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REVIEW

Role of 15-lipoxygenase/15-hydroxyeicosatetraenoic acid in hypoxia-induced pulmonary hypertension

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Abstract Pulmonary arterial hypertension (PAH) is a rare disease with a complex aetiology characterized by elevated pulmonary artery resistance, which leads to right heart ventricular afterload and ultimately progressing to right ventricular failure and often death. In addition to other factors, metabolites of arachidonic acid cascade play an important role in the pulmonary vasculature, and disruption of signaling pathways of arachidonic acid plays a central role in the pathogenesis of PAH. 15-Lipoxygenase (15-LO) is upregulated in pulmonary artery endothelial cells and smooth muscle cells of PAH patients, and its metabolite 15-hydroxyeicosatetraenoic acid (15-HETE) in particular seems to play a central role in the contractile machinery, and in the initiation and propagation of cell proliferation via its effects on signal pathways, mitogens, and cell cycle components. Here, we focus on our important research into the role played by 15-LO/15-HETE, which promotes a proliferative, antiapoptotic, and vasoconstrictive physiological milieu leading to hypoxic pulmonary hypertension.

Keywords Hypoxic pulmonary hypertension · 15-Lipoxygenase · 15-Hydroxyeicosatetraenoic acid · Vasoconstriction · Remodeling

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Introduction

Pulmonary hypertension (PH) is a severe and frequently fatal disease characterized by elevated mean pulmonary arterial (PA) pressure greater than 25 mmHg at rest or greater than 30 mmHg with exercise [1], and which contributes to the morbidity and mortality of adult and pediatric patients with various lung and heart diseases. According to the Venice Classification of Pulmonary Hypertension in 2003, PH is currently classified into five categories as listed in Table 1. Importantly, many of these diseases or conditions are associated with persistent or intermittent hypoxia, either globally or regionally, within confined areas of the lung [2]. The acute hypoxia-induced pulmonary vasoconstriction (HPV) is an important mechanism that aids in matching ventilation with perfusion by directing blood flow from poorly ventilated regions of the lung to areas with normal or relatively high ventilation. Although acute HPV benefits gas exchange and maximizes oxygenation of venous blood in the pulmonary artery, sustained HPV or chronic exposure to hypoxia is a major cause for the elevated pulmonary vascular resistance and pulmonary arterial remodeling (PAR) in patients with pulmonary arterial hypertension (PAH) associated with hypoxic cardiopulmonary diseases [3]. Vascular remodeling is characterized largely by medial hypertrophy and hyperplasia due to enhanced vascular smooth muscle cell (VSMC) proliferation or attenuated apoptosis and endothelial cell over-proliferation [4, 5]. However, the mechanism of pulmonary vascular remodeling (PVR) and pulmonary hypertension is still unknown.

The arachidonic acid cascade plays a vital role in homeostasis of the endothelium and VSMCs, and has been observed in dysregulation of downstream pathways of arachidonic acid in patients with PAH and in animal

Table 1 Clinical classification of pulmonary hypertension

Venice Classification of Pulmonary Hypertension (2003)
1. Pulmonary arterial hypertension (PAH)
1.1. Idiopathic (IPAH)
1.2. Familial (FPAH)
1.3. Associated with (APAH)
1.3.1. Collagen vascular disease
1.3.2. Congenital systemic-to-pulmonary shunts
1.3.3. Portal hypertension
1.3.4. HIV infection
1.3.5. Drugs and toxins
1.3.6. Other (thyroid disorders, glycogen storage disease, Gaucher disease, hereditary hemorrhagic telangiectasia, hemoglobinopathies, myeloproliferative disorders, splenectomy)
1.4. Associated with significant venous or capillary involvement
1.4.1. Pulmonary veno-occlusive disease (PVOD)
1.4.2. Pulmonary capillary hemangiomatosis (PCH)
1.5. Persistent pulmonary hypertension of the newborn
2. Pulmonary hypertension with left heart disease
2.1. Left-sided atrial or ventricular heart disease
2.2. Left-sided valvular heart disease
3. Pulmonary hypertension associated with lung diseases and/or hypoxemia
3.1. Chronic obstructive pulmonary disease
3.2. Interstitial lung disease
3.3. Sleep-disordered breathing
3.4. Alveolar hypoventilation disorders
3.5. Chronic exposure to high altitude
3.6. Developmental abnormalities
4. Pulmonary hypertension owing to chronic thrombotic and/or embolic disease
4.1. Thromboembolic obstruction of proximal pulmonary arteries
4.2. Thromboembolic obstruction of distal pulmonary arteries
4.3. Nonthrombotic pulmonary embolism (tumor, parasites, foreign material)
5. Miscellaneous: sarcoidosis, histiocytosis X, lymphangiomatosis, compression of pulmonary vessels (adenopathy, tumor, fibrosing mediastinitis)

models. More and more biological data suggest that arachidonic acid metabolites of lipoxygenases (LOs) play pivotal roles in the pathological development of PAH. LOs are a family of non-heme iron-containing enzymes which dioxygenate polyunsaturated fatty acids to hydroperoxyl metabolites. As shown in Table 2, LOs mainly include four isoforms, 5-lipoxygenase (5-LO) [6], 8-lipoxygenase (8-LO) [7, 8], 12-lipoxygenase (12-LO) [9], and 15-lipoxygenase (15-LO) [10], which correspond to the carbon position of arachidonic acid oxygenation, whereas 8-LO was not found to be expressed in human tissues. LOs are involved in biosynthesis of vasoactive mediators, growth factors, adhesion molecules, and cytokines [10–12], and hence are important targets for the atherogenesis, vasoconstriction, and vascular remodeling. Moreover, LOs-derived compounds impact the metabolic characteristics of vascular cells, particularly those of endothelial cells (EC) and smooth muscles cells (SMC) [13, 14]. In our laboratory, we have reported that 15-LO/15-HETE played an important role in hypoxic pulmonary hypertension (HPH). Therefore, this review is intended to provide a comprehensive overview of the effect of 15-LO/15-HETE on HPH, and also to propose underlying the important aspects of 5-LO and 12-LO in PAH.

Basic properties of LOs

Two distinct types of 15-LO have been identified in humans: reticulocyte type of 15-LO-1 [15] and epidermis type of 15-LO-2 [16]. 15-LO-1 was initially discovered in a rabbit reticulocytes mass at a 75-kD, single-polypeptide chain [17]; the enzyme has a two-domain structure [18] with one non-heme iron per molecule. 15-LO-2 was subsequently identified, and its expression has been reported in human prostate, skin, and cornea [17]. Both enzymes convert arachidonic acid to 15-hydroperoxyeicosatetraenoic acid (15(S)-HPETE). 15(S)-HPETE is unstable and can be reduced by peroxidases to the corresponding

Table 2 Basic properties of LOs

Gene name	Alternative nomenclature	Predominant enzyme products	Main distribution
ALOX15	15-LOX-1	15(S)-HPETE, 15(S)-HETE	Reticulocyte, eosinophils, bronchial epithelial cells
	15-LOX-2	15(S)-HPETE, 15(S)-HETE	Prostate, skin, lung, cornea
ALOX12	Platelet-type lipoxygenase 12	12(S)-HPETE, 12(S)-HETE	In various cell types
	Epidermis-type lipoxygenase 12	12(R)-HPETE, 12(R)-HETE	In various cell types
	Leukocyte-type lipoxygenase 12	12(S)-HPETE, 12(S)-HETE	In various cell types
ALOX8	8-LOX	8(S)-HPETE, 8(S)-HETE	Only in mice and rats tissues
ALOX5	5-LOX	5-HETE, leukotrienes	In various cell types

15-hydroxyeicosatetraenoic acid (15-HETE), but they share only 40% amino acid homology, and there are two major differences between the isozymes. The first difference is that 15-LO-1 converts AA to 15(S)-HPETE (90%) and lesser amounts of 12(S)-HPETE (10%), whereas 15-LO-2 produces exclusively 15(S)-HPETE [17]. The second difference is that, although both arachidonic acid and linoleic acid are preferred substrates for 15-LO-1, only arachidonic acid is a substrate for 15-LO-2 [19, 20].

The human 5-LO consists of 673 amino acids and a non-heme iron, and the sequence is highly homologous with those of other mammalian LOs and soybean 15-LO [21]. 5-LO catalyzes conversion of arachidonic acid to leukotriene (LT) A4, which can be subsequently converted into the potent chemoattractant LTB4, or into cysteinyl leukotrienes (Cys-LTs: LTC4, LTD4, and LTE4) [22]. 8-LO is consisted of 677 amino acids, which displays 78% sequence identity to human 15-LO-2 considered to be its human orthologue. 8-LO expression or activity has only been detected in tissues of mice and rats [7, 8, 23], and is predominantly expressed in epithelia of mice, hair follicle, forestomach, and footsole. 8-HETE is a major arachidonic acid metabolite for 8-LO [24]. There are three isoforms of 12-LO named after the cells where they were first identified; platelet, leukocyte, and epidermis. The leukocyte-type enzyme is widely distributed among cells, but the tissue distribution varies substantially from species to species. The platelet and epidermal enzymes are present in only a relatively limited number of cell types, but murine epidermis-type 12-LOX is not a functional human gene [9]. The 12-LO pathway metabolizes AA to a variety of products with numerous biological activities, and the major products of this pathway are 12(S)-HETE, hydroxyepoxy-containing hepoxilins, and trihydroxy-containing trioxilins [25].

Biological role of 15-LO/15-HETE

15-LOs are lipid peroxidizing enzymes that catalyze the stereoselective introduction of molecular dioxygen at carbon 15 (C-15) of arachidonic acid [26, 27], and their expression and arachidonic acid metabolites are implicated in several important inflammatory conditions, cell differentiation, carcinogenesis, atherogenesis, and other potential functions. The physiologic roles of the enzymes and arachidonic acid metabolites are dependent on the context in which (tissue- and species-specificity) they are expressed.

Tissue levels of 15-LOs and 15-HETE are often elevated during inflammation condition. Further evidence for a role of 15-HETE is that it is increased in asthma and chronic bronchitis patients and animals [28–31]. Several lines of ex vivo studies have documented that 15-LOs product 15-HETE might have anti-inflammatory properties. 15-HETE inhibits the activity of 5-LO and LT production by neutrophils [32–34]. The expression of 15-LO induced by interleukin (IL)-4 in monocytes significantly decreased the LTB4 expression [35]. 15-HETE can inhibit neutrophil migration across cytokine-activated (interleukin-1 beta or tumor necrosis factor-alpha) endothelium [36]. Transfection of rat kidney with human 15-LO can suppress inflammation [37]. According to these studies, the in vivo activity of 15-LO/15-HETE may be regarded as a protective response to limit or reverse inflammatory symptoms and to maintain basic cell function [38].

Also, it has been found that 15-LO is implicated in the maturation of rabbit reticulocytes by inhibiting mitochondria degradation [39–41]. 15-HETE improves the proliferation of Friend erythroleukemia cells, rat aortic smooth muscle cells, and calf PASMCs [42–44], and even plays an important role in differentiation and function of macrophage [45]. Moreover, 15-LO plays a role in pro- and anti-carcinogenesis [46–48], 15-HETE also triggers cell death through the release of cytochrome C, activation of caspase-3, and PARP-1 (poly (ADP) ribose polymerase-1) cleavage in the K-562 cell line [49]. Chronic inflammation plays an important role in atherogenesis. Furthermore, there is evidence for a pro-atherosclerotic effect and anti-atherosclerotic effect of 15-LO [50–52].

Although 15-LO/15-HETE participates in various physiological and pathological processes, yet the exact role and mechanism in HPH have not been explained, so here we will describe in detail the effect of 15-LO/15-HETE on hypoxic pulmonary vasoconstriction and vascular remodeling.

15-LO/15-HETE and HPH

Distribution and expression of 15-LO/15-HETE in HPH

It is reported that only 15-LO-1 is expressed in normoxic lung tissues, and that 15-LO-1 and 15-LO-2 are upregulated by hypoxia. Moreover, in hypoxic lungs, 15-LO is concentrated in the microsomes, whereas in normoxic lungs, 15-LO is localized in the cytosol, suggesting that activation of 15-LO is associated with translocation of the enzyme from the cytosol to membrane under hypoxic conditions [53]. Moreover, the 15-LO-1 mRNA and protein were localized in pulmonary artery endothelial cells (PAECs), while the 15-LO-2 mRNA and protein were localized in both PAECs and pulmonary smooth muscle cells (PASMCs) [54]. Furthermore, both 15-LO-1 and 15-LO-2 were up-regulated and localized in PASMCs and PAECs of pulmonary vessels from patients displaying severe PH [44]. Using a combination of high-pressure liquid chromatography (HPLC) and gas chromatography/mass spectrometry (GC/MS), the synthesis of 15-HETE

was increased in microsomes from hypoxic lungs and this effect is dependent on the lipoxygenase pathway [53].

15-LO/15-HETE and hypoxic pulmonary vasoconstriction

15-HETE increases intracellular Ca^{2+} and contracts pulmonary arteries

15-HETE increases the tension of PA from hypoxic rats in a concentration-dependent manner [53]. After inhibiting the endogenous production of 15-HETE, the reaction of hypoxic pulmonary artery rings to phenylephrine (a vasoconstrictor and used to detect the viability of vessels) was markedly decreased, suggesting that endogenous 15-HETE is involved in pulmonary vasoconstriction. PA vasoconstriction induced by 15-HETE is triggered by an increase in intracellular Ca^{2+} concentration($[Ca^{2+}]_i$) in PASMCs, which is in turn caused by Ca^{2+} release from intracellular Ca^{2+} stores, or an influx of Ca^{2+} through ion channels such as L-type and store-operated calcium channels (SOCCs) [55].

More and more studies are showing that there is an important role for SOCCs in the chronic hypoxia-induced increase in resting $[Ca^{2+}]_i$ which is responsible for HPV but eliminates a role for voltage-dependent calcium channels (VDCCs) during this procedure [56]. Our data also show that transient receptor potential channel 1 (TRPC1), one candidate of SOCCs, was up-regulated by 15-HETE, leading to elevation of capacitative calcium entry (CCE) via SOCCs in PASMCs [57]. It has been suggested that the mechanism of 15-HETE mobilizes $[Ca^{2+}]_i$ signaling through Ca^{2+} release from intracellular Ca^{2+} stores via IP3 receptor- and ryanodine receptor-operated Ca^{2+} channels. After depletion of sarcoplasmic reticulum Ca^{2+} stores, the resulting Ca^{2+} influx, known as CCE, is mediated by SOCCs, and consists of up-regulated TRPC1. The other mechanism is directly activating Ca^{2+} entry from extracellular solution via L-type Ca^{2+} channels (VDCCs). PASMCs are required to maintain active vascular tones, then the increased $[Ca^{2+}]_i$ can form complexes with calmodulin, which activates myosin light chain (MLC) kinase (MLCK), causing phosphorylation of MLC. Phosphorylated MLC (P-MLC) then facilitates stimulation of myosin ATPase activity by actin leading to cross-bridge cycling and contraction [58].

15-HETE induces pulmonary vasoconstriction by inhibiting Kv channels

The resting membrane potential (E_m) is primarily determined by K^+ permeability and K^+ concentration gradient across the plasma membrane, and therefore the activity of K^+ channels in the plasma membrane is a critical determinant of E_m. Inhibition of K^+ channels causes membrane depolarization, opens VDCCs, promotes Ca^{2+} influx, increases $[Ca^{2+}]_i$, and triggers PASMCs contraction. Studies have suggested that K^+ channels in PASMCs are inhibited by subacute hypoxia, leading to depolarization, an increase in $[Ca^{2+}]_i$, and constriction of pulmonary arteries [59, 60]. These K^+ channels are voltage-gated and sensitive to 4-aminopyridine (4-AP) [61–63]. However, how the K^+ channels are inhibited after subacute hypoxia remains elusive. Both direct and indirect effects have been proposed for the channel inhibition.

Our studies have shown that inhibiting K_{ATP} and BK_{Ca} channels could not affect 15-HETE induced vasoconstriction, but once inhibited Kv channels can completely block the effect of 15-HETE on pulmonary arteries [64], 15-HETE can inhibit the Kv currents of PASMCs [65]. Recent studies also identified that subacute hypoxia down-regulates Kv1.5, Kv2.1, and Kv3.4 channel expression and suppresses IK current through endogenous 15-HETE. 15-HETE was found to be more potent than 5-HETE and 12-HETE in mediating hypoxia-induced down-regulation of Kv3.4 channel expression. These results fill the lacunae which define how subacute hypoxia inhibits Kv channels leading to membrane depolarization and an increase in $[Ca^{2+}]_i$ level in PASMCs, and demonstrate the link between 15-LO, 15-HETE formation, and pulmonary vasoconstriction after subacute hypoxia [66–68].

Effects of pulmonary artery endothelial cells on 15-HETE induced pulmonary vasoconstriction

PAECs can release several kinds of vascular activity factors such as PGE_2, NO, and some others, which have important contributions to vasoconstriction. Hypoxia reduces the activity of eNOS to decrease the production of NO, then contracts PAs [69]. In in vitro tension studies, denuded endothelial and inhibiting the NO production of vascular rings increased the effect of 15-HETE on PAs contraction. Blockage of endogenous 15-HETE can induce the production of NO in PAECs. Moreover, 15-HETE phosphorylated eNOS at Thr495, causing reduced activity of eNOS [70]. In addition, the immunoprecipitation (IP) supported there were 15-LO, Hsp90, and Akt in an eNOS complex in PAECs, and therefore these data must be interpreted with 15-HETE overcoming the protein network of eNOS process through phosphorylating or de-phosphorylating to inactivate some sites of eNOS resulting in reduced NO, thereby contracting PAs.

15-HETE regulates pulmonary vessels rhythm by protein kinase pathways

Protein kinase pathways play an important role in HPV, such as PKC, Rho kinase, Rho-associated serine/threonine

kinase (ROCK), and extracellular signal-regulated kinase 1/2 (ERK1/2). Tension measurements of responsiveness of rat PA rings have demonstrated that incubation with specific protein kinase inhibitors significantly attenuated the constriction of PA rings to 15-HETE under hypoxic conditions. 15-HETE can activate the translocation of PKC isoforms, PKC-delta and PKC-varepsilon, from the cytoplasm to the membranes of PASMCs, then down-regulate expression of Kv1.5, Kv2.1, and Kv3.4 channels to protect the effect of 15-HETE on PAs [71]. Also, 15-HETE mediated the up-regulation of ROCK expression and promoted the translocation of ROCK2 from the nucleus to the cytoplasm through G-protein and tyrosine kinase pathways under hypoxic conditions, then leading to PA vasoconstriction [72]. Furthermore, the ERK1/2 pathway was involved in 15-HETE-induced PA vasoconstriction, the ERK1/2 phosphorylation was also upregulated by 15-HETE in a dose-dependent manner, and this phosphorylation was detected in cytosol as well as in nucleus [73]. These data shown above suggested that 15-HETE mediated HPV by activating different signal transduction pathways.

15-LO/15-HETE and HPVR

Hypoxia induces pathological changes of the pulmonary vasculature mainly including: extracellular matrix components such as increase in collagen fibers and elastic fibers, smooth muscle cell hypertrophy and hyperplasia, endothelial cell swelling, hypertrophy, thereby resulting in pulmonary tube wall thickening, and luminal stenosis, reducing blood vessel flexibility in the vessel wall cavity volume level. Under conditions of prolonged hypoxia, although recovering the PO_2 to normoxic conditions, the pulmonary artery pressure is still higher than normal values. It can be concluded that hypoxic pulmonary vascular remodeling (HPVR) is the main mechanism of HPH [74].

It has been reported that PA remodeling induced by hypoxia in vivo is mediated by the 15-LO/15-HETE pathway at least in part. Moreover, both 15-LO-1 and 15-LO-2 were overexpressed in the pulmonary vessels of human PH lungs and localized in PASMCs and PAECs from pulmonary vessels of patients displaying severe PH [44]. Intragastric administration of rats with 15-LO inhibitor (nordihydroguaiaretic acid, NDGA) under hypoxic conditions decreased the formation of the endogenous 15-HETE level, which also reversed all the pathological changes of PAs induced by hypoxia, including the deposition of collagen and medial thickening [44, 54].

15-HETE and PASMCs apoptosis

Especially considering the fact that maintaining the proper balance between cell apoptosis and proliferation is closely related to tissue homeostasis, when this balance is disrupted, diseases such as PAH can result [75]. The enhanced VSMC proliferation and suppressed normal VSMC apoptosis are likely the major reasons leading to medial hypertrophy, arterial remodeling, and vascular lumen narrowing [76]. Indeed, the inadequate apoptosis has been implicated in the development and maintenance of severe pulmonary hypertension. We found subtle thickening of proximal media/adventitia of the PA in rats exposed to hypoxia, which was associated with an up-regulation of the anti-apoptotic Bcl-2 expression and down-regulation of activated caspase-3 and Bax expression in PA homogenates [54].

The effects of hypoxia on PASMCs apoptosis are well known; however, whether 15-HETE acts on the apoptotic responses in PASMCs remains unclear. Studies have shown that 15-HETE induced anti-apoptotic Bcl-2 expression, and down-regulated apoptotic caspase-3, Bax, FasL, Bad and caspase-9 expression to prevent PASMCs from apoptosis via ROCK, HSP90, PI3K/Akt, ERK1/2, and iNOS pathways. Some methods such as cell viability measurement, nuclear morphology determination, TUNEL assay, and mitochondrial potential analysis have also demonstrated that 15-HETE suppressed PASMC apoptosis and improved cell survival, contributing to HPVR including morphological alterations, mitochondrial depolarization, and the expression of anti-apoptotic proteins [77–80].

Activity of Kv channels also plays a major role in regulating the PASMCs population in the pulmonary vasculature, as they are involved in cell apoptosis, survival, and proliferation [3, 75, 76]. PASMCs from PAH patients demonstrate many cellular abnormalities linked to Kv channels, including decreased Kv current, down-regulated expression of various Kv channels, and inhibited apoptosis [81, 82]. It is well known that hypoxia can inhibit Kv channels, inhibiting cell apoptosis, but it remains unclear whether K^+ channels participate in the 15-HETE anti-apoptotic process under hypoxic conditions. Data have also shown that 15-HETE enhanced cell survival, suppressed the expression and activity of caspase-3, up-regulated Bcl-2 and attenuated mitochondrial depolarization, prevented chromatin condensation, and partly reversed K^+ channel opener-induced apoptosis in PASMCs under serum-deprived conditions, indicating that 15-HETE inhibited the apoptosis in PASMCs through, at least in part, inactivating K^+ channels [83].

15-HETE and PASMCs proliferation

Studies on the exact mechanism of 15-HETE on HPVR are currently in progress. Until now, it has been found that proliferating cell nuclear antigen (PCNA) and Cyclin A were up-regulated by endogenous 15-HETE induced by hypoxia and exogenous 15-HETE in PASMCs, and that the

effect was also abolished in the presence of cinnamyl-3,4-dihydroxy-α-cyanocinnamate (CDC), the inhibitor of 15-LO. Moreover, hypoxia and exogenous 15-HETE significantly enhanced 5-bromodeoxyuridine (BrdU) incorporation and microtubule formation by α-tubulin, and made more PASMCs accumulation at the G2/M+S phase, respectively. CDC also suppressed the cell proliferation, α-tubulin polymerizatio,n and made more PASMCs arrest at the G0/G1 phase of the cell cycle. Our results also clarified that the ROCK pathway was responsible for 15-HETE-induced PASMCs proliferation, which contributed to media hypertrophy. These findings appeared in favor of the potential relevance of ROCK pathway and 15-HETE inhibition in the treatment of human PH [44].

15-HETE and angiogenesis

Most studies conclude that the structural changes that are thought to underlie the increased vascular resistance can be broadly classified into two processes: firstly, remodeling of the walls of the pulmonary resistance vessels, and, secondly, a reduction in the total number of blood vessels in the lung [74, 84]. The second major structural alteration caused by chronic hypoxia is loss of small blood vessels, sometimes termed rarefaction or pruning, which is said to increase vascular resistance by reducing the extent of parallel vascular pathways [85]. But some other researchers question this widely accepted paradigm, and hypoxia-induced angiogenesis in the mature pulmonary circulation, a structural adaptation that have important beneficial results for gas exchange, has recently been reported [86]. The specific vascular endothelial mitogen, the vascular endothelial growth factor (VEGF) family, plays an important role in the development of PH, and chronic hypoxia can lead to increased VEGF, PIGF, and their receptor expression in the lung [87, 88]. The ROCK pathway also mediates hypoxia-induced capillary angiogenesis [89]. However, the mechanism by which hypoxia induced the angiogenesis in PH needs to be explored further.

Our study also showed that hypoxia could induce angiogenesis, and that this effect can be inhibited by intragastric administration of NDGA in vivo in rats, indicating that endogenous 15-HETE was involved in hypoxia-induced angiogenesis. It has also been found that exogenous 15-HETE can induce bovine PAECs migration, tube formation in Matrigel, or vessel density in chick chorioallantoic membrane under normoxic conditions [44]. Meanwhile, hypoxia induced significant PAECs migration and tube formation was blocked by CDC. However, the inhibitory effect was partly diminished in the presence of exogenous 15-HETE [44], and also ROCK pathway mediated the effect of 15-HETE-induced PAECs migration, tube formation in vitro, and chick chorioallantoic membrane angiogenesis in vivo. These results indicated that 15-HETE was a potent mediator involved in hypoxia-induced pulmonary vascular angiogenesis. Also, we are studying whether 15-HETE could regulate other pathways to

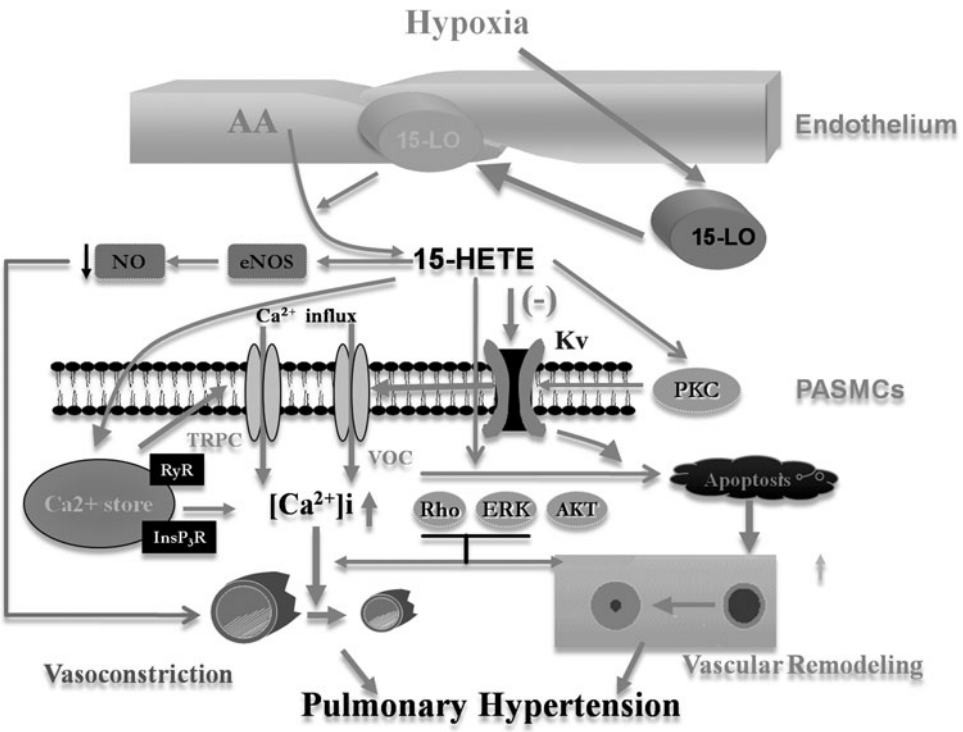

Fig. 1 Role of 15-LO/15-HETE in hypoxia-induced pulmonary hypertension. Hypoxia-induced 15-LO/15-HETE function and expression stimulates pulmonary arteries constriction, promotes pulmonary artery endothelial cell (PAEC) and smooth muscle cell (PASMC) proliferation and inhibits PASMC apoptosis. The increased vascular pressure, proliferation and inhibited apoptosis in PASMC may play an important role in initiation and/or progression of pulmonary vascular remodeling

promote angiogenesis, such as VEGF and PIGF. From all the results, we can conclude 15-LO/15-HETE plays an important role in HPH as in Fig. 1.

5-LO/12-LO and PH

In addition to the 15-LO pathway, many other data suggest that arachidonic acid metabolism via 5-LO and 12-LO also play pivotal roles in PH, since in one of these studies, 5-LO expression was unregulated in PAECs from patients with PAH [90], in rats subjected to chronic hypoxia [91], and in mice challenged with antigen [92]. Cys-LTs was also found to be in the lung lavage fluid of neonates with persistent pulmonary hypertension [93], patients with chronic obstructive pulmonary disease [94], and animals subjected to acute hypoxia, which may increase vascular permeability and accelerate PAECs proliferation after injuring endothelial cells [95]. Targeted disruption of the 5-LO gene and protein [96] or treatment of animals with diethylcarbamazine, MK-886, an inhibitor of five-lipoxygenase-activating protein (FLAP) [91], or LT antagonists [97], reduced hypoxia and monocrotaline (MCT)-induced pulmonary hypertension, while overexpression of 5-LO accelerated the development of MCT-induced PH in rats, respectively [98]. More recently, it has been shown that adenoviral overexpression of 5-LO in BMPR2+/− exposed to MCT resulted in increased inflammation and exacerbation of PH compared with wild-type mice [99, 100].

12-LO and 12-HETE have also contributed to PASMCs proliferation and have participated in PVR. 12-LO gene and protein expression was elevated in lung homogenates of rats exposed to chronic hypoxia. 12(S)-HETE at concentrations as low as 10^{-5} μM stimulated proliferation of PASMCs, most likely via the ERK1/ERK2, MAPK pathway and apparently without the involvement of p38 MAPK, while inhibition 12-LO with baicalein blocked PASMCs proliferation, suggesting a potential role for 12-LO and its metabolite 12(S)-HETE in hypoxia-induced pulmonary hypertension [101].

Summary

It is clear that PAH has a complex, multifactorial pathobiology and it is unlikely that one factor or gene mutation will explain in all forms and aspects of PAH. In addition, we have shown that hypoxic exposure promoted the expression and activity of 15-LO, catalyzed arachidonic acid to the production of 15-HETE which played a significant role in HPV and vascular remodeling. So the signal transduction pathway and mechanism of 15-HETE may be an important mechanism underlying the treatment of PAH and provide a novel therapeutic in sight for the future. Although 15-LO/15-HETE exerted an integral role in the development of pulmonary hypertension, the mechanism by which 15-LO was regulated under hypoxic conditions is still not clear. So further studies will need to evaluate the precise mechanism of hypoxia-+regulated 15-LO expression and activity.

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