15-Lipoxygenase-1 in the Vasculature
Expanding Roles in Angiogenesis

Naoki Mochizuki, Young-Guen Kwon

Lipoxygenases (LOs) constitute a heterogeneous family of enzymes that catalyze the stereoselective dioxygenation of polyunsaturated fatty acids to their corresponding hydroperoxy derivatives.1,2 In mammals, LOs are categorized with respect to their positional specificity of arachidonic acid oxygenation into 5-, 8-, 12-, and 15-LOs.1–3 These enzymes induce structural and metabolic changes in cells during a wide variety of physiological and pathological processes, such as differentiation, carcinogenesis, inflammation, and atherogenesis. Accumulating studies have suggested diverse and opposing roles for the various LO pathways in the pathogenesis of human diseases, particularly cancer and atherosclerosis.2–5 Consistent with the existence of multiple pathways and opposing roles for the various LO pathways in the pathogenesis of human diseases, including differentiation, carcinogenesis, inflammation, and atherogenesis. Accumulating studies have suggested diverse and opposing roles for the various LO pathways in the pathogenesis of human diseases, particularly cancer and atherosclerosis.2–5 Consistent with the existence of multiple isoforms of LOs, a variety of intermediate and end products of arachidonic acids are found in various cell types, and they in turn activate diverse signaling cascades, resulting in diverse outcomes. Therefore, sophisticated understanding of the expression pattern of individual isozymes in the target cells and biological actions of the corresponding metabolites should be prerequisite for predicting their roles in disease processes.

Among the 4 mammalian LO subfamilies, 15-lipoxygenase-1 (also known as 12/15-lipoxygenase in mice) catalyzes the transformation of free arachidonic acid to 12-hydroperoxy-eicosatetraenoic acid and 15-hydroperoxyeicosatetraenoic acid and the transformation of linoleic acid to 13-hydroperoxyoctadecenoic acid (13-HPODE) (the reduced product is 13-HODE).3,4 Both human 15-LO-1 and mouse 12/15-LO enzymes also metabolize more complex lipids, including phospholipids, cholesterol esters, and plasma lipoproteins. 15-LO-1 was considered initially to be the reticuloocyte LO that can react with mitochondrial membrane lipids,6 but subsequently, a constitutive expression of 15-LO-1 has been reported in various types of cells, such as immature red blood cells, eosinophils, and airway epithelial cells.3 In addition, the eicosanoid products of 12/15-LO have been detected in vascular cells, and 15-LO-1 protein has been shown to be localized to aortic atherosclerotic lesions in rabbits and in humans.3,5 Moreover, its induction has been observed in human monocytes, macrophages, and human lung carcinoma cell line A549 by stimulation with interleukin (IL)-4 or IL-13.3 The level of 15-LO-1 appears to be altered during the tumor development.4 Such expression profiles in a wide variety of cells, together with its enzymatic potential to produce reactive oxygen species and lipid hydroperoxides, have raised the possibility that 15-LO-1 and its products may be crucially involved in many pathological conditions, such as asthma, inflammation, atherogenesis, and carcinogenesis. However, the evidence to date is controversial if not contradictory, and the underlying molecular mechanisms of 15-LO-1 in certain disease processes remain far from clear.

The biological relevance of 15-LO-1 in the vascular system has been widely explored and supported by its expression in vascular cells and the results from functional studies. Specifically, expression and function of 15-LO-1 have been studied in endothelial cells (ECs), smooth muscle cells, and monocytes as well as atherosclerotic animal models, and 15-LO-1 has been shown to play active roles in vascular remodeling and the progression of atherosclerosis.2,3,5,7,8 Although the directly relevant data are limited, 15-LO-1 has also been implicated for its role in angiogenesis. In this issue of Circulation Research, Viita et al assign a new role for 15-LO-1 as a regulator of angiogenesis by demonstrating its antiangiogenic action in rabbit skeletal muscle system.9 They have previously developed an adenovirus-mediated gene transfer method into the rabbit hindlimb model and shown that vascular endothelial growth factor (VEGF)-A induces strong angiogenic effects in skeletal muscles.10 In this study, they show that coadministration of 15-LO-1 significantly decreases all angiogenic effects induced by VEGF-A and placental growth factor (PIGF), including capillary perfusion, vascular permeability, vasodilatation, and the increase in capillary number.

However, the role of 15-LO-1 in angiogenesis still remains controversial and requires careful scrutiny. Several studies in tumor models have shown that the enzyme and its products could promote or inhibit neovascularization. The PC-3 human prostate cancer cell line, which overexpresses 15-LO-1, secretes high levels of VEGF and enhances tumor growth and angiogenesis as compared with the parental PC-3 cell lines.11 In contrast, angiogenesis and tumor formation in 2 xenograft models is inhibited in transgenic mice overexpressing 15-LO-1 in ECs under the control of preproendothelin promoter.12 The angiogenic process is complex and occurs in multiple steps coordinated by integrated action of various cells including ECs and their surrounding cells. Considering the complexity of angiogenic process, elucidation of the precise mechanism of action of 15-LO-1 in angiogenesis probably requires evaluating signaling and secondary chem-
expression of VEGFR2 and other angiogenic signaling molecules. The dotted arrow indicates the potential involvement of transcriptional regulation.

Figure. Overview of potential roles of 15-LO-1 in the process of neovascularization. 15-LO-1 appears to regulate neovascularization in multiple ways, working in ECs and neighboring cells. Based on the findings obtained by Viita et al., 9 15-LO-1 in neighboring cells is likely to regulate the mRNA levels of angiogenic factors such as VEGF. In ECs, 15-LO-1 blocks VEGF-induced angiogenesis and vascular permeability by reducing eNOS expression and bioavailability of NO. Conversely, the enzymatic activity of 15-LO-1 can be modulated by NO. Furthermore, 15-LO-1 and its products inhibit VEGF-induced expression and activity of PPAR-γ, which modulates expression of VEGFR2 and other angiogenic signaling molecules.

Viita et al have further examined the mechanisms of action with biochemical and histological analyses and proposed 3 plausible mechanisms to explain the anti-angiogenic action of 15-LO-1 in their animal studies (Figure). They first demonstrate that 15-LO-1 suppresses the expression of VEGF-A and PlGF mRNAs. An RT-PCR analysis showed that the expression of the transduced VEGF-A165 and PlGF-2 mRNAs in both the rabbit muscles and cultured human ECs were significantly reduced when transduced in combination with 15-LO-1 compared with when transduced alone. It is intriguing that coadministration of 15-LO-1 reduced human VEGF-A165 and mouse PlGF-2 mRNAs generated from constructs containing only the protein-coding regions under the control of the cytomegalovirus promoter but not the endogenous promoters. As suggested by the authors, the most likely explanation is that 15-LO-1 expression leads to destabilization of the coding sequences of the corresponding transcripts. Given that 15-LO-1 overexpressed in PC-3 cells has been shown to increase VEGF expression,11 further determination of the precise underlying mechanism is required for clarifying the role of 15-LO-1 in regulating expression of angiogenic factors. Interestingly, it has been shown that 15-LO-1 and 13-HODE levels are decreased in various human cancer tissues, including colon, breast, and pancreas, compared with the corresponding normal tissues.4,13,14 Considering that in most cases, VEGF expression is correlated with tumor malignancy and increased under the hypoxic conditions at tumor and ischemic sites, it should be important to determine whether the level of 15-LO-1 is correlated with the level of VEGF in such hypoxic environments.

The present study also addresses the potential link between 15-LO-1 and endothelial nitric oxide synthase (eNOS) expression and NO bioavailability in the vascular wall. Endothelium-derived NO promotes angiogenesis by directly stimulating proliferation, migration, and tube formation of ECs and plays an important role in vascular remodeling and the maintenance of vascular integrity.15,16 In this article, the authors propose a potential role of 15-LO-1 in regulating endothelium-derived NO, based on the data that Adh15-LO-1 inhibits AdhVEGF-A–induced eNOS expression and reduces bioavailability of NO. Given that the level of 15-LO-1 is induced by other cytokines, such as IL-4 and IL-13, and is altered under certain cellular contexts, it seems possible that the expression level and activity of 15-LO-1 in ECs may act as a potential NO barometer by modulating the level of eNOS enzyme and bioactive free NO in ECs. The finding that 15-LO-1 reduces eNOS expression is predictable because 15-LO-1 could block expression of VEGF, which is known to increase eNOS transcription in ECs. An alternative explanation is that the products of 15-LO-1 may suppress signaling to the promoter of the eNOS gene, which can also account for the reduction of eNOS activity. It is notable that the reciprocal relationship between 15-LO-1 and NO in the endothelium. 12/15-LO is known to be present in mammalian cells as inactive ferrous enzyme species and requires activation to the ferric form by hydroperoxy activators.3 Biochemical studies have shown that NO reacts with the 12/15-LO–bound lipid peroxyl radical. In this reaction, 2 moles of NO are consumed per mole of enzyme, and interaction of 12/15-LO with NO is most likely to result in the inhibition of its enzymatic activity.3 Therefore, it is very appealing that 15-LO-1 in human ECs can inhibit angiogenesis and vascular permeability by removing free NO, and its activity can in turn be modulated by the cytoplasmic NO level.

Another important mechanistic implication of the results from this study is the regulatory effect of the products of 15-LO-1 on peroxisome proliferator-activated receptor (PPAR)-γ and VEGFR2 expression. 12/15-LO has been shown to generate endogenous ligands for PPAR-γ.17 Endogenous and chemical ligands of PPAR-γ have been reported for their roles in angiogenesis, but the results have been controversial. In bovine ECs and cultured cardiac myofibroblasts, the PPAR-γ activators induce VEGF and VEGFR2 expression, thereby stimulating angiogenesis in vitro.18,19 In contrast, choroidal neovascularization induced by VEGF is inhibited by the PPAR-γ ligands.20 Xin et al.21 have also shown that PPAR-γ ligands suppress VEGFR1 and VEGFR2 expression in human umbilical vein ECs (HUVECs). In contrast, Meissner et al.22 have reported that VEGFR2 expression is inhibited by PPAR-α but not by PPAR-γ agonists in HUVECs. The present study by Viita et al further demonstrates that VEGF induces PPAR-γ expression and hypothesizes that 13-HODE, a product of 15-LO-1, becomes a ligand

critical reactions mediated by the 15-LO-1 products in the endothelium of the vessel wall and in neighboring cells in exquisite detail.

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for PPAR-γ and inhibits VEGF-induced VEGFR2 expression presumably through activated PPAR-γ binding to the promoter region of VEGFR2. Therefore, overexpression of 15-LO-1 would result in reduction of VEGFR2 expression and inhibition of angiogenesis. Given that the products of the 12/15-LO stimulate both transactivation and transrepression activity of PPAR-γ in a target promoter context–dependent manner,17 the hypothesis by the authors is appealing but is still limited in resolving the present discrepancy. It should be noted that ligand-induced PPAR-γ activity can be modulated by the existence of other PPARs and that pathological angiogenesis is a complex multicellular event involving ECs, smooth muscle cells/pericytes, and macrophages. Therefore, how 15-LO-1 is regulated in these cells, as well as how PPAR-γ is implicated in the transcription of specific angiogenic molecules, should be further dissected. Also, identification of the native ligand should help in understanding LO-regulated PPARs. Because the chemical PPAR-γ ligands thiazolidinediones have been used widely for the treatment of type 2 diabetic patients, many of whom experience vascular diseases, clarifying the precise role of PPAR-γ in neovascularization should be clinically valuable.

In summary, Viita et al have demonstrated succinctly the inhibitory effect of 15-LO-1 on VEGF- or PI GF-induced angiogenesis. Further analyses of upstream signals that activate LO family members in vascular cells and inflammatory cells will contribute to understanding how LOs function in physiological and pathological angiogenesis.

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Disclosures

None.

References


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