solely ascribed to either 5- or 15-LOX due to the nonspecific inhibition by NDGA. The expression of 15-LOX-2 in lung carcinoma biopsies was explored as well [22]. In benign lung tissue, 15-LOX-2 immunostaining was confined to type II pneumocytes (known to express PPARγ), especially in reactive areas. Among the various lung tumor types, 15-LOX-2 expression was detected only in NSCLC, and not in small cell carcinoma, which is more aggressive biologically. Tumor stage and patient survival, however, were not significantly correlated with 15-LOX-2 expression [22].

A murine model of Lewis lung carcinoma (LLC) was used by Harats et al. [23] to study the effect of endothelial 15-LOX-1 expression on lung cancer. Transgenic mice harboring endothelial cells overexpressing 15-LOX-1 under regulation of the preproendothelin-1 promoter were injected with LLC cells. Primary tumor and metastasis growth rates were diminished by 15-LOX-1 overexpression, as compared to control mice, with evident necrosis and apoptosis of tumor cells. 15-LOX-1 may act as a tumor suppressor in lung carcinoma through p53. After treatment with IL-4, A549 human lung carcinoma cells showed an increased expression of 15-LOX-1, with a concomitant rise in the expression of downstream targets of p53, a well-established tumor suppressor [24]. This is possibly achieved through binding of DNA-dependent protein kinase (DNA-PK), which was shown to precipitate with 15-LOX-1. Enhanced phosphorylation of p53 ensued, and this was reduced by knockdown of DNA-PK.

The levels of the 15-LOX metabolites, 13-HODE and 15-HETr, were shown to be reduced in NSCLC biopsies [25]. In a murine model of a tobacco carcinogen-induced lung carcinoma, a drop in mRNA and protein levels of 12/15-LOX as well as the levels of 15-HETr were noted prior to the development of pronounced lung tumors. A similar decrease in PPARγ activity was found in this model, again preceding a significant rise in tumor numbers. The NSCLC cell line A549 also express upregulated levels of 15-LOX-1.
and 15-LOX-2 mRNA after exposure to white tea extract (WTE) [26]. Apoptosis of NSCLC cells treated with WTE was demonstrated using enzyme-specific immunoassay (EIA), and was partially blocked by NDGA and the PPARγ inhibitor GW9662. The expression of 15-LOX-1 was found during terminal differentiation of human bronchial epithelial cells in air–liquid interface cultures, but was missing from lung cancer cell lines [27]. Along with more elaborate differentiation events missing in colonic cancer cell lines following 15-LOX-1 RNA knockdown, this suggested the involvement of 15-LOX-1 in differentiation processes abolished by malignant transformation.

Although both 15-LOX isozymes are expressed in normal lung tissue, a tumor suppressor role has been demonstrated more extensively for 15-LOX-1. In contrast to prostate carcinoma, opposing effects of 15-LOX-1 and 15-LOX-2 have not been suggested.

5. Colorectal carcinoma

The growth rate of murine colon adenocarcinoma tumors decreased upon inhibition of 5-LOX, an enzyme that harbors countering properties to 15-LOX [28]. It is not clear whether 15-LOX was associated with this phenomenon, but its expression is induced in the colon carcinoma cell line HTB 38 following treatment with IL-4 [19]. Expression of 15-LOX-1 in colorectal carcinoma tumor samples was found in high percentage and was more prominent than in adjacent normal tissue, using both Western blotting and immunohistochemistry as detection methods [29]. Conversely, Shureiqi et al. [30] reported a markedly reduced expression of 15-LOX-1 in both colorectal tumors and transformed colonic cell lines, detected by immunohistochemical staining along with diminished levels of 13-HODE, a major 15-LOX-1 metabolite. By Western blotting no difference in 15-LOX-1 expression was found. 13-HODE was also found to induce apoptosis in CRC cell lines [31], and its levels increased following treatment with apoptosis-inducing non-steroidal anti-inflammatory drugs (NSAID) [31]. Along these lines, pharmacologic inhibition of 15-LOX-1 blocked the effect of NSAIDs [31].

In the CRC cell line HCT-116, overexpression of 15-LOX-1 led to down-regulation of p21, a target of the tumor-suppressor gene p53, which was inhibited by NDGA and restored by 13-HODE [32]. This down-regulation was accompanied by increased cell growth, suggesting a pro-tumorigenic role for 15-LOX-1 metabolites. Of note, Zhu et al. countered this notion by demonstrating up-regulation of p53 and its targets following transfection of the same cell line with enzymatically inactive 15-LOX-1 [24]. Thus, 15-LOX might exert opposing actions pending on the presence of catalytic activity. Inhibition of cyclooxygenase-1 (COX-1) induces apoptosis in CRC cells in vitro with a concomitant rise in 15-LOX-1 expression [33]. This increased expression may depend on increased intracellular levels of cyclic guanylyl monophosphate (cGMP), with subsequent activation of protein kinase G (PKG) [33]. The decreased expression of 15-LOX-1 in CRC tissue may stem from transcriptional regulation of the GATA-6 transcription factor. Increased expression of GATA-6 mRNA was found in a CRC cell line, and a GATA binding site was demonstrated in the 15-LOX-1 promoter region in these cells [34]. When CRC tissue from biopsy samples was inspected, GATA-6 was over-expressed as compared to normal colonic tissue [35]. GATA-6 knockdown increased 15-LOX-1 expression and apoptosis in CRC cell lines treated with sodium butyrate (a histone deacetylase inhibitor) or NSAIDs [35]. Other mechanisms proposed by the same group for transcriptional silencing of 15-LOX-1 in CRC include the association of DNA methyltransferases (DNMTs) with the 15-LOX-1 promoter [36], and the nucleosome remodeling and histone deacetylase (NuRD) repression complex [37].

Adenoviral vector delivery was used to enhance 15-LOX-1 expression in various CRC cell lines, with subsequent restoration of gene expression and enzymatic activity [38]. CRC cell survival in vitro and growth of CRC xenografts in vivo were significantly inhibited, alongside down-regulation of anti-apoptotic proteins and induction of apoptosis [38]. These findings suggested for the first time that 15-LOX-1 might be used as a molecular target for cancer therapy. To establish the therapeutic potential of 15-LOX-1 in CRC, a mouse model with stable human transgene expression was created [39]. Intestinal epithelial targeting was achieved through the villin promoter, and was confirmed by RT-PCR and immunoblotting. Experimental tumorigenesis with azoxymethane was significantly suppressed in 15-LOX-1 transgenic mice in comparison with wild-type mice [39]. Moreover, the expression of tumor necrosis factor (TNF)-α and its target, inducible nitric oxide synthase (iNOS), were decreased in colonic cells, as was the activation of nuclear factor-kappa B (NF-κB) [39]. The reduction in the activity of NF-κB, a critical component in inflammation, was found to directly emanate from 15-LOX-1 overexpression, and thus underscores the important role of 15-LOX-1 in the resolution of inflammation [40]. Similarly, 15-LOX might be involved in the termination of pathologic processes that upon extension may lead to terminal cell de-differentiation and ultimately tumorigenesis [40]. The consistent findings on tumor suppression by 15-LOX-1 in CRC have led the Shureiqi group to conduct a clinical trial probing the association between the effect of celecoxib, a selective COX-2 inhibitor, on colorectal endothelial polyps, and the expression of 15-LOX-1 and GATA-6. This study is currently ongoing (NCT00503035, see references under National Cancer Institute).

6. Breast cancer

The interactions between 15-LOX and COX enzymes also affect the outcome of breast cancer. Indomethacin, a COX-1 and COX-2 inhibitor, decreased the growth of human breast cancer (BC) cells in nude mice, as well as slowed the rate of lung metastasis formation [41]. The levels of 12-HETE, but not 5- or 15-HETE, were elevated following indomethacin treatment, suggesting up-regulation of 12/15-LOX activity, and possibly a pro-tumorigenic role for this enzyme, in line with the stimulated metastatic growth rate induced by a high linoleic acid diet [41]. This accelerated metastatic rate was also achieved in a similar model by the addition of 12-HETE [42]. Other reports attributed pro-tumorigenic actions to 15-LOX metabolites in BC cells [43], possibly through epidermal growth factor (EGF)/transforming growth factor (TGF)-α regulation of 13-HODE production. 15-HPETE, on the other hand, was reported to have a cytotoxic effect on BC cells [44]. Also, treatment with EGF up-regulated both the activity and the expression of 12-LOX in a BC cell line [45].

A prospective study examined 120 human BC tumor biopsies for the expression of various LOX types using RT-PCR. The levels of 15-LOX-1 were shown to be reduced, whereas 5- and 12-LOX levels were increased [46]. Specifically, tissues from BC tumors with positive lymph node involvement showed a decreased 15-LOX-1 expression compared to lymph node-negative tumors. Moreover, 15-LOX-1 expression was reduced in patients who developed metastatic disease, local recurrence, or death, compared to patients with a more favorable outcome [46]. Interestingly, Western blotting showed increased expression of 15-LOX-2 in a BC cell line, in the absence of 15-LOX-1 [47]. Overexpression of 15-LOX-2 resulted in enhanced p38 MAPK phosphorylation in these cells following treatment with arachidonic acid [47]. Moreover, treatment with exogenous 15-HETE increased cell adhesion to collagen type IV, in keeping with previous reports on the importance of p38 MAPK in cell adhesion [47]. However, when tested in tumor biopsy samples, the levels of 15-LOX-2 were found to be reduced [46]. A thorough research into the effect of 15-LOX-1 on BC metastasis formation.
was published recently by Kerjaschki et al. [48]. To modulate lymphatic invasion by breast tumors an in vitro model was established using BC cell spheroid coculture in monolayers with lymphatic endothelial cells (LEC). Defects were documented in the organization of LEC monolayers cultured in proximity to BC spheroids, where 15-LOX, but not 12-LOX, expression was demonstrated [48]. Treatment with NDGA and baicalein, another pan-LOX inhibitor, inhibited the formation of these circular defects. Blocking 12-HETE or knocking down ALOX15 (the 15-LOX-1 gene) restored monolayer integrity, whereas knocking in ALOX12 (the 12-LOX gene) reestablished defect formation in ALOX15 knocked-down cells [48]. In the same study, 13 tumor samples were examined by tissue microarray and showed colocalization of both 15-LOX and 12-HETE to sentinel lymph nodes. Moreover, enzyme localization matched tumor stage. 12-LOX staining was not reported in tumor biopsies [48]. Taken together, the role of 15-LOX in BC has not been fully ascertained yet. While the deleterious effect of 12-HETE is consensual, it is not clear whether 15-LOX is in fact the enzyme responsible for its production in mammary tumor beds, or whether the culprit is 12-LOX.

7. Hematologic malignancies

Examination of leukemic cells for various LOXs preceded much of the research on 15-LOX in solid tumors. This resulted from the expression of LOXs in human peripheral blood cells, both from erythroid and myeloid lineages, which are easily attainable. In an early report, bone marrow samples from chronic myelocytic leukemia (CML) were probed for the presence of LTBA and 12-HETE using high-pressure liquid chromatography (HPLC). An increased production of LTBA by CML bone marrow cells was found when compared to cells from healthy donors, which were skewed toward 12-HETE production [49]. Another early report suggested a role for 15-LOX in the maintenance of DNA synthesis in an erythroleukemia cell line [50]. Nonspecific inhibitors of LOXs blocked DNA synthesis, whereas 15-HETE increased it. Lipoxins also augmented DNA synthesis in the same cell line [51]. A contrasting anti-carcinogenic role for 12/15-LOX was implicated in a murine model of myeloproliferative neoplasm (MPN) progressing to leukemia. In 12/15-LOX-deficient mice, increased activation of the phosphatidylinositol 3 kinase (PI3K) pathway was demonstrated, as manifested by augmented Akt phosphorylation [52]. Elevated levels of the oncoprotein Bcl-2 were also demonstrated in 12/15-LOX-deficient mice, and these levels were reduced by PI3K inhibition. Moreover, 12/15-LOX overexpression suppressed the growth of a CML cell line [52]. Along these lines, treatment with 15-HPETE induced the generation of reactive oxygen species in a CML cell line, with subsequent apoptotic features [53].

Human leukemic blasts from both AML and ALL patients demonstrated 5-, 12-, and 15-LOX expression [54]; however, using quantitative PCR, 5-LOX was much more prevalent than 15-LOX. When 15-HETE, 12-HETE, and LTBA were tested for a direct effect on leukemic blasts, none induced apoptosis [54]. In contrast, the human T-cell leukemia cell line Jurkat was sensitive to 15-HETE and 15-HPETE, showing both enhanced apoptotic markers and reactive oxygen species formation [55]. In an apoptosis-resistant EBV-converted Burkitt’s lymphoma cell line, increased expression of 5-LOX was detected [56]. Addition of 5-LOX inhibitors to the cell culture induced apoptosis. Both 5- and 15-HETE counteracted the induction of apoptosis by 5-LOX inhibitors, suggesting that 5-LOX, rather than 15-LOX, may be involved in lymphomagenesis [56].

Biopsies of both non-Hodgkin lymphoma (NHL) and classical Hodgkin lymphoma (HL) were tested for the expression of 15-LOX-1 by immunohistochemistry [57]. No expression was detected in the NHL samples, whereas in 85% of the HL samples the actual Reed–Sternberg cells (tumor cells of classical HL) stained positive [57]. In addition, an HL cell line (L1236) showed mRNA expression of 15-LOX-1. The enzyme was localized to the cytosol, and translocated to the cell membrane upon increase in intracellular calcium levels [57]. The same research group also tested a primary medullary B-cell lymphoma (PMBCL) cell line for 15-LOX-1 expression. Untreated cells did not produce 15-HETE after incubation with arachidonic acid [58], consistent with lack of 15-LOX-1 protein in PMBCL cell lines and in tumor biopsies, although 15-LOX-1 mRNA was documented by RT-PCR [59]. After treatment with IL-4 (as well as IL-13), both 15-HETE production (in the presence of AA) and 15-LOX-1 expression were detected [58]. These findings were not corroborated in a different PMBCL cell line [58]. Interestingly, the common expression of 15-LOX-1 reiterates the many phenotypic similarities between PMBCL and HL.

The role of 15-LOX in multiple myeloma (MM) has not been studied extensively. An early publication reported arachidonic acid incorporation into the membranes of three MM cell lines; however, LTBA, LXA4 and LTB4, and 12- and 15-HETE had no effect on cell proliferation [60]. In summary, the role of 15-LOX in hematologic malignancies awaits further elucidation.

8. Discussion

The impact of 15-LOX expression and activity has been studied in various pathological states, including the microvascular complications of diabetes mellitus, obesity, atherosclerosis, hypertension, renal dysfunction, cerebrovascular disease, Alzheimer’s disease, and Parkinson’s disease [61]. In complex processes, such as the formation of atherosclerotic plaques, evidence points to a dual role for 15-LOX, i.e., both protective against, and promoting, atherogenesis [61]. Similar conflicting evidence points to a protective role against acute and perhaps even chronic kidney injury, alongside a vasoconstrictive and pro-inflammatory effect promoting renal hypertension and vascular injury [61]. Moreover, discussing the role of 15-LOX in mediating inflammation is challenging due to conflicting findings in different inflammatory settings. Whereas in experimental inflammation and fibrosis several studies point to beneficial actions exerted by this enzyme [62–64]. In airway inflammation there is active debate whether 15-LOX exerts an anti-inflammatory effect [65], or a pathological, pro-inflammatory one [66].

Therefore, it is not surprising that opposing findings on the function and significance of 15-LOX in human malignant diseases surface in the current review. Can two isozymes with considerable resemblance be on the one hand devastating in promoting neoplastic transformation and metastasis formation, and on the other protective in keeping cancer at bay? Clearly, it is difficult to come up with a plausible answer to such a conundrum using non-comparable scientific models. For example, the availability and usage of different substrates by 15-LOX is reflected in the net biologic effect. Substrates such as arachidonic acid and DHA are oxygenated by 15-LOX to different degrees. This may lead to the production of different metabolites at varying concentrations with conflicting actions. Metabolism of DHA, an ω-3 polyunsaturated fatty acid from foodstuffs, may lead to the production of protectin D1, or resolvins of the D series [6]. These potent anti-inflammatory mediators dampen deleterious tissue injury in inflammatory settings and thus may hinder tumor growth in the malignant milieu, which is supported by chronic inflammation [67]. Conversely, metabolism of arachidonic acid by eicosanoids and mast cells may lead to the production of eoxins, proinflammatory mediators that are structurally related to the cysteiny1-leukotrienes, and enhance vascular permeability [68]. Protecins, resolvins, and eoxins exemplify the myriad of bioactive products generated by 15-LOX in
various tissues. At the same token, they represent the wide range of actions exerted by 15-LOX enzymatic activity. This versatility is seldom explored by researchers who focus on the readily produced and abundant metabolites, such as 15-HETE and 13-HODE.

The ambiguity surrounding the role played by the 15-LOXs in malignant biological processes stems also from the limitations of current animal models, and especially the usage of its 12/15-LOX murine homolog, which can also catalyze the production of 12-HETE. Indeed, the proinflammatory effect of 12-HETE on TNF-α production was partially reversed by creating transgenic mice expressing human 15-LOX-1, which does not produce high concentrations of 12-HETE, and thus produced more 13-HODE [69]. However, this expression was limited to epithelial cells, and may not reflect the full effect of 15-LOX expression and function in stromal cells and macrophages in the tumor bed. Moreover, the interplay between 15-LOX-1 and 15-LOX-2 is not reflected in such maneuvers. Part of the gap in our understanding of the role played by the different 15-LOX enzymes in cancerous tissue may stem from both incomplete methodology to measure their distinct activity, and restricted availability of instrumentation to that end, e.g., high-pressure liquid chromatography and liquid chromatography/mass spectrometry. Any attempt to create a solidifying concept regarding the role of the 15-LOXs in the neoplastic milieu is, therefore, premature. Presumably, much of the controversy stems from incomplete understanding of the molecular mechanisms and signaling cascades through which 15-LOX metabolites influence cell fates. Such understanding demands further research.

Disclosure statements

A.J. Kil-Drori wrote the manuscript. A. Ariel edited the manuscript and proofed it.

Conflict of interest statement

No conflict of interest exists for any of the authors.

Acknowledgements

This work was supported by grants from the Israel Science Foundation (number 534/09), the Nutricia Research Foundation, and the Marc Rich Foundation (to A. Ariel). A. Ariel is a recipient of the young scientist award from Teva Pharmaceuticals Ltd.

References

[38] Wu Y, Fang B, Yang XQ, et al. Therapeutic molecular targeting of 15-
[41] Beckman BS, Despinasse BP, Spriggs L. Actions of lipoxins A4 and B4 on B3
[44] Vincent C, Gianfette R, Donnad M, et al. 5-LLOX, 12-LLOX and 15-LLOX in immu-
[50] Desplaat V, Duley C, Praloran V, Denizot Y. Incorporation and effect of arachi-
[53] Arial A, Timor O. Hanging in the balance: endogenous anti-inflammatory mech-
[54] Serhan CN, Jain A, Marleau S, et al. Reduced inflammation and tissue damage in beaglenic rabbits overexpressing 15-lipooxygenase and endogenous anti-
[55] Morin C, Fortin S, Cantin AM, Rousseau E. Docosahexaenoic acid deriva-
tive prevents inflammation and hyperreactivity in lung: implication of PKC–potentiated inhibitory protein for heterotrimeric myosin light chain phos-
[57] Chw MT, Morley A, Smyth MJ. Inflammation and immune surveillance in can-