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Authors
Sharma, Salil
Ruffenach, Grégoire
Umar, Soban
et al.

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Role of oxidized lipids in pulmonary arterial hypertension

Salil Sharma,[1] Grégoire Ruffenach,[1] Soban Umar,[1] Negar Motayagheni,[1] Srinivasa T. Reddy,[2] Mansoureh Eghbali[1]

[1]Division of Molecular Medicine, Department of Anesthesiology, David Geffen School of Medicine at UCLA, Los Angeles, California, USA; [2]Division of Cardiology, Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, California, USA

Abstract: Pulmonary arterial hypertension (PAH) is a multifactorial disease characterized by interplay of many cellular, molecular, and genetic events that lead to excessive proliferation of pulmonary cells, including smooth muscle and endothelial cells; inflammation; and extracellular matrix remodeling. Abnormal vascular changes and structural remodeling associated with PAH culminate in vasoconstriction and obstruction of pulmonary arteries, contributing to increased pulmonary vascular resistance, pulmonary hypertension, and right ventricular failure. The complex molecular mechanisms involved in the pathobiology of PAH are the limiting factors in the development of potential therapeutic interventions for PAH. Over the years, our group and others have demonstrated the critical implication of lipids in the pathogenesis of PAH. This review specifically focuses on the current understanding of the role of oxidized lipids, lipid metabolism, peroxidation, and oxidative stress in the progression of PAH. This review also discusses the relevance of apolipoprotein A-I mimetic peptides and microRNA-193, which are known to regulate the levels of oxidized lipids, as potential therapeutics in PAH.

Keywords: metabolism, oxidative stress, pulmonary hypertension.

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Pulmonary arterial hypertension (PAH) is a rare but fatal disease, characterized by persistent elevation in pulmonary artery pressures.[1,2] PAH is also a serious complication of several connective-tissue diseases, including systemic lupus erythematosus, progressive systemic sclerosis, and rheumatoid arthritis.[3,4] PAH could also be associated with pulmonary thromboembolism, portal hypertension, HIV infection, hepatitis C infection, intravenous drug abuse, and various other pulmonary disorders.[5-9] Many cellular and genetic events are involved in the pathogenesis of PAH. PAH is associated with marked vascular injury caused by endothelial dysfunction of small pulmonary arteries, promoting vasoconstriction. In addition, structural abnormalities, excessive hypertrophy of smooth muscle cells lining the arterioles, endothelial cell proliferation resulting in plexiform lesions, extracellular matrix remodeling leading to fibrosis, and activation of inflammatory cells vastly contribute to the pathogenesis of the disease. All these vascular changes increase the afterload on the right ventricle, leading to right ventricular (RV) hypertrophy, decompensation, and failure.[1] There are many complex processes and events that culminate in the development and progression of PAH. This has resulted in the advancement of many therapies targeting the endothelin 1, phosphodiesterase, nitric oxide, and prostacyclin pathways to slow the progression of the disease.[10-14] This review specifically focuses on the role of oxidative stress, lipid oxidation, and peroxidation as contributing factors in PAH. Finally, this review discusses the emerging potential of high-density lipoprotein (HDL) mimetic peptides, which bind to oxidized lipids with high affinity, as well as microRNA-193, which targets oxidized lipids, as novel therapeutics.

LIPID AND LIPOPROTEIN METABOLISM IN PAH

PAH is associated with an increase in oxidative stress participating in the oxidation of lipids. Oxidized lipids participate in many pathophysiological hallmarks of PAH, including smooth muscle cell (SMC) proliferation, endothelial cell (EC) apoptosis, and inflammation (Table 1).[53-55]

Role of oxidized fatty acids produced from arachidonic acid via the lipoxygenase pathway in PAH

In this section, we focus on oxidized lipids in PAH generated from linoleic acid (LA) and arachidonic acid (AA). LA can be oxidized by 5-lipoxygenase (5-LOX) and 15-LOX to form 9-hydroxyoctadecadienoic acid (9-HODE) and 13-HODE, respectively (Fig. 1*A*). Involvement of these oxidized products as contributing factors in oxidative stress in PAH has recently been demonstrated.[16,28,29,56,57] LA is also the precursor of AA, which by enzymatic oxidation gives rise to different oxidized lipids, including 5-hydroxyeicosatetraenoic acid (5-HETE), 12-HETE, and 15-HETE (Fig. 1*A*). These three oxidized metabolites are found to be elevated in the lung tissue samples obtained from patients with primary pulmonary hypertension (PH).[58] The 12-HETE levels are increased in the lung SMCs cultured from hypoxia-treated rats, and exogenous 12-HETE treatment stimulated proliferation of SMCs.[17] Similarly, 15-HETE plays an important role in hypoxic PAH.[59] Increased activity of 15-LOX in pulmonary arteries upon exposure to hypoxia catalyzes and enhances 15-HETE production.[57] Vascular remodeling in hypoxic PAH is in part mediated by a positive feedback regulatory loop between 15-HETE and hypoxia-inducible

Address correspondence to Dr. Mansoureh Eghbali, UCLA School of Medicine, Department of Anesthesiology, BH-160CHS, Los Angeles, CA 90095-7115, USA. E-mail: meghbali@ucla.edu.

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Table 1. Role of oxidized lipids and lipid metabolism in pulmonary hypertension

PAH hallmarks	Effect	References
HETEs and HODEs		
Vascular remodeling	Activation of PDGF/15-LOX/15-HETE axis	15, 16
PASMC proliferation	12-LOX/12-HETE and 15-LOX/15-HETE axis activates ERK1/2 pathway	17, 18
Resistance to apoptosis	15-HETE activates ERK1/2, PI3K/Akt, and ROCK/iNOS pathways	19–22
Vasoconstriction	15-HETE inhibits expression of $K_v1.5$, $K_v2.1$, and $K_v3.4$ and activates Rho/ROCK signaling	23–27
Angiogenesis	15-HETE activates ROCK pathways promoting angiogenesis	16
Fibrosis	15-HETE activates TGF-β/Smad2/3 axis	28
Inflammation	Increased LDL/HDL inflammatory index	29
Leukotrienes		
PASMC proliferation	LTB_4 activates BLT1 receptor	30
Inflammation	Macrophages LTB activates LOX enzyme	31, 32
Epoxyeicosatrienoic acid (EET)	8,9-, 11,12-, 14,15-EET promote JNK1/2 activation in PAECs	33
Resistance to apoptosis		
Endothelial dysfunction		
Plexiform lesions		
Lipids metabolism		
Vascular remodeling	Impaired PPARγ signaling	34–38
Mitochondrial dysfunction	Inhibition of fatty acid oxidation promotes mitochondria hyperpolarization	39–46
Vasoconstriction		16, 47–51
Endothelial dysfunction	Oxidized LDL activates NF-κB pathways	52

Note: ERK: extracellular signal–regulated kinase; HDL: high-density lipoprotein; HETE: hydroxyeicosatetraenoic acid; HODE: hydroxyoctadecadienoic acid; iNOS: nitric oxide synthase; JNK: c-Jun N-terminal kinase; LDL: low-density lipoprotein; LOX: lipoxygenase; LTB: leukotriene B; PAECs: pulmonary artery endothelial cells; PAH: pulmonary arterial hypertension; PASMC: pulmonary artery smooth muscle cell; PDGF: platelet-derived growth factor; PI3K: phosphoinositide 3-kinase; PPARγ: peroxisome proliferator–activated receptor γ; ROCK: Rho-associated protein kinase; TGF: transforming growth factor.

factor 1α (HIF-1α), a critical oxygen-sensing transcription factor in PAH.[60] Increased levels of 15-HETE stimulate proliferation of pulmonary artery SMCs (PASMCs);[18] pulmonary arterial vasoconstriction via K^+ channels and the protein kinase C (PKC) signal transduction pathway;[23-26] the Rho/Rho-associated protein kinase (Rho/ROCK) signaling pathway;[27] inhibition of apoptosis in PASMCs mediated via several signaling pathways, such as ERK1/2 (extracellular signal–regulated kinases),[19] PI3K (phosphatidylinositol 3-kinase)/Akt,[20] the ROCK pathway,[21] and the nitric oxide synthase (iNOS) pathway;[22] and pulmonary vascular remodeling mediated via platelet-derived growth factor.[15] In addition, 15-LOX/15-HETE induces the p38 MAPK (mitogen-activated protein kinase)–dependent transforming growth factor (TGF) β1/Smad2/3 intracellular signaling pathways to mediate vascular fibrosis in the adventitia of the pulmonary arterial wall, resulting in pulmonary artery remodeling.[28] Also, 15-HETE has been shown to mediate vascular medial hypertrophy and EC migration and angiogenesis contributing to hypoxic PH.[16] A mutual positive regulatory mechanism exists between telomerase reverse transcriptase and the 15-LOX/15-HETE pathway that could mediate migration, proliferation, and cell cycle distribution of PASMCs in hypoxia-induced pulmonary vascular remodeling.[61] Inhibition of 5-LOX by diethlycarbamazine, an enzyme responsible for 5-HETE and leukotriene synthesis (Fig 1*B*), has been demonstrated to improve PAH in the Sugen/hypoxia rat model.[62] Indeed, inhibition of 5-LOX improves RV function by decreasing RV systolic pressure (RVSP) and hypertrophy. Al-Husseini et al.[62] demonstrated that this improvement is mediated by a decrease in inflammation and pulmonary vascular wall thickness. Furthermore, we recently demonstrated that PH is associated with elevated plasma levels of 5-HETE, 12-HETE, 15-HETE, 9-HODE, and 13-HODE in the monocrotaline (MCT)-induced PH model in rodents.[56] We also reported significantly higher levels of plasma HETEs and HODEs in PAH patients

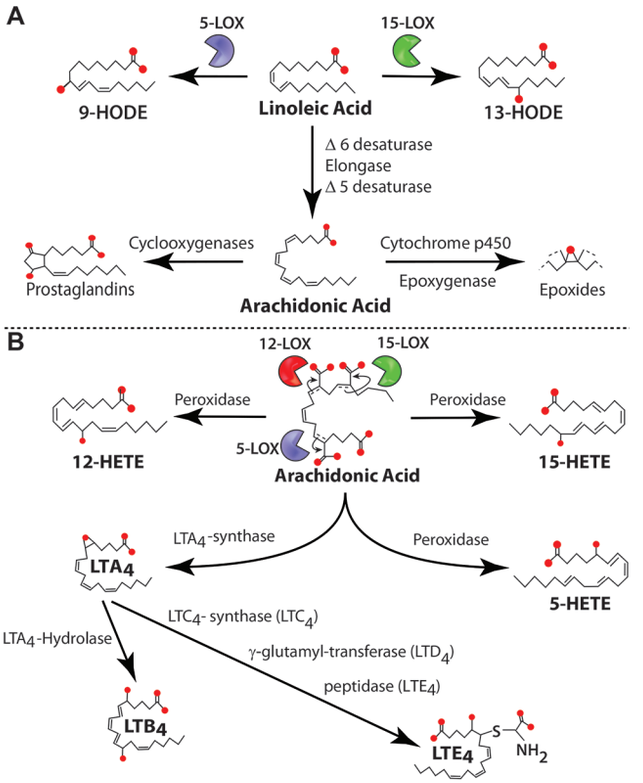

Figure 1. Pathways involved in linoleic and arachidonic acid metabolism. *A*, Linoleic acid is metabolized by 5- and 15-lipoxygenase (LOX) to form 9-hydroxyoctadecadienoic acid (9-HODE) and 13-HODE, respectively. Linoleic acid can also be metabolized to arachidonic acid. In turn, arachidonic acid is used to form prostaglandins (by cyclooxygenases) and epoxides (by the cytochrome p450 epoxygenase pathway). *B*, Arachidonic acid can also be metabolized to 5-hydroxyeicosatetraenoic acid (5-HETE), 12-HETE, and 15-HETE by LOXs and to leukotrienes (LTs) such as LTA_4 (by LTA_4-synthase), LTB_4 (by LTA_4-hydrolase), LTC_4 (by LTC_4-synthase), LTD_4 (by γ-glutamyl-transferase), and LTE_4 (by peptidase).

(idiopathic and associated PAH secondary to connective-tissue disease).[29] Conversely, we showed that increasing oxidized lipids in vivo by feeding mice with 15-HETE leads to PH, establishing a strong connection between the levels of oxidized lipids and the pathophysiology of PH.[56] Collectively, these studies reinforce a major role of oxidized lipids in the development of PH (Fig. 2).

Role of other metabolites of AA in PAH

Many by-products of AA play an important role as mediators in PAH. For instance, epoxyeicosatrienoic acid (EET), which is derived from AA by cytochrome p450 epoxygenase (Fig. 1*A*), plays an essential role in vasoconstriction and the modulation of proliferative and angiogenic properties in pulmonary artery ECs (PAECs) in PH and other diseases.[63,64] It has been shown that the EET and JNK/c-Jun pathways are involved in pulmonary vascular remodeling caused by proliferation of ECs, inhibition of apoptosis, and stimulation of angiogenesis, culminating in pulmonary artery endothelial plexiform lesions (Fig. 2*B*).[33] Kandhi et al.[65] demonstrated that jugular administration of EET led to an increase in RVSP in a dose-dependent manner. Thus, the effect of EET on RVSP seems synergic with hypoxia. Wang et al.[66] have clearly demonstrated that hypoxic pulmonary vasoconstriction response, which is impaired in PH, is modulated by endothelium calcium signaling via activation of EETs, further supporting the essential role of EETs in PH.

Leukotrienes (LTs) are another class of lipid mediators derived from the 5-LOX pathway of AA metabolism (Fig. 1*B*).[67,68] They trigger immune response by recruitment and activation of leukocytes and play an essential role as mediators in pulmonary inflammation.[31,32] Several studies have shown that leukotriene B_4 (LTB_4) is involved in PAH pathogenesis.[69-71] Rodent models of PH, including MCT- and SU5416 (a VEGFR2 [vascular endothelial growth factor receptor 2] inhibitor)-treated rat models, have shown elevated levels of LTB_4.[30,72] LTB_4 induces apoptosis of PAECs, proliferation of PASMCs, and fibroblast activation, three major pathologic events leading to PAH.[30,73] We have recently demonstrated that plasma levels of LTB_4, but not those of LTC_4 and LTE_4, are increased in the rat model of MCT-induced PH (Fig. 2*B*).[56] Taken together, these data strengthen a critical role of oxidized lipids in the pathophysiological mechanism of PH (Table 1).

Plasma HDL and PAH

HDL cholesterol (HDL-C) is protective in coronary artery disease because of its antioxidant and anti-inflammatory properties.[74,75] Apolipoprotein A-I (ApoA-I), the major protein component of HDL-C, is present at lower levels in PAH, which correlates with increased endothelial dysfunction.[76] Metabolic syndrome and insulin resistance are associated with low circulating HDL-C levels and may predispose to the development of pulmonary vascular disease.[77,78] Indeed, insulin resistance and RV hypertrophy with pulmonary vascular remodeling are observed in an apolipoprotein E–deficient mouse model.[79] In addition, depressed circulating levels of HDL-C are associated with worse clinical outcomes in PAH patients and are independent of other cardiovascular risk factors.[80] This observation is further supported by another independent study showing that circulating HDL cholesterol levels are depressed in a cohort of patients with idiopathic PAH (IPAH) and are associated with worse clinical outcomes.[81] On the whole, these data suggest a potential role of circulating HDL in metabolic syndrome and PH and are supported by the prevalence of subclinical PH in patients with metabolic syndrome.[82]

Role of lysophosphatidic acid

Studies have investigated the effects of lysophosphatidic acid (LPA) signaling and metabolism on vascular SMCs and ECs, indicating that LPA may also have implications for the remodeling of pulmonary vasculature.[83,84] LPA is a bioactive lipid molecule produced by the plasma lysophospholipase D enzyme autotoxin.[85,86] LPA has been shown to stimulate migration and proliferation of SMCs and to alter EC function; consequently, it plays a critical role in vascular development.[87-89] Mouse models with loss-of-function mutations in genes required for LPA production and signaling have been used to investigate the pathophysiological role of LPA metabolism and signaling in diverse settings, including pulmonary inflammation and hypoxia-induced vascular remodeling.[90,91]

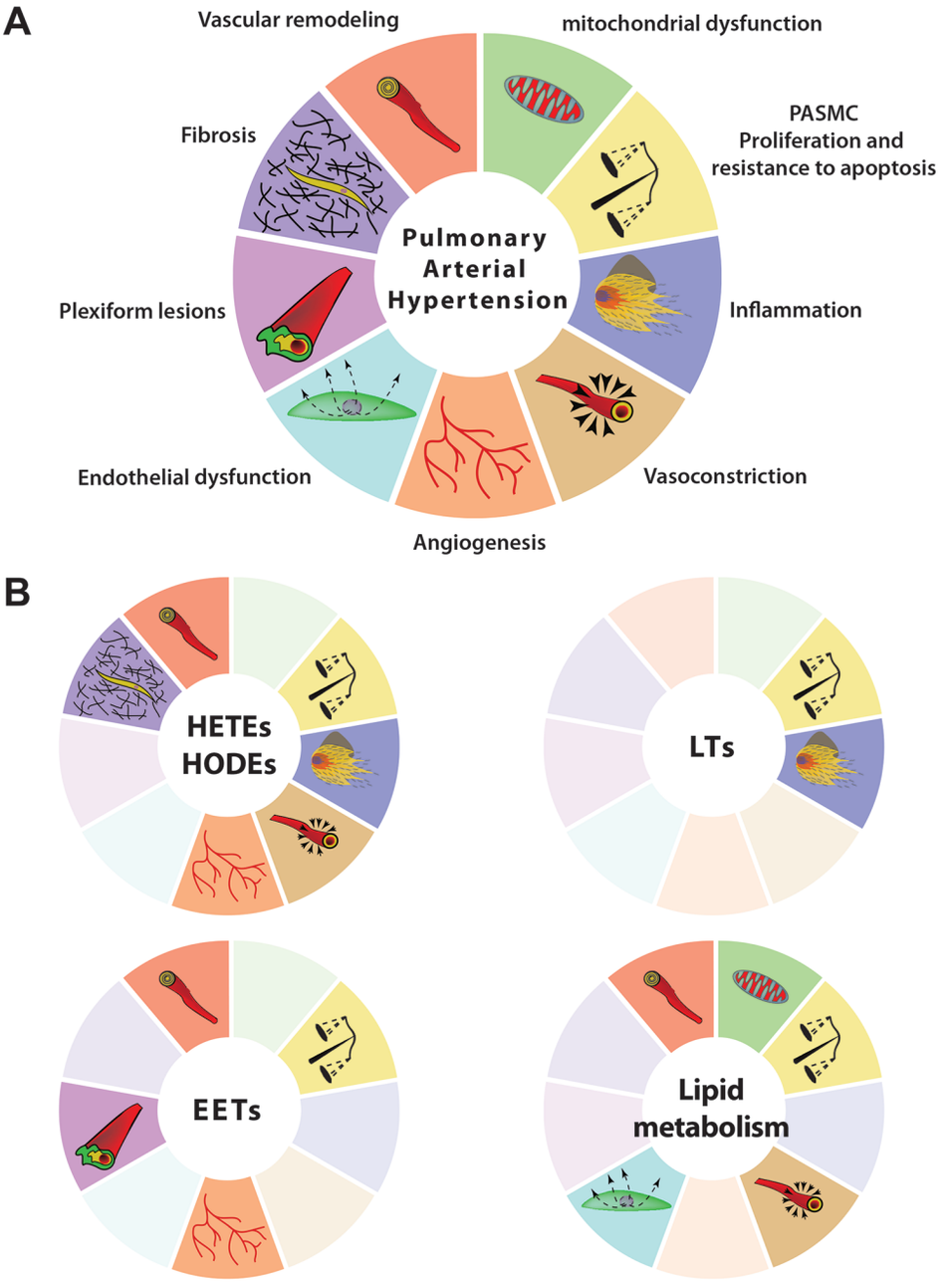

Figure 2. Role of oxidized lipids, oxidative stress, and lipid metabolism in promoting pulmonary arterial hypertension (PAH). *A*, Many altered pathways and abnormalities are involved in the progression and development of PAH. PASMC: pulmonary artery smooth muscle cell. *B*, Oxidized lipids (hydroxyeicosatetraenoic acids [HETE]s and hydroxyoctadecadienoic acids [HODEs]), leukotrienes (LTs), epoxyeicosatrienoic acids (EETs), and lipid metabolism contribute only to a selective subset of pathways (highlighted); pathways that are not involved in the pathogenesis of PAH are shaded (See Table 1 for references).

PAH is associated with oxidative stress and lipid peroxidation

PAH is associated with increased oxidative stress. This leads to tissue damage by oxidation of many important biological molecules, including DNA damage and lipid peroxidation.[58] Increased reactive oxygen species (ROS) production is involved in the pathogenesis of PH in various animal models. Chronic hypoxia–induced PH in mice is associated with increased intrapulmonary superoxide levels and other pathophysiological changes, which are abolished by the antioxidant xanthine oxidase inhibitor allopurinol.[92,93] Elevated RV superoxide levels are also observed in the MCT rat model of PH. Antioxidant therapies with intratracheal administration of

antioxidant superoxide dismutase or resveratrol suppressed the progression of PH.[94-97] Tissue hypoxia and an increase of inflammatory cytokines in the lungs of animal models of PH lead to elevated levels of ROSs.[58,97] PAH is associated with oxidative stress arising from the accumulation of ROSs, including superoxide and peroxide. Reaction of these highly reactive molecules with functional groups in membrane lipids and proteins can produce harmful oxidative-breakdown products.[98] One such class of metabolites is the chemically stable bioactive lipid peroxidation product of AA known as "isoprostanes." Levels of isoprostanes are elevated in many pulmonary vascular diseases, including PAH.[99-104] Isoprostanes not only serve as biomarkers of the disease but also act as signal transduction molecules exerting multiple biological effects through prostanoid receptors and other signaling pathways.[47,105-107] They can exert their effects on pulmonary vasculature in many ways, including pulmonary vasoconstriction,[48,108,109] induction of pulmonary endothelium to release endothelin 1, vasoconstriction in general,[49,110] and nonspecific effects on smooth muscle, such as hyperresponsiveness and hypertrophy,[50,51] thereby serving as important mediators in many lung pathologies including PAH.

Lipid peroxidation products of AA, including isoprostanes as well as other end products, such as malondialdehyde, are increased in patients with PAH.[103,111,112] Lungs of transgenic mice with bone morphogenetic protein receptor II (BMPR2) mutation showed an increase in isoprostanes, indicative of a rise in oxidative stress that was of mitochondrial origin and specific to vasculature.[113] Chronic hypoxic exposure of rats results in the production of ROSs, including phosphatidylcholine hydroperoxide, a primary peroxidation product of phosphatidylcholine, and serves as a contributing factor for pulmonary vascular thickening and development of PH.[93] A reduction in the levels of antioxidants, including β-carotene and α-tocopherol, is observed in patients with IPAH compared to control subjects.[114] Studies have evaluated the role of NADPH oxidase in the development of PH. In a lamb model of PH of the newborn, it has been shown that the NADPH oxidase enzyme complex may contribute to proliferation of SMCs by producing increasing superoxide levels.[115,116] Moreover, the proliferative effect of endothelin 1 on fetal PASMCs is a consequence of increased generation of ROSs.[117] Regulation of the growth of PASMCs by the transcription factor GATA4 is inhibited by antioxidant serotonin via suppression of NADPH oxidase or monoamine oxidase A, further reinforcing the critical role of ROSs in altering signaling pathways involved in PH.[118,119] Several other studies have suggested that antioxidant therapy can suppress the progression of PH. Many compounds with antioxidant properties, including the superoxide dismutase mimetic tempol and resveratrol, are effective in preventing the development of PH in various animal models.[97,120] In addition, cell culture studies, transgenic mouse models, and human samples confirm that the heritable form of PAH caused by mutations in the BMPR2 pathway is also associated with increased oxidative stress that is very vascular specific and most likely of mitochondrial origin. Lectin-like oxidized low-density lipoprotein (oxLDLs) is involved in endothelial dysfunction and injury upon stimulation by many factors, including inflammation and shear stress;[52] oxLDL plays an important role in cardiovascular diseases such as myocardial infarction and atherosclerosis.[121,122] Overexpression of lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1), an endothelial receptor of oxLDL in the lungs of transgenic mice, promotes oxidative stress by ROS generation and induces PH in chronic hypoxia.[123] Thus, oxidative stress and lipid peroxidation could make a major contribution to the pathogenesis of PAH (Fig. 2; Table 1).

OXIDATIVE STRESS AND IMPAIRED MITOCHONDRIAL FUNCTION IN THE PATHOGENESIS OF PAH AND ASSOCIATED RV FAILURE

There is a growing interest in the potential involvement of abnormal cellular metabolism and impaired mitochondrial function in the pathogenesis of PAH and associated RV failure. These changes may participate in the factors involved in the resistance to apoptosis and increased vascular cell proliferation, which are characteristics of PAH.[39] RV failure is associated with many metabolic transformations at the cellular and molecular levels affecting glucose and fatty acid metabolism. Glycolytic shift is observed in the right ventricles (RVs) of both humans with PAH[40] and rat models of PH induced by MCT or RV pressure overload by pulmonary artery banding.[41] Previous studies have suggested that limitation of the energy supply due to a mitochondrial metabolic switch from the energy-rich oxidative metabolism of glucose to glycolysis, arising from pathological pyruvate dehydrogenase kinase (PDK) activation, leads to RV failure.[41,42] Involvement of dysregulated fat metabolism in the failing RV during the progression of PAH has also been highlighted. In a transgenic rodent model of BMPR2 mutation, dysfunctional BMPR2 signaling in the RV results in triglyceride and ceramide deposition and potential fat toxicity.[43,44] Indeed, mutations in the gene for BMPR2 were identified to cause familial primary PH.[113,124,125] Fessel et al.[126] have provided an extensive analysis of widespread metabolomic and transcriptomic changes affected by BMPR2 mutations in the pathogenesis of PH. The role of fatty acid oxidation in PAH is further emphasized in a study showing that mice lacking the gene for the metabolic enzyme malonyl-coenzyme A decarboxylase (MCD), an enzyme involved in fatty acid oxidation, do not develop PAH during chronic hypoxia.[45] Studies have shown that serum levels of secreted glycoproteins involved in lipid metabolism and angiogenesis, such as angiopoietin-like protein 3 (ANGPTL3), are positively correlated with RVSPs and could contribute to PAH in systemic sclerosis.[46] In summary, abnormal cellular metabolism and impaired mitochondrial function could play an important role in the pathogenesis of PAH (Fig. 2).

VENTRICULAR DYSFUNCTION ASSOCIATED WITH PULMONARY ARTERY ATHEROSCLEROSIS

It has been observed that patients with obstructive sleep apnea, which is characterized by episodes of hypoxia and hypercapnia during sleep, are susceptible to atherosclerotic disease in the pulmonary vasculature. This was demonstrated in LDL receptor–deficient mice by exposing them to intermittent hypoxia/hypercapnia for periods of

8 or 16 weeks. Intermittent hypoxia/hypercapnia resulted in marked increase in atherosclerotic lesions in the pulmonary artery, accompanied by RV and left ventricular dysfunction.[127] In addition, pulmonary artery atherosclerosis is accelerated in patients with hypertensive pulmonary disease and shows significant correlation with RV dilation and hypertrophy.[128] It has been reported that pulmonary artery atherosclerosis is also characterized by increased lipid peroxidation in pulmonary artery lesions.[129]

ROLE OF OXIDIZED LIPIDS IN PAH INFLAMMATION

Recruitment of inflammatory cells and an increase in inflammatory mediators are hallmarks of PAH.[130] The pathogenesis of PH includes an inflammatory response, resulting in a higher circulating levels of monocyte chemoattractant protein 1 (MCP-1), interleukin (IL) 6, IL-8, and tumor necrosis factor α (TNF-α) in patients with IPAH and chronic thromboembolic PH than in healthy controls.[131-134] Oxidized lipids are known to promote inflammatory processes in many diseases such as atherosclerosis.[135] For example, oxidized LDL has been demonstrated to promote MCP-1 expression (Fig. 3).[136] Growing evidence demonstrates the implication of oxidized lipids derived from LA and AA in the inflammatory mechanism in PAH. Indeed, 5-HETE promotes neutrophil recruitment.[137] Interestingly, 9-HODE and 13-HODE are capable of exerting both pro- and anti-inflammatory reactions by regulation of monocyte/macrophage activation.[138] Enzymatic synthesis of 9-HODE and 13-HODE leads to the production of 9-(S)-HODE and 13-(S)-HODE. On the one hand, these two enantiomers are known to have anti-inflammatory properties by virtue of binding and activating peroxisome proliferator–activated receptor γ (PPARγ), leading to downregulation of inflammatory mediators such as IL-12, interferon α, and TNF-α and thus inhibiting inflammatory cell activation.[138-140] On the other hand, nonenzymatic production of HODEs in the case of oxidative stress, as observed in PAH, leads to the synthesis of 9-/13-(S)-HODEs and 9-/13-(R)-HODEs in the same proportion.[141] Recently, it was demonstrated that the production of 9-/13-HODEs by nonenzymatic pathways leads to monocyte/macrophage activation and inflammation in atherosclerosis disease.[141] In addition to an enhancement of inflammation arising from oxidative stress in PAH, downregulation of PPARγ in PAH impairs the anti-inflammatory effect of oxidized lipids. Nonetheless, more studies are required to fully understand the precise role of HODEs in PAH pathophysiology with respect to inflammation. Furthermore, oxidative stress can also promote inflammation by leading to the synthesis of compounds, such as isoprostanes, known to promote inflammatory cell recruitment.[142] Finally, LTs produced from AA also exert a proinflammatory effect. Among these, LTB_4 has been demonstrated to be a chemoattractive compound for neutrophil and to promote expression of the ICAM-1 protein by ECs, leading to leukocyte recruitment.[69] Moreover, LTC_4 and LTE_4 could also promote inflammation by activating the expression of TGF-β1.[143-145] Taken together, these data give evidence of major involvement of oxidized lipids in inflammation observed in PAH and make them attractive pharmacological targets to counteract PAH pathology.

NOVEL THERAPEUTIC STRATEGIES IN PAH

Role of HDL (ApoA-I) mimetic peptides

HDL is a major lipid carrier in the bloodstream and plays a critical role in vascular disease. It is known that HDL protects against atherosclerosis through several mechanisms, including the ability to extract cholesterol and phospholipids from peripheral cells and transfer them to the liver for excretion. Moreover, HDL also protects against lipid oxidation and inflammation. However, under certain pathological conditions, as in PAH, these antioxidant and anti-inflammatory properties of HDL decrease, accompanied by a drastic increase in the levels of oxidized lipids. Therefore, HDL can act as both an anti- and a proinflammatory molecule, depending on the context and environment. Indeed, we have determined the "inflammatory indices" of HDL and LDL in IPAH and associated PAH (APAH) patients.[29] We found that LDL inflammatory indices were significantly higher in IPAH and APAH patients than in controls. Furthermore, HDL was proinflammatory in both IPAH and APAH.[29]

The major component of HDL in plasma is ApoA-I, which possesses anti-atherosclerotic, anti-inflammatory, and antioxidant properties.[146] The mechanistic relationship between ApoA-I and pulmonary function was highlighted by genetic deletion of ApoA-I in mice. ApoA-I-null (ApoA-$I^{-/-}$) mice show an increase in proinflammatory HDL, indicative of high oxidative stress and increased airway hyperresponsiveness as well as impaired pulmonary vascular function.[147] PH in patients with sickle cell disease is associated with altered expression of ApoA-I, contributing to sickle cell disease–associated vasculopathy.[148] ApoA-I concentrations were decreased in the lungs of idiopathic pulmonary fibrosis patients and in an experimental bleomycin-induced fibrosis model.[149] The local treatment with ApoA-I has been shown to be very effective against the development of experimental lung injury and fibrosis.[149] ApoA-I appears to be a promising therapeutic molecule, considering its therapeutic potential in reducing inflammation and fibrosis in the animal model of bleomycin-induced pulmonary fibrosis.[150]

Several HDL mimetic peptides, which mimic the lipid-binding properties of ApoA-I, have been engineered to mimic the anti-inflammatory and antioxidant properties of HDL. Among these, the 4F peptide (18 amino acids with 4 phenylalanines, at positions 3, 6, 14, and 18) has received the most attention over the past decade. The 4F peptide is highly effective in improving vascular dysfunction implicated in the pathogenesis of many diseases and disorders, including atherosclerosis, diabetes, hypercholesterolemia, and sickle cell disease.[151-153] In the context of lung disease, 4F decreases airway hyperresponsiveness, inflammation, and oxidative stress in a murine model of asthma.[154] We have recently demonstrated that the levels of oxidized lipids are elevated in the plasma of PH rats,[56] as well as in PAH patients,[29] and may contribute to the inflammatory response and vascular changes involved in the progression of PH. Therapy with 4F has been shown to be very effective in restoring the levels of oxidized lipids and rescue of preexisting PH in animal models.[56] We have examined the effect of 4F on HDL and LDL inflammatory indices in an arterial wall model and a monocyte migration assay in IPAH and APAH patients.[29] HDL, as well as

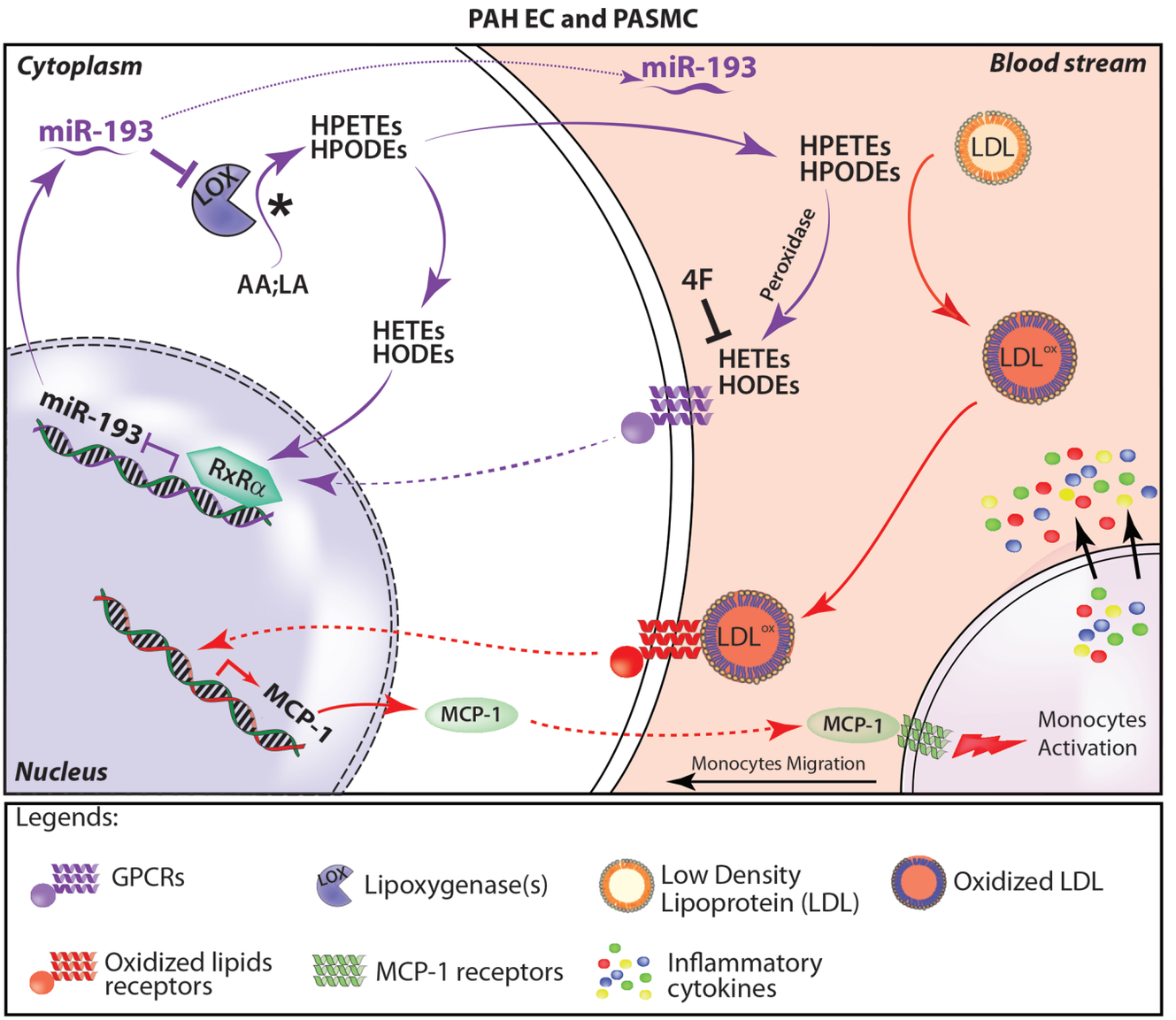

Figure 3. Hypothetical scheme of production and effects of oxidized fatty acids. In the oxidized fatty acid pathway (purple arrows) linoleic acid (LA) and arachidonic acid (AA) in the cytoplasm (shown with an asterisk to indicate the starting point) are enzymatically cleaved through lipoxygenases (LOXs) into hydroperoxyeicosatetraenoid acids (HPETEs) and hydroperoxyoctadecadienoic acids (HPODEs) that are further oxidized into hydroxyeicosatetraenoic acids (HETEs) and hydroxyoctadecadienoic acids (HODEs). The apolipoprotein A-I mimetic peptide 4F inhibits production of HETEs and HODEs. HETEs and HODEs in the blood bind to G protein–coupled receptors (GPCRs) and induce intracellular pathways, leading to the activation of transcription repressor retinoid X receptor alpha (RXR-α), which inhibits microRNA-193 (miR193) expression. Inhibition of miR193 increases HETE and HODE production by targeting the LOX pathway; miR193 is also secreted in the blood. In the inflammation pathway (red arrows), HPETEs and HPODEs, the reactive intermediates of HETEs and HODEs, oxidize LDL, which initiates the inflammatory response, including initiating the transcription of monocyte chemoattractant protein 1 (MCP-1), monocyte migration, and aggravation of pulmonary arterial hypertension (PAH) symptoms. EC: endothelial cells; PASMC: pulmonary artery smooth muscle cells.

LDL, inflammatory indices were decreased significantly after ex vivo treatment with 4F to levels comparable to healthy controls.[29]

Role of the microRNA 193–oxidized lipids axis in PH

MicroRNAs (miRNAs) are small, regulatory, noncoding, single-stranded RNA molecules involved in the regulation of several physiological pathways, including apoptosis, cell migration, vascular development, and cell proliferation, via modulation of target genes.[155,156] Altered expression of miRNAs could result in a dysregulated expression of their target genes, consequently causing or exacerbating several pathological conditions, including cardiovascular diseases and PH.[157,158] Several miRNAs, including miR-21, miR-204, and miR-328,

have been reported to regulate pathogenic signaling in the development and progression of PH.[159-161] We have recently demonstrated that PH is associated with increased plasma levels of oxidized lipids in rodents as well as in IPAH patients.[29] Therapy with 4F was very effective in restoring their levels and led to rescue of preexisting PH in models of both hypoxia and MCT. Mechanistically, we identified the miRNA miR-193-3p (miR193) as a downstream effector molecule whose expression was significantly downregulated in the lungs in two experimental animal models of PH. The 4F therapy fully restored expression of miR193 to its level in the control group.[56] Overexpression of miR193 in the lungs rescued preexisting PH induced by either MCT or hypoxia in animal models.[56] We also found that overexpression of miR193 in SMCs isolated from small pulmonary arteries of PAH patients (confirmed by right catheterization) reduces proliferation, whereas knockdown of miR193 in SMCs isolated from small pulmonary arteries of control subjects with no PAH (discarded nondonor lungs) increases proliferation.[56] Our data highlight the therapeutic role of miR193 in reversing pulmonary vascular remodeling.

We further showed that oxidized lipids regulate expression of miR193 through the transcriptional factor retinoid X receptor alpha (RXR-α).[56] Oxidized lipids induce the expression of RXR-α in PASMCs. This induction results in an increased binding of RXR-α on the promoter of miR193, thus causing its subsequent downregulation and a net increase in the expression of lipoxygenases, the enzymes responsible for the production of oxidized lipids (Fig. 3). However, 4F can decrease the overall content and binding of RXR-α to an miR193 promoter by sequestering oxidized lipids, ultimately leading to miR193 induction.[56] This study explored an essential aspect of oxidized lipid–induced pathology of PH, wherein miRNA (i.e., miR193) modulation is involved in the molecular and functional outcome. Future studies on the role of miRNAs in the oxidized lipid–mediated induction of PH with insight into novel targets are warranted to develop a multiple-miRNA therapeutic approach to tackle the disease effectively.

OXIDIZED LIPIDS AND miR193 AS POTENTIAL BIOMARKERS OF PH

Several tests are currently used in clinical practice for evaluation of PAH, including 6-minute walk distance (6MWD); hemodynamic parameters, such as pulmonary artery pressure and cardiac output/cardiac index; B-type natriuretic peptide (BNP) and N-terminal-pro-BNP (NT-proBNP); and New York Heart Association functional class. Unfortunately, all of these parameters have significant limitations, since they are either invasive (pulmonary artery pressure using direct catheterization) or not very specific, as BNP/NT-proBNP can be influenced by left heart dysfunction and/or renal impairment and 6MWD by arthritis or myositis. Hence, there is an urgent need for discovering new, reliable, specific biomarkers that can predict disease progression and survival in PAH. Our recent work shows that oxidized lipids are significantly elevated in the plasma of rodents with PH. In the same setting, miR193 expression is significantly downregulated. Similarly, downregulation of miR193 was also observed in plasma samples obtained from PAH patients.[56] These data raise the possibility that a combination approach using both oxidized lipids and miR193 expression may be exploited as a potential biomarker panel in PAH to assess the disease severity or response to therapy in PAH patients (Fig. 3). Analysis of a large cohort of human samples is needed to affirm the reliability and development of oxidized lipids and miR193 as a biomarker panel for PAH.

SUMMARY

PAH is a multifactorial and heterogeneous disease associated with dysregulation of many molecular mechanisms contributing to the pathogenesis of the disease. The underlying causes of PAH include structural changes such as vascular remodeling, induction of proproliferative pathways, increased inflammation and oxidative stress, altered metabolic signaling, and genetic mutations. In this review, we have mainly focused on the involvement of oxidized lipids, lipid peroxidation, and impaired cellular mechanisms, including metabolism and oxidative stress, in the pathophysiology of PAH (Fig. 2). We have also elucidated the emerging role of HDL in the context of PH and the downstream mechanisms, including miR193, involved in the therapeutic potential of the ApoA-I mimetic peptide 4F in PH (Fig. 3). Given the heterogeneity of PAH, it is important to explore in depth the molecular mechanisms involved in the cause and consequence of the disease. Understanding the involvement of oxidized lipids in the pathophysiology of PAH may help in the development of more effective therapeutics and would increase the existing repertoire of potential therapeutics for this rare disease.

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