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The role of lipoxygenases in pathophysiology; new insights and future perspectives

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ABSTRACT

Lipoxygenases (LOXs) are dioxygenases that catalyze the formation of corresponding hydroperoxides from polyunsaturated fatty acids such as linoleic acid and arachidonic acid. LOX enzymes are expressed in immune, epithelial, and tumor cells that display a variety of physiological functions, including inflammation, skin disorder, and tumorigenesis. In the humans and mice, six LOX isoforms have been known. 15-LOX, a prototypical enzyme originally found in reticulocytes shares the similarity of amino acid sequence as well as the biochemical property to plant LOX enzymes. 15-LOX-2, which is expressed in epithelial cells and leukocytes, has different substrate specificity in the humans and mice, therefore, the role of them in mammals has not been established. 12-LOX is an isoform expressed in epithelial cells and myeloid cells including platelets. Many mutations in this isoform are found in epithelial cancers, suggesting a potential link between 12-LOX and tumorigenesis. 12R-LOX can be found in the epithelial cells of the skin. Defects in this gene result in ichthyosis, a cutaneous disorder characterized by pathophysiologically dried skin due to abnormal loss of water from its epithelial cell layer. Similarly, eLOX-3, which is also expressed in the skin epithelial cells acting downstream 12R-LOX, is another causative factor for ichthyosis. 5-LOX is a distinct isoform playing an important role in asthma and inflammation. This isoform causes the constriction of bronchioles in response to cysteinyl leukotrienes such as LTC_4, thus leading to asthma. It also induces neutrophilic inflammation by its recruitment in response to LTB_4. Importantly, 5-LOX activity is strictly regulated by 5-LOX activating protein (FLAP) though the distribution of 5-LOX in the nucleus. Currently, pharmacological drugs targeting FLAP are actively developing. This review summarized these functions of LOX enzymes under pathophysiological conditions in mammals.

Contents

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1. Introduction

Lipoxygenases (LOXs) catalyze the oxygenation of polyunsaturated fatty acids such as arachidonic acid and linoleic acid [1,2]. The oxygenated lipids initiate subsequent biological reactions, activate cellular signaling mechanisms through specific cell surface receptors, or are further metabolized into potent lipid mediators. LOX can be found not only in mammals, but also in plants. Historically, biochemical characterizations have been performed mainly on soybean LOX isoforms. While the overall structure of mammalian LOX enzymes seems to be similar, each isoform has unique properties, such as substrate specificity (Table 1, reviewed in [3]). In most cases, the structure depends on the shape of the substrate cavity and the coordination of histidine residues or alternatives to a non-heme iron atom at the catalytic center [4,5]. Importantly, LOX enzymes require a lag period for the activation of enzymes from an inactive ferrous form to an active ferric form by either molecular oxygen or lipid hydroperoxides. Enzymatic activity is also regulated by the N-terminal β-barrel region of polypeptides, where this region has a similar amino acid sequence to the C2-like domain; thus, Ca^{2+}-mediated activation via interaction with the plasma membrane has been proposed. Earlier studies have shown that LOX enzymatic activity can be inhibited by phenolic antioxidants such as nordihydroguaiaretic acid and caffeic acid, suggesting a beneficial role of dietary polyphenol intake [6]. Alternatively, synthesized drugs for LOX are relatively limited thus far. The 5-LOX inhibitor zileuton has been accepted and used successfully for the control of asthma. Currently, inhibitors for 5-LOX activating protein are actively developed by many pharmaceutical companies [7]. These inhibitors essentially modulate the transportation of 5-LOX from the nucleus to the cytoplasm, leading to suppressive 5-hydroperoxyeicosatetraenoic acid (5-HPETE) production. This mode of action of 5-LOX inhibitor is unique, and there are no similar regulatory mechanisms and drugs for other LOX isoforms.

From a genetic point of view, the alignment of LOX isoform nucleotides encoded by arachidonate lipoxygenase (*ALOX* in humans and *Alox* in mice) genes has revealed that *ALOX5* and other *ALOX* genes have separate origins. The other *ALOX* genes seem to have originated from fewer genes, as human *ALOX* genes are found in a cluster in chromosome 17p13.1 and murine *Alox* genes are found in chromosome 11 as active enzymes [8]. The expression levels of *ALOX* genes are partially controlled by cytokines, such as *ALOX15*, whose expression increases in response to Th2 cytokines. *ALOX* enzymatic activity is also regulated by tissue distribution and cell type. *ALOX12B*, *ALOXE3*, and *ALOX15B* are expressed mainly in the skin and other epithelial cells, whereas *ALOX15*, *ALOX12*, and *ALOX5* are expressed in hematopoietic/immune cells. They are involved in atherosclerosis, neuronal disorder, immune modulation, skin diseases, and maintenance of the epithelium. The roles of human enzymes (Table 2) seem to be slightly different from what is expected from phenotypes of knockout mice (Table 3), which shows that these oxygenated lipids are uniquely and finely regulated in humans and mice.

2. 15-Lipoxygenase (15-LOX)

15-LOX is a prototypical enzyme catalyzing oxygenation of polyunsaturated fatty acids. Among various mammalian species, rabbit reticulocyte LOX has been characterized from earlier studies and often used as standard for biochemical assays. When the

Table 1
Properties of LOX enzymes.

Proteins	15-LOX	15-LOX-2	12-LOX	12R-LOX	eLOX-3	5-LOX	FLAP
Human							
Gene	*ALOX15*	*ALOX15B*	*ALOX12*	*ALOX12B*	*ALOXE3*	*ALOX5*	*ALOX5AP*
Products[a]	15S-HPETE	15S-HPETE	12S-HPETE	12R-HPETE	Epoxyalcohols	5S-HPETE	NA
Expression	Leukocytes	Epithelium, leukocytes	Myeloids, skin, epithelium	Skin, epithelium	Skin, epithelium	Leukocytes	Leukocytes
Mouse							
Gene	*Alox15*	*Alox15b*	*Alox12*	*Alox12b*	*Aloxe3*	*Alox5*	*Alox5ap*
Products[a]	12S-, 15S-HPETE	8R-HPETE, epoxyalcohols	15S-, 12S-HPETE	12R-HPETE	Epoxyalcohols	5S-HPETE	NA
Expression	Leukocytes	Skin, epithelium, leukocytes	Platelet, skin, epithelium	Skin, epithelium	Skin, epithelium	Leukocytes, epithelium	Leukocytes

NA, not available. [a]Arachidonic acid as a substrate except eLOX-3 where 12R-HPETE as a substrate.

Table 2
Human diseases that potentially links to lipoxygenase genes.

Genes	Atherosclerosis/heart disease	Immune response	Neurological disorder	Cancer	Skin disease	Others
ALOX15	A near null mutant (T560M) in coronary artery disease [140]			Rectal cancer [100] Colon cancer [141] Adenoma recurrence [142] Breast cancer [39] Prostate cancer [12]		Bone mineral density↓[56,143–144]
ALOX15B	↑ in carotid plaque with thrombosis [43] ↑ in carotid lesion [32] Variants in coronary artery disease [145]			Esophageal cancer [35] Adrenocortical tumor [36] Breast cancer [39] Epithelial tumors [37] Prostate cancer cells [38] Head and neck carcinoma [40] ↑ in TAMs isolated from renal cell carcinoma [42]		
ALOX12	Variants in subclinical atherosclerosis [146]	Variants in Toxoplasmosis [147]	Bipolar disorder [49] Schizophrenia [50]	Methylation in AML[61] Esophageal squamous cell carcinoma [64] Rectal cancer [100] Adenoma recurrence [142] Colorectal cancer [148] E261R mutation Breast cancer [48] Colon cancer [47] Colorectal cancer [65] Colorectal adenoma [149]		Bone mineral density↓[54–57,150] Fat mass↑[53]
ALOX12B					ARCI [81–82,94,151] NCIE [84–86]	
ALOXE3					ARCI [82,94,151] NCIE [84,86]	
ALOX5	Variants in atherosclerosis [152] ↑ in atherosclerosis [153] Variants in subclinical atherosclerosis [146]	Variants in asthma [97,154,155] Variants in AHR [98,99]		Inverse correlation Rectal cancer [100] Ovarian cancer [101] Colon cancer [47]		
ALOX5AP	Variants in subclinical atherosclerosis [146]	Variants in asthma [126,156]		Rectal cancer [100] Adenoma [100]		

AHR, airway hyperresponsiveness; AML, acute myeloid lymphoma; ARCI, autosomal recessive congenital ichthyosis; NCIE, nonbullous congenital ichthyosiform erythroderma; TAM, tumor-associated macrophages.

potential link between atherosclerosis and its inhibition with antioxidants was explored, 15-LOX has been hypothesized to initiate and/or promote atherosclerosis through low density lipoprotein (LDL) oxidation. This is based on the "oxidative LDL theory" that oxidation of lipids induces atherosclerosis and its inhibition by antioxidants prevents atherogenesis. To initiate oxidation in vivo, there must be some initiators for lipid peroxidation. One potential candidate includes carbon-centered free radicals, which react with molecular oxygen to give rise to peroxyl radicals. Since these radicals trigger oxidation of lipids continuously, therefore, generation of such free radical-generating initiators must be tightly regulated in vivo. Lipid hydroperoxides generated from polyunsaturated fatty acids by LOX enzyme can induce this reaction since these oxidation products further decompose into other free radicals in the presence of metal ions. 15-LOX can be found in macrophages and other immune cells as well as epithelial cells. Human and mouse enzymes are known to be induced by Th2 cytokines such as IL-4 and IL-13 via STAT6-dependent manner [9,10].

2.1. *ALOX15*

It is widely accepted that cyclooxygenase (COX) inhibitor nonsteroidal anti-inflammatory drugs (NSAIDs) induce colon cancer in humans [11]. One suggested reason is that the balance between COX and LOX determines tumorigenesis critically. Under low COX activity, arachidonic acid released from cell membranes in response to external stimuli is preferentially metabolized by LOX

Table 3
Phenotypes of LOX-deficient mice.

Genes	Atherosclerosis	Immune response	Neurological disorder	Others
Alox15	↓ in ApoE KO [19–21] ↓ in LDLR KO [22,23] VSMC response↓[24]	LTC4↑[26] Arthritis↑[157] *Schistosoma mansoni* infection→[158] Th1↓[159] IL-12 ↓, TNF-α → [160] Acute lung injury↓[161] Phagocytosis↑[162]	Peripheral diabetic neuropathy↓[163] Diabetic autonomic neuropathy→[163]	Osteoclast development↑[28] Insulin resistance↓[164] Angiogenesis↓[165] Myeloid differentiation↓[27] Erythrocyte development→[26] Angiogenesis↓[166] ER stress↓[167] Hypertension↓[168] Inflammatory neovascularization↓[169] Nonalcoholic fatty liver disease↓[170] Diabetes associated pp38 and pErk↓[171] Obesity↓[29] Ischemic cardioprotection↓[25] Airway epithelial injury in asthma↓[172]
Alox15b				Epidermal permeability barrier↓ [44]
Alox12		Platelet sensitivity↑[67]		Carcinoma (B6/129)↓[68] Papilloma (SENCAR)↓[68] Basal transepidermal water loss↑[69]
Alox12b				Skin barrier↓[87,95] Ichthyosiform↑[88]
Aloxe3				Skin barrier↓[95]
Alox5	↓ in LDLR KO [114]	PAF-induced lethal shock↓[102,103] OVA-induced asthma↓[108] *Schistosoma mansoni* infection↓[158] Early female mortality (MRL-lpr/lpr)↓[109] Toxoplasma gondii elimination↓[107] Tumor-infiltrating macrophages↑[121] OVA/alum-induced Th2↓[159] Peritonitis↓[105] LTB4↓[173] Acute pancreatits↓[104] *Borrelia burgdorferi* elimination↓[106] *Histoplasma capsulatum* elimination↓[174]	Anxiety-like behavior (C57BL/6)↑[117] Synaptic dysfunction↓[119] Anxiety-like behavior (B6/129)↓[175]	Inflammatory neovascularization↓[169] Endotoxin-induced Hypoxic pulmonary vasoconstriction↓[176] $Apc^{\Delta 468}$-induced intestinal polyposis↓[121–123].
Alox5ap	↓ in COX-2 KO [115]	PAF-induced shock↓[127] Zymozan-induced peritonitis↓[127] Collagen-induced arthritis↓[129] Cerebral inflammation↓[128]	Improved Alzheimer's disease-like phenotype [133] Anxiety-like behavior↑[134]	

ApoE, apolipoprotein E; ARCI, autosomal recessive congenital ichthyosis; KO, knockout; LDLR, low-density lipoprotein receptor.

enzymes. There is evidence that a 15-LOX metabolite 13S-HPODE (13S-hydroperoxyoctadecaenoic acid) generated from linoleic acid induces apoptosis in colon cancer cells; thus, defective expression of *ALOX15* in colon cancers could promote tumorigenesis [11]. *ALOX15* expression itself is controlled, at least in part, by the epigenetic process, as an alteration of methylation in the *ALOX15* promoter has been observed in prostate cancer patients [12]. Furthermore, the expression of 15-LOX in epithelial cancer cells is tightly regulated by additional mechanisms. As mentioned, STAT6 is a critical regulator of *ALOX15* expression regulated by its phosphorylation and acetylation, as well as histone modification [13,14]. Recent studies have also shown the *ALOX15* expression can be modulated by the chromatin-dependent STAT6-independent mechanism [15,16]. Biochemically, the produced 13 S-HPETE interacts with PPAR-δ, followed by the induction of apoptosis prior to carcinogenic conditions [17]. The importance of 15-LOX-derived metabolites has also been defined by its aberrant failure in conditional transgenic mice, expressing it in the mouse prostate, inducing prostatic intraepithelial neoplasia once the apoptotic function is dysregulated [18].

2.2. *Alox15*

Atherosclerosis is an inflammatory disease characterized by an accumulation of lipid-loaded macrophages in blood vessels. Evidence has suggested a close link between *Alox15* expression and atherosclerosis in the mouse, characterized mostly on the atherosclerosis-prone genetic background of mice lacking the *ApoE* and/or LDL receptor [19–23]. In both cases, the initiation and/or development of atherosclerosis depends on 15-LOX enzymatic

activity. Recent studies have suggested that atherosclerosis is involved, at least in part, in sterile inflammation characterized by augmented IL-1β and the activation of caspases. The accumulation of lipids in blood vessels causes a failure of vascular function; therefore, disruption of *Alox15* shows impaired migration of vascular smooth muscle cells [24]. In addition, ischemia-induced cardioprotection plays an important role in maintaining proper heart function. A previous study showed that *Alox15* deficiency caused its impairment after reperfusion-mediated preconditioning by PKC activation in the heart [25]. These results suggest that this enzyme might also contribute to the pathogenesis of cardiovascular disease.

Lipid hydroperoxides, the primary reaction products of *Alox15* from polyunsaturated fatty acids, readily induce oxidative stress through their decomposition to free radicals. Thus, *Alox15*-deficient mice might produce less oxidative stress under physiological conditions. However, *Alox15*-deficient mice have shown an increase in oxidative stress markers such as isoprostane 8-epi-prostaglandin F2a in a zymosan-induced peritonitis model [26]. These apparently paradoxical results might be explained by the concomitantly enhanced 5-LOX-, but not 15-LOX-, dependent mechanism, which facilitates the accumulation of neutrophils by 5-HPETE and leukotrienes, thereby leading to enhanced oxidative stress at the site of inflammation in *Alox15*-deficient mice.

Accumulating evidence suggests that *Alox15* is critically involved in the regulation of cell differentiation. For example, the development of hematopoietic stem cells into the myeloid lineage is impaired in *Alox15*-deficient mice [27]. Alternatively, the formation of osteoclasts in *Alox15*-deficient mice and mice treated with enzymatic inhibitors has indicated its attenuation [28]. This impaired bone-desorbing osteoclastogenesis is negatively regulated by osteoblast formation, which derives from mesenchymal stem cells. Given that this bone-absorption is impaired in *Alox15*-deficient mice, bone-generating osteoblast formation is likely to be enhanced. In this case, adipocytes, which also stem from mesenchymal stem cells, would be impaired. Consistent with this speculation, *Alox15*-deficient mice have displayed impaired obesity [29], indicating a critical role of *Alox15* in cell differentiation.

3. 15-Lipoxygenase, type B (15-LOX-2)

15-LOX-2 shows higher similarity to 15-LOX, initially discovered in the skin in humans [30]. Human 15-LOX-2 generates 12R-HPETE from arachidonic acid specifically, showing unusual specificity for the regioisomeric lipid mediators in contrast to most isozymes, which produce S-regioisomers. Furthermore, mouse ortholog of 15-LOX-2 generates 8S-HETE and 8S-, 15S-diHPETE from arachidonic acid, thus enzymologically murine *Alox15b* is considered to be similar to human *ALOX12* [31]. Due to this, there are almost no overlapping diseases/phenotypes in the human and mouse in this isoform.

3.1. ALOX15B

Although a link between atherogenesis and *ALOX15* expression has long been hypothesized, it is known that the expression of *ALOX15B* in human carotid plaque macrophages is higher compared to *ALOX15* [32,33]. An in vitro experiment of *ALOX15B* silencing reported an attenuated lipid accumulation in human macrophages, indicating that it is functional for lipid uptake into the cells [34]. Thus, it is suggested that *ALOX15B* plays an important role in the initiation and development of atherosclerosis in humans.

Several studies have shown the downregulation of *ALOX15B* in epithelial tumors, suggesting that *ALOX15B* has an antiproliferative role [35–40]. This reduction of 15-LOX-2 in tumor cells was restored by an inhibitor for COX enzyme, demonstrating that its expression is negatively regulated by prostaglandins, at least in part [35]. PPAR-γ, a nuclear receptor regulated by endogenous LOX products, was upregulated in some epithelial tumors, suggesting that the downregulation of *ALOX15B* is autonomously controlled by PPAR-γ in epithelial cancer cells [37]. In prostate epithelial cells, *ALOX15* expression is positively regulated by transcription factor Sp1, whereas transcription factor Sp3, which is closely related to Sp1, negatively regulates its expression, suggesting that *ALOX15B* expression is critically regulated by multiple regulators [41]. Apart from epithelial cells, a separate study demonstrated increased *ALOX15B* expression in tumor-associated macrophages from renal cell carcinoma, suggesting that *ALOX15B* expression is distinctly regulated in epithelial cancer cells and macrophages [42]. Similar to tumor-associated macrophages, the upregulation of *ALOX15B* in carotid plaque macrophages has also been described [32,43].

3.2. Alox15b

There are a limited number of studies involving *Alox15b*. In chimeric mice transplanted with *Alox15b*-silenced bone marrow cells in mice lacking LDL receptor showed defective atherogenesis, suggesting that 15-LOX-2 is required in the murine atherosclerotic model [34]. As mentioned previously, the enzymatic action of murine 15-LOX-2 preferentially generates 12S-HPETE rather than 15S-HPETE from arachidonic acid, suggesting that mouse *Alox15b* and human *ALOX12* have similar roles in vivo. The skin of *Alox15b*-deficient mice has been shown to display an ichthyosiform appearance, as in *Alox12b*- and *Aloxe3*-deficient mice [44]. This example clearly shows that the skin phenotype found in *Alox15b*-deficient mice seems to require specific oxidation products from polyunsaturated fatty acids. Furthermore, this result suggests that *Alox15b* could be a functional alternative for *Alox12b* and *Aloxe3*.

4. 12-Lipoxygenase (12-LOX)

12-LOX enzymes derived from *ALOX12* in humans and its murine ortholog *Alox12* (also known as platelet-typed 12S-LOX) produce 12S-HPETE from arachidonic acid. These enzymes are expressed in leukocytes in humans and in platelets, megakaryocytes, and skin in mice [45,46]. Genetic studies have suggested that *ALOX12* gene polymorphisms are associated with cancers [47,48], neurological disorders [49,50], hypertension [51,52], fat mass [53], and bone mineral density [54–57]. The mechanism of regulation of 12-LOX expression has been studied in several models. The *ALOX12* promoter region contains at least four binding sites for RUNX1, leading to its suppressive effect in human erythroleukemic cells [58]. *Alox12* expression is also regulated by transcription factor p63 in the skin, which plays a key role in the development and terminal differentiation of the epidermis [59]. Similar to other isozymes, human 12-LOX has a catalytic domain in the C-terminal. Truncation of the N-terminal domain (which needs to be explored in terms of function) decreases its enzymatic activity at approximately 20% without altering substrate specificity [60].

4.1. ALOX12

Since the discovery of the high expression of LOX in platelets, the mechanism of its expression has been studied in detail. A previous study reported that *ALOX12* expression was attenuated in platelets by the haplodeficiency of RUNX1, a hematopoietic transcription factor associated with familial thrombocytopenia, platelet dysfunction, and a predisposition to acute leukemia in patients

with thrombocytopenia [58]. *ALOX12* expression is also regulated epigenetically, as indicated by the increase in DNA methylation of *ALOX12* genes in myelodysplastic syndrome and acute myeloid leukemia patients with megakaryocytic dysplasia [61,62]. It has been suggested that *ALOX12* is associated with diminished bone mineral density as well [54–57]. Given that 12-LOX produces endogenous lipid ligands for nuclear receptors, such as PPAR-γ, which facilitate adipocyte differentiation from mesenchymal stem cells, the number of osteoblasts decreases, followed by impairment of bone mineral density [55].

An earlier study suggested that the 12-LOX-mediated pathway is associated with the risk of colorectal cancer [63]. The best-studied example includes mutation of E261R (835A > G), which causes an increase in 12-LOX activity, with a potential link to esophageal squamous cell carcinoma [64]. This mutation has also been associated with colorectal cancer [47,65] and breast cancer [48]. An in vitro and in vivo study has shown that 12-LOX plays a role in the proliferation and antiapoptosis of hepatocellular cells, suggesting that this carcinogenic function of *ALOX12* requires endogenously generated lipid mediators [66].

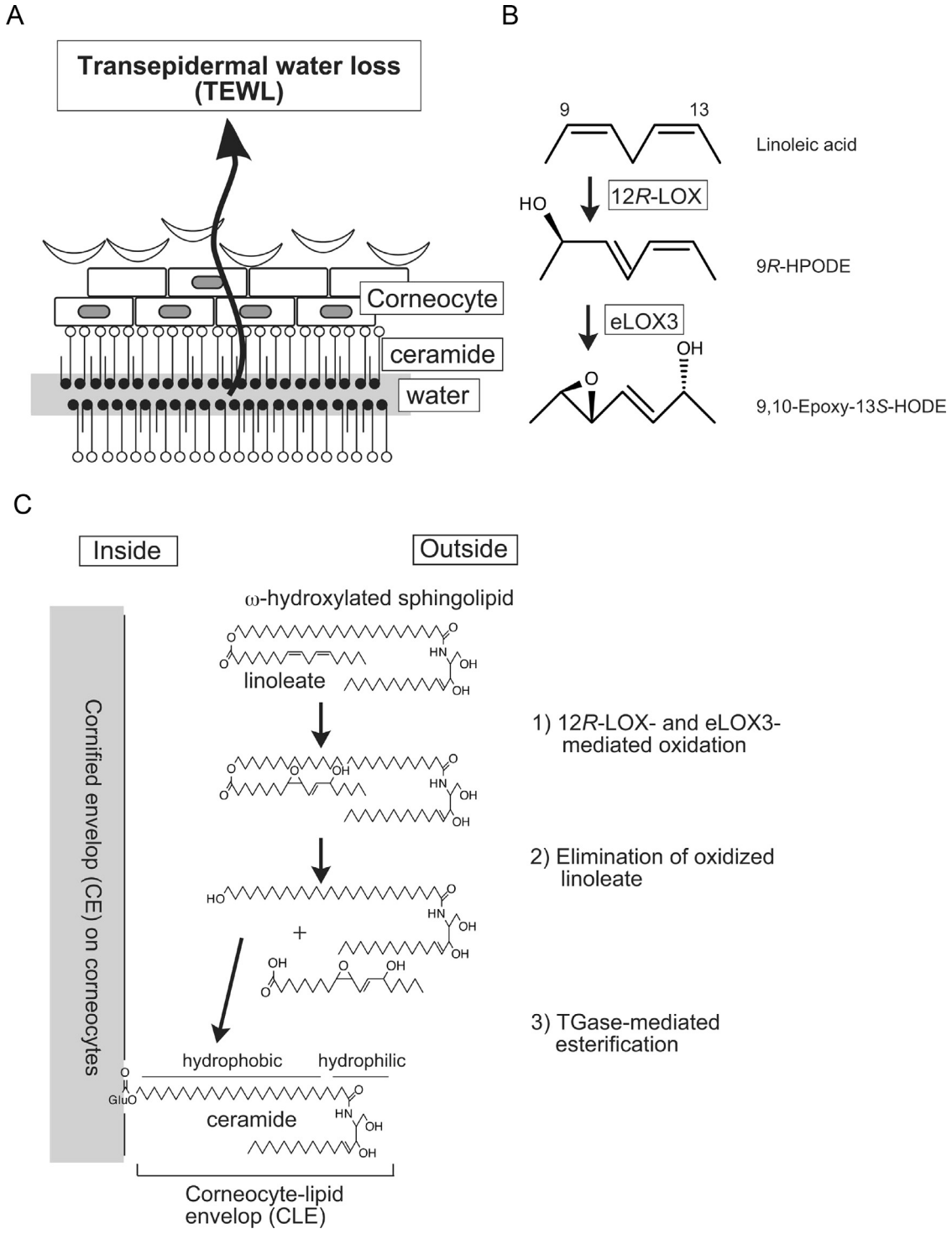

Fig. 1. A. Schematic representation of transepidermal water loss (TEWL) from the skin. B. Reaction of 12R-LOX and eLOX-3. C. Development of corneocyte-lipid envelop (CLE) over cornified envelop (CE) on corneocyetes.

4.2. Alox12

Consistent with the expression of *ALOX12* in the platelets of humans, mice lacking *Alox12* have shown increased platelet sensitivity and mortality due to thrombosis in response to the administration of adenosine diphosphate, whereas aggregation and secretion in response to most agonizts seemed normal [67]. *Alox12* deficiency has led to a reduced incidence of carcinoma in a C57BL6/129 genetic background and of papilloma in a tumor-sensitive SENCAR genetic background, showing that *Alox12* is involved in tumorigenesis in the skin in a context-dependent manner [68]. Most notably, *Alox12* deficiency has caused basal transepidermal water loss in the skin with unaltered inflammatory responses in *Alox12b*- and *Aloxe3*-deficient mice [69]. This finding suggests that *Alox12* has a critical role in the maintenance of the skin barrier in association with other isoforms, as explained in detail later.

5. 12-Lipoxygenase, 12R type (12R-LOX)

In humans, 12R-LOX has been detected in keratinocytes, tonsil squamous epithelial cells, bronchial epithelial cells, and psoriasis scales, as well as in B cells [8,70–74]. In mice, its expression has been induced at embryonic day (E) 15.5 in the epidermis, nasal epithelium, and surface of the tongue, suggesting that 12R-LOX is required for the proper development of neonates [71].

The physiological role of 12R-LOX is rather specific, in conjunction with its limited expression profile in epithelial cells. The best-characterized example is the skin. The important physiological role in the skin is to maintain appropriate moisture by preventing unnecessary water evaporation through epithelial cells, called transepithelial water loss (TEWL) (Fig. 1A). TEWL results in a loss of water from the skin, leading to excessively dry skin, as found in ichthyosis. In humans, this inherited disease is known as autosomal recessive congenital ichthyosis (ARCI) (MIM#s 190195, 242100, 242300). Biochemically, 12R-LOX reacts readily with linoleate rather than arachidonate to produce 9R-HPODE (Fig. 1B). This hydroperoxide is converted into its associated epoxide derivatives through the isomerase activity of eLOX-3 [75]. In the skin, the best substrate for these enzymes is linoleate, which is esterified with ω-hydroxylated sphingolipids, usually found outside corneocytes during the immature stage of skin development (Fig. 1C). As mentioned previously, this esterified linoleate is further converted into oxygenated linoleate, followed by elimination from sphingolipids by hydrolysis. The newly formed ceramide is ω-terminally hydroxylated; therefore, it is subsequently linked covalently to a carboxyl acid moiety of glutamine in cornified envelope (CE) proteins. The established lipid layer is called a corneocyte-lipid envelope (CLE), and it plays a crucial role in holding water in the hydrophobic group, in ceramides in the CLE. The completion of CLE formation requires transglutaminase-1, which catalyzes the cross-linking of the ceramides with the carboxylic acid in the side chain of glutamate in CE proteins, as many mutations of 12R-LOX enzymes cause ARCI in humans [76]. Mice lacking the transglutaminase-1 gene have consistently exhibited defective skin formation and high TEWL [77]. Both enzymes act critically prior to this transglutamination, as previous observations have suggested that a deficiency of 12R-LOX, as well as eLOX-3, causes failure of the skin barrier due to defective formation of enzymatic lipid oxidation products [78–80].

5.1. ALOX12B

Genetic failure of the above process leads to ARCI, a heterogeneous skin disease characterized by rough and scaly skin, with a prevalence of one in 200,000 newborns throughout the world [81–86]. The affected skin generally improves during either childhood or puberty, and these patients have a normal life span. Among various ARCI diseases, *ALOX12B* is mainly, but not solely, involved in nonbullous congenital ichthyosiform erythroderma. Mutations, mostly found as missense, termination, and frameshift in *ALOX12B*, are widely found in its entire molecule, involving both in a C-terminal catalytic domain and an N-terminal β-barrel structure [82]. Some mutants have lost enzymatic activity, as confirmed by biochemical assays.

5.2. Alox12b

Disruption of the *Alox12b* gene in the murine model provides an effective means of studying human ichthyosis. *Alox12b*-deficient mice suffer postnatal death characterized by a severely impaired barrier function of the skin [87]. This defective epidermal barrier appears around E17.5 in wild-type (WT) controls, prior to which the expression of *Alox12b* reaches its maximal level beginning at E15.5 and continues after birth. Thus, there is a strong correlation between *Alox12b* expression and the formation of functional epidermis.

A study using skin transplantation from *Alox12b*-deficient neonates into nude mice revealed ichthyosiform formation, typically characterized by a thickening of the epidermis and severe hyperkeratosis, with a phenotype similar to that of patients with *ALOX12B* mutations in the grafted mice [88]. Essentially, the skin grafted from the neonates became thicker than that from the WT controls, with a hyperplastic histology displaying epidermal acanthosis and severe hyperkeratosis. Further investigation of this hyperkeratosis by electron microscopy revealed that the stratum corneum of skin grafted from *Alox12b*-deficient mice was abnormally overlaid, indicative of aberrant proliferation. In addition, both the size and number of keratohyaline granules increased, indicating hypergranulosis in mutant skin grafts. Functional assays that measured TEWL identified a marked increase in the skin from the neonates, as well as a marginal but significant increase in mature skin grafted in these mice, demonstrating that *Alox12b* plays a critical role in the maintenance of barrier function in the skin (Fig. 1A). Among genetically manipulated mice with defects in barrier function in the skin, such as *KLF4*- and *Claudin*-deficient mice [89,90], *Alox12b*-deficient mice displayed an extremely defective phenotype in epidermal barrier function, but not in tight junctions.

6. Epidermal lipoxygenase 3 (eLOX-3)

As mentioned previously, the conversion of linoleoyl ceramide in CLE into CE plays a crucial role in the proper maintenance of epidermal barrier formation. The eLOX-3 enzyme plays a major role in the second step, which involves the conversion of 9R-HPODE esterified with ω-hydroxyacyl-sphingosine into its related epoxylated derivative (Fig. 1B) [91–93]. Biochemical reactions catalyzed by eLOX-3 are essential, and their failure leads to ARCI.

6.1. ALOXE3

Genetically, many mutations in *ALOXE3*, together with *ALOX12B*, have been found in ichthyosis [81–84,86,94]. The outcome of disease in *ALOXE3* variants seems to be similar to that found in *ALOX12B* variants, showing clearly that this sequential oxidation by *ALOXE3* and *ALOX12B* are equally important. As a substrate, it is known that eLOX-3 favors oxygenated lipids like 12R-HPETE rather than unoxidized compound such as arachidonic acid (Fig. 1B). Other than ARCI, other diseases associated with *ALOXE3* variants

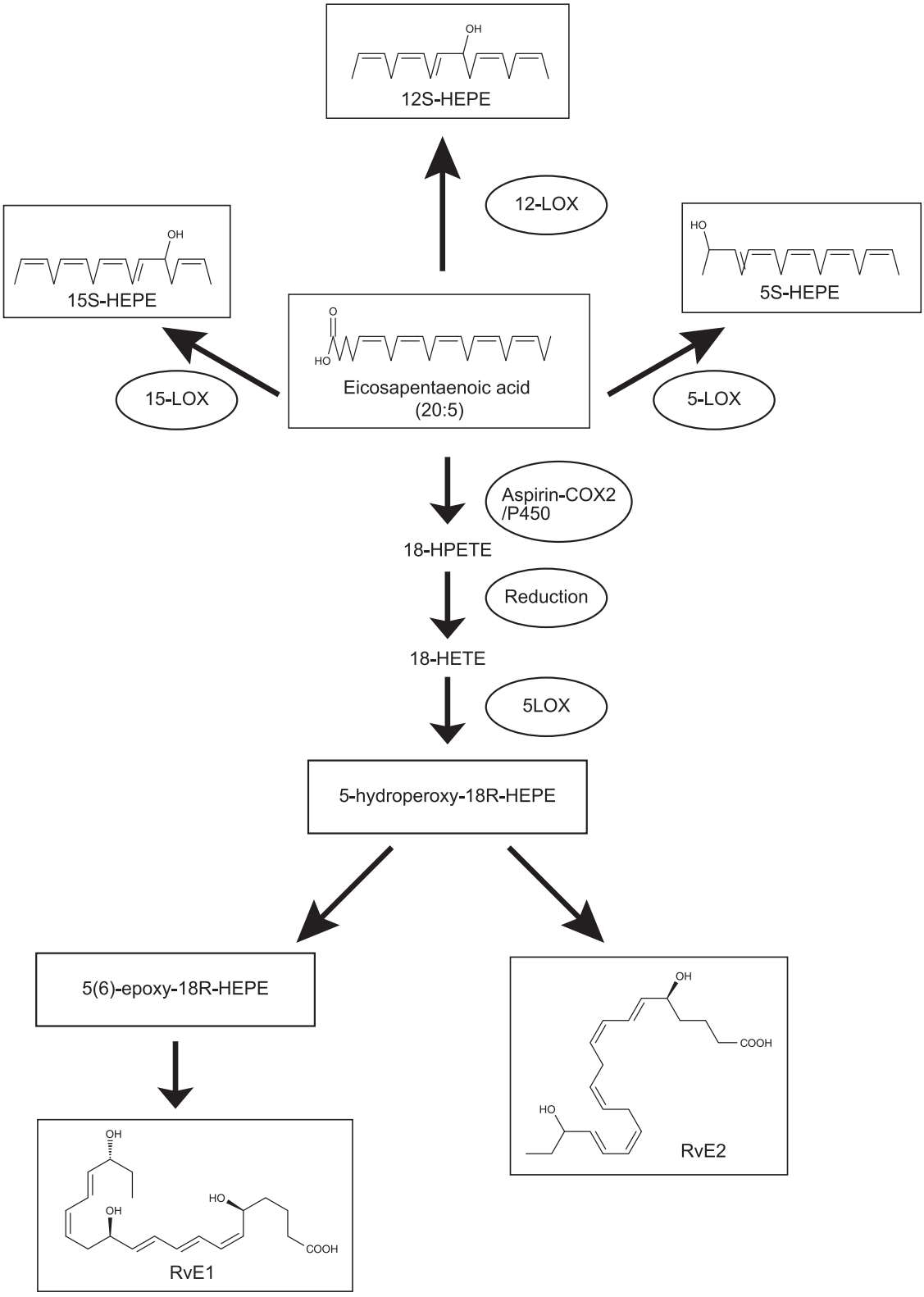

Fig. 2. Formation of resolvins from eicosapentaenoic acid (20:5).

have not been reported.

6.2. *Aloxe3*

A previous study showed that *Aloxe3*-deficient mice also exhibit a similarly severe ichthyosis phenotype with the loss of covalently bound ceramides and impaired CLE development [86]. As expected from its hepoxilin synthase activity of eLOX-3, in vivo results also showed a marked reduction of its metabolites in the skin. Similar to *Alox12b*-deficient mice, *Aloxe3*-deficient mice displayed a postnatal lethal phenotype [95].

7. 5-Lipoxygenase (5-LOX)

5-LOX plays an important role in the control of asthma. Under asthmatic conditions, activated immune cells first produce arachidonic acid by an enzymatic action of phospholipase A_2 from the plasma membrane, followed by 5-HPETE production through the 5-LOX enzyme. The produced 5-HPETE then converts into leukotrienes that have a potent biological effect on the constriction of bronchioles through cysteinyl leukotriene receptor 1, which is expressed exclusively onto bronchiolar smooth muscle cells, but not epithelial cells [96]. Conversely, cysteinyl leukotriene receptor 2 is expressed strongly in pulmonary interstitial macrophages and weakly in smooth muscle cells.

7.1. ALOX5

The expression of the *ALOX5* gene is transcriptionally regulated at a basal level and regulated by external stimuli such as Ca^{2+}. Numerous agents have been developed as antiasthmatics. A potent LOX-5 inhibitor zileuton introduced in the United States blocks a considerable amount of cysteinyl leukotriene production for a short period of time. Although the prevalence is low, a genetic E254K (760G > A) mutation in *ALOX5* has been reported in bronchiolar asthma patients [97]. This mutation causes an alteration in the electronic charge of the C-terminal catalytic domain from negative to positive, implicating defective changes in enzymatic activity and protein interaction. Mutations in the Sp1 binding site in the *ALOX5* promoter has been associated with airway hyperresponsiveness, but not with asthma [98,99]. Some evidence suggests that low 5-LOX expression in tumors found in humans might lead to greater 15-LOX expression followed by cancer formation through impaired apoptotic activity [47,100,101].

7.2. Alox5

7.2.1. Inflammation

As mentioned previously, immune cells express leukotriene receptor 1 and 2 as well as cysteinyl leukotriene receptor 2; as such, the role of *Alox5* could account for the migration of these immune cells. As expected, neutrophilic inflammation is one of the apparent phenotypes in *Alox5*-deficient mice. These mice have been shown to be resistant to anaphylaxis induced by platelet-activating factor (PAF), showing that *Alox5* seems to be closely involved in this process [102,103]. Similarly, *Alox5*-deficient mice exhibit a suppressed response to chemically induced local inflammation [104,105]. These mice are also susceptible to *Borrelia burgdorferi*-induced arthritis [106]. In this study, the authors showed that enzymatic activity of 5-LOX is not required for the initiation of infection, but it is required for earlier joint swelling and retarded arthritis recovery, suggesting a potential increase in the accumulation of neutrophils. Similarly, in a *Toxoplasma gondii* infection model, *Alox5*-deficient mice exhibited suppressed leukotriene A_4 production and increased interleukin-12 and interferon-γ production, followed by an increase in mortality rate due to marked encephalitis [107]. Such altered cytokine production seems to be explained, at least in part, by impaired neutrophilic inflammation.

In an ovalbumin-induced asthma model, *Alox5*-deficient mice exhibited a suppressed methacholine-induced response to airway hyperresponsiveness with impaired eosinophilic inflammation in the lung [108]. Thus, the production of lipid products from 5-LOX plays an important role under physiological conditions, and its level is tightly regulated by the balance between LOX and other enzymes, such as COX, as shown in an earlier study. Another study reported an increased mortality rate in *Alox5*-deficient male mice with an autoimmune-prone MRL-lpr/lpr genetic background, raising the possibility that an enhanced renal autoimmune inflammation could be involved in this process [109].

Recently, the roles of oxidation products derived from eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6) have been studied extensively (Fig. 2). As seen in their chemical structures, 20:5 and 22:6 have four and five bisallylic carbon atoms, respectively, in their molecules, providing a variety of oxidation products through free radical-mediated mechanisms. For example, 20:5 is a primary substrate for conventional LOX enzymes such as 15-LOX, 12-LOX, and 5-LOX. In addition, 18-hydroperoxy-5,8,11,14,16-eicosapentaenoic acid (18-HPETE), a free radical-mediated oxidation product of 20:5, can be further metabolized by 5-LOX to produce a novel class of oxidation product collectively called resolvins (Fig. 2) [110,111]. Emerging evidence has shown that resolvins assist in terminating inflammation through specific GPCR ChemR23 at nM concentrations in vitro [112]. The formation of resolvins seems to be critically regulated by local O_2 concentration, as well as the expression and activity of multiple LOX enzymes, both of which influence the final yield of resolvins from its initial substrate 20:5. Due to its anti-inflammatory function, whether these oxidized lipids might modulate the functions of microRNAs is under investigation [113].

7.2.2. Atherosclerosis

Apart from reactions in asthma and neutrophilic inflammation, there is some research showing that 5-LOX plays a key role in the initiation and/or development of atherosclerosis. Impaired expression of functional 5-LOX in LDL receptor-deficient mice has revealed suppressed atherogenesis, suggesting that 5-LOX plays a causative role in this disease [114]. Consistently, another atherosclerotic model induced by COX-2 disruption attenuated disease formation in *Alox5*-deficient mice [115]. Given that LOX-15 is involved in atherosclerosis, these studies provide examples that atherosclerosis can be induced by lipid peroxidation products formed from any isoforms. This finding is entirely consistent with observations indicating that antioxidants generally exhibit protective effects in experimental models.

7.2.3. Neuronal disorder

Alox5 is known to be highly expressed in neuronal tissue, particularly in Alzheimer's disease; thus, its role in neuronal disorders has been actively characterized [116]. A recent study reported that aged female *Alox5*-deficient mice exhibited protective effects against anxiety-like behavior on a C57BL/6 genetic background, raising the possibility that *Alox5* could modulate neuronal function [117]. A subsequent study using a transgenic mouse model of Alzheimer's disease demonstrated the efficacy of a 5-LOX inhibitor zileuton and hypothesized that *Alox5* could facilitate the initiation or progression of this disease [118]. Using such a disease model, *Alox5* deficiency consistently improved disease phenotypes [119].

7.2.4. Tumor

Colorectal cancer is often caused by mutation of the tumor suppressor Adenomatous polyposis coli (*APC*) gene. Among many mouse models generated by *Apc* mutations, $Apc^{\Delta 468}$ mice specifically bear a truncated *Apc* gene that develops severe polyposis by four months [120]. Interestingly, immunohistochemistry showed an increase in *Alox5* expression in $Apc^{\Delta 468}$ mice, suggesting that LOX-5 might contribute to tumorigenesis in colorectal cancer [121–123]. Mast cells play an important role in the development of colorectal cancer in this animal model, as they induce epithelial proliferation [122]. Consistently, the number of mast cells increased in $APC^{\Delta 468}$ mice compared to WT controls. In this model, a deficiency in *Alox5* led to impairment, implying that LOX5 acts as an important role in colorectal tumorigenesis [122].

8. 5-Lipoxygenase activating protein (FLAP)

FLAP is a small protein that activates 5-LOX through protein interaction. The formed complex of 5-LOX and FLAP in the nucleus efficiently generates 5-HEPE from arachidonic acid, which is subsequently converted into various leukotrienes. FLAP protein stays on the nuclear membrane and acts as a transporter for 5-LOX. FLAP expression is limited in myeloid cells.

8.1. *ALOX5AP*

Drugs targeting FLAP protein have been actively developed in humans. DG-031 (veliflapon, BAY x 1005), first licensed by DeCode Genetics and then developed by Bayer, is one example. This compound also reduces the incidence of ischemic myocardial infarction by reducing LTB_4 production [124]. A recently developed AM-103/GSK2190914 was designed based on the three-dimensional structure of FLAP protein [125]. In asthma, two intronic single-nucleotide polymorphisms have been associated with *ALOX5AP*, suggesting that these mutations can be used for diagnostic markers [126].

8.2. *Alox5ap*

Alox5ap-deficient mice exhibited unique phenotypes similarly observed in *Alox5*-deficient mice, such as an impaired response to PAF-induced anaphylaxis and zymosan-induced peritonitis [127]. In a collagen-induced arthritis model, *Alox5ap*-deficient mice displayed impaired arthritis, whereas the accumulation of antibody against collagen remained unchanged, suggesting that 5-LOX positively regulated inflammation without affecting the immune response. In a cerebral artery occlusion model, disruption of the *Alox5ap* gene caused impaired median infarct size and a better functional score, demonstrating that FLAP protein positively regulates cerebral inflammation [128]. The expression of *Alox5ap* is independent of the expression of 5-LOX. *Alox5ap* induces 12S-HETE production in the 12-LOX-induced signaling pathway, suggesting that 5-HPETE or downstream leukotriene metabolites might be involved in this process [129].

In atherosclerosis, FLAP inhibitor MK-886 and BAY x 1005 effectively attenuated disease formation in mice lacking *ApoE* and LDL receptors [130,131]. Similarly, another atherosclerotic model developed by transgenic mice expressing a dominant negative form of TGFβ receptor II in *ApoE*-deficient mice was suppressed by MK-886 [132]. These examples strongly suggested that FLAP protein is required for atherogenesis in these mouse models. Consistently, *Alox5ap*-deficient mice exhibited attenuated disease formation in an experimental model generated by *Cox-2*-deficient mice [115].

Alox5ap-deficient mice displayed an improved Alzheimer's disease-like phenotype [133] and an apparent increase in anxiety-like behavior in aged mice [134]. These results supported the phenotype of *Alox5*-deficient mice, showing that both 5-LOX and FLAP mutually and collaboratively play critical roles in the leukotriene pathway in neurological disorders.

9. Clinical Trials

There are many studies reporting synthesis and characterization of lipoxygenase inhibitors (reviewed in [135,136]). Generally, substances sharing similarity to either lipids or phenolic antioxidants have lower inhibitory activity for LOX enzyme. Furthermore, due to the presence of multiple isoforms, the development of selective inhibitor seems to be challenging. Therefore, there is a limited number of successful drugs that can be used for therapeutic purposes. One clinically applicable example includes zileuton that acts as anti-asthmatic agent by inhibiting 5-LOX. Apart from direct regulation of enzymes, there are some studies targeting FLAP for modulating 5-LOX under pathophysiological conditions in clinical trials.

9.1. *GSK2190915*

This is a FLAP inhibitor that inhibits the production of LTB_4 and other cysteinyl leukotrienes. The results of Phase I study showed that there was no clear difference in adverse events between placebo and drug-treated subjects in Western Europe (EUDRACT2007-00484872) and Japan (NCT00955383) [137]. Plasma concentration of GSK2190915 reaches maximal at two hours after oral administration. Consistently, LTB_4 production in drug-treated subjects was significantly impaired (EC_{50} in plasma is approximately 85 nM). Its beneficial effect is proven in adults and adolescents with persistent asthma (NCT01147744) [138]. Comparison between GSK2190915 and established asthma treatment such as montelukast and the inhaled corticosteroid fluticasone propionate revealed that GSK2190915 30 mg once daily has similar effect compared to montelukast 10 mg once daily as assessed by forced expiratory volume in 1 s (FEV_1), a widely used measure for asthma evaluation. A subsequent study reported that GSK2190915 50 mg daily showed clear attenuation in early asthmatic response induced by inhaled allergens in a placebo-controlled double-blind randomized study in UK (NCT00812773) [139].

10. Conclusions

The physiological roles of LOX enzymes have been studied extensively, due to the close link between them and various diseases. Apparently, the best characterized example includes the relation between 5-LOX and asthma, because cysteinyl leukotriene receptor 1, a GPCR activated by leukotrienes produced by 5-LOX, is widely expressed on bronchiolar smooth muscle cells. Genetically, both *ALOX12B* and *ALOXE3* play a critical role in the development of ichthyosis through TEWL. In humans, some mutations in *ALOX12* are found in tumor cells, suggesting this isoform might have anti-tumor effect. An increased expression of LOX enzymes in response to Th2 cytokines has been well established; how their expression is controlled and its consequences need to be investigated in the future.

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