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REVIEW

Role of ROS/Kv/HIF Axis in the Development of Hypoxia-Induced Pulmonary Hypertension

Wen Wu¹, Yan Li², Dunquan Xu^{3*}

¹Department of Ultrasound, ²Medical Examination Center, General Hospital of the Army, Beijing 100007, China

³Clinical Laboratory, the Xigaze Branch of Xinqiao Hospital, the Army Medical University, Xigaze, Tibet 857000, China

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Abstract Hypoxic pulmonary hypertension (HPH) is a common complication in patients with chronic obstructive pulmonary disease (COPD), sleep-disordered breathing, or dwellers in high altitude. The exact mechanisms underlying the development of HPH still remain unclear. Reactive oxygen species (ROS), hypoxia inducible factors (HIF), and potassium channels (K_v) are believed as the main factors during the development of HPH. We propose that the “ROS/Kv/HIF axis” may play an important initiating role in the development of HPH. Being formed under a hypoxic condition, ROS affects the expression and function of HIFs or K_v, and consequently triggers multiple downstream signaling pathways and genes expression that participate in promoting pulmonary vasoconstriction and arterial remodeling. Thus, further study determining the initiating role of “ROS/Kv/HIF axis” in the development of HPH could provide theoretic evidences to better understand the underlying mechanisms of HPH, and help identify new potential targets in the treatment of HPH.

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PULMONARY hypertension, a condition associated with increased mortality, is a severe comorbidity in many diseases. Clinically it is characterized by progressive increase in pulmonary arterial pressure and pulmonary vascular resistance.¹ According to the latest clinical classification of the guidelines for the diagnosis and treatment of pulmonary hypertension,² pulmonary hyper-

tension is classified into 5 groups, where hypoxic pulmonary hypertension (HPH) is a subtype of pulmonary hypertension due to lung diseases and/or hypoxia. Totally, there are 7 etiologies for HPH: chronic obstructive pulmonary disease, interstitial lung disease, other pulmonary diseases with mixed restrictive and obstructive pattern, sleep-disordered breathing, alveolar hypoven-tilation disorders, chronic exposure to high altitude, and developmental abnormalities.

Hypoxic vasoconstriction, one of the important physio-

logical responses to hypoxia, known as the von Euler-Liljestrand mechanism, is meaningful for maintaining ventilation/perfusion ratio.³ However, generalized pulmonary arteriole constriction results in elevated pulmonary arterial pressure, and long-term elevation of pulmonary arterial pressure promotes thickening of pulmonary arterial wall. Thus pulmonary vascular remodeling together with enhanced pulmonary vascular resistance eventually lead to pulmonary hypertension.

PATHOGENESIS FOR HYPOXIC PULMONARY HYPERTENSION

The mechanisms underlying the pulmonary vascular remodeling and vasoconstriction during hypoxic pulmonary hypertension remain to be fully explored. To date, there exist several cellular and molecular mechanisms explaining pulmonary vascular remodeling and vasoconstriction during HPH. Stenmark *et al* summarized that the cellular and molecular mechanisms are varied, and depend on the cellular composition of vessels at particular sites along the longitudinal axis of the pulmonary vasculature, as well as on local environmental factors.⁴ They also elucidated that the resident vascular cell types play specific roles in the overall remodeling response, which undergo site and time-dependent changes in proliferation, matrix protein production, expression of growth factors, *etc.*⁴ In another review, Stenmark and colleagues proposed that the adventitia played critical roles in hypoxia-induced pulmonary vascular remodeling, during which adventitial fibroblasts were activated and underwent phenotypic changes including proliferation, differentiation, recruitment of inflammatory and progenitor cells to the vessel wall, *etc.*⁵

ROS, HIFs, AND K_v IN HYPOXIC PULMONARY HYPERTENSION

Reactive oxygen species (ROS)

Sommer *et al* exhibited that the mechanisms responsible for hypoxic pulmonary vasoconstriction include ion channels, reactive oxygen species (ROS), and redox couples.⁶ According to recent research, hypoxia leads to exaggerated increase of ROS in the pulmonary arterial smooth muscle cells.⁷⁻⁹ Accumulating data have shown that oxidative stress plays important roles in mediating pathological changes in the pulmonary arterioles and the right ventricle.¹⁰⁻¹² Additionally, ROS affects cells' sensitivity to oxidative stress, cell migration, proliferation, apoptosis, and matrix protein deposition, all of which are

related with vasoconstriction and vascular remodeling.^{10,13} Lately, another team reported that ROS mediated physiological angiogenesis due to aerobic endurance exercise, in which HIF may play as an exercise-sensitive oxygen sensor and a redox regulator.¹⁴ Taken together, ROS is believed to be an initiating factor for vascular response under hypoxia exposure. Thus, oxidative stress is becoming a new target for treating pulmonary hypertension.¹⁵

Hypoxia inducible factors (HIFs)

Hypoxia inducible factors (HIFs) are demonstrated to be the main modulators in cellular response to hypoxia, and play key roles in the development of organs and the progression of diseases.^{16,17} Recently, a controversy about whether ROS participates in HIF-1 α modulating has arisen.¹⁸ An opinion considers that hypoxia induces superoxide production in compound III of the mitochondrial electron transport chain, and the activity of prolyl hydroxylase (PHD) is inhibited by oxidation of the nonheme Fe II, which stabilizes HIF-1 α .¹⁹ On the contrary, another opinion propose that the decreased activity of mitochondrial electron transport chain can result in elevation of oxygen concentration in cytoplasm.²⁰ Consequently, PHD can be reactivated, and then HIF-1 α degradation occurs. HIF-2 α is also believed to be regulated via this mechanism.²¹ Calvani M *et al* reported that hypoxia induces the production of ROS in mitochondria, and inhibition of PHD stabilizes HIF-1 α .²² Consequently, the expression of vascular endothelial growth factor (VEGF) increases. When VEGF binds to VEGF receptor-2, nicotinamide adenine dinucleotide phosphate (NADPH), an oxidase, is activated, resulting in a secondary increase of ROS. As HIF-1 α is further stabilized, cells acclimatize to oxidative stress. Besides, Prabhakar and colleagues also demonstrated that, besides by hypoxia, HIF-1 α could be activated by nitric oxide (NO) and ROS.²³ Furthermore, study showed that the mechanism for ROS increase under chronic intermittent hypoxia is related with the imbalance of the pro-oxidation and anti-oxidation genes encoded by HIF-1 α and HIF-2 α .²⁴

Potassium channels (K_v)

Research showed that oxygen-sensitive potassium channels (K_v) are predominantly expressed in resistant pulmonary arterioles, and are responsible for hypoxia induced pulmonary vasoconstriction occurring mainly in this area.²⁵ A team found that the ROS-K⁺ signaling pathway modulates NO, endothelin 1 (ET-1), and VEGF secretion in human pulmonary arterial endothelium under oxidative stress. They also demonstrated that Kv1.5 may

play an important role in ROS-K⁺ signaling pathway, and intracellular Ca²⁺ may be related with the secretion regulation of endothelium by ROS-K⁺.²⁶ Yasui *et al* reported that H₂O₂ directly inhibits the activity of K_{ATP} at the concentration of 10 μM.²⁷

ROS/KV/HIF AXIS

Based on the research findings and questions mentioned above, we believe that HIFs and K_v may exert key roles during the development of HPH. Consequently, we propose the concept of "ROS/K_v/HIF axis". Firstly, hypoxia causes exaggerated increase of ROS in pulmonary arterioles. The increased ROS then inhibits the activity of K_v located in pulmonary arterioles, meanwhile, stabilizes HIFs activity. The decreased K_v activity could explain the early pulmonary vascular constriction reaction under hypoxia exposure. On the other hand, the stabilized HIF axis could activate its downstream genes, and subsequently cause the expression of various hypoxia-related proteins that participate in pulmonary vascular remodeling during HPH. We hypothesize that during the development of HPH, the production of ROS inhibits K_v activity, stabilizes HIF axis, and further leads to imbalances of pulmonary vascular constriction/dilation and proliferation/apoptosis.

Nevertheless, does the "ROS/K_v/HIF axis" really exist in pulmonary circulation during the development of HPH? What is the exact nature of the "ROS/K_v/HIF axis", and what is the underlying molecular mechanism? These questions need further investigation.

The characteristics of HPH are remarkable pulmonary arterial remodeling and elevated vascular resistance. The specific pathological changes in resistant arterioles during HPH are exhibited as: thickening of pulmonary arteriole wall with proliferated and hypertrophic medial smooth muscle cells, inflammatory cell infiltration, and enlarged adventitia along with extracellular matrix accumulation. The pulmonary vasoconstriction response to hypoxia is initially a physiological adaptation to maintain ventilation/perfusion ratio, which eventually leads to elevation of vascular resistance. Chronic hypoxia exposure induces durable vasoconstriction and vascular resistance, which synergistically promote pulmonary arteriole remodeling. As a consequence, pulmonary arteriole remodeling inversely promotes vasoconstriction and vascular resistance. The positive feedback path during chronic hypoxia exposure further exacerbates HPH. However, the exact mechanisms underlying HPH still

remain further exploration.

Studies showed that intracellular redox reaction could affect cellular signaling transduction and genes expression, which exerts important function during cell proliferation, growth inhibition, and apoptotic pathophysiological processes. ROS are produced during intracellular redox reaction. Mitochondrial electron transport chain and NADPH oxygenase (Nox) are the sources of ROS in pulmonary arterial smooth muscle cells (PASMCs) under the hypoxic condition. Wang and colleagues showed that hypoxia leads to increase of ROS in PASMCs, along with significant elevation of intracellular [Ca²⁺]_i, followed by the constriction of pulmonary arterial smooth muscle cells.²⁸ Sommer *et al* demonstrated that endothelial cells regulated the vascular tone partially through regulating the ROS production in cells.⁶ In the early phase of hypoxia, decreasing of hydrogen carriers accompanied with reducing of oxygen radical causes elevation of GSH/GSSG and NADPH/ NADP. Next, the intracellular deoxidation tendency increases, and the calcium-dependent potassium channels are inhibited. Transmembrane influx of Ca²⁺ increases afterwards, followed by pulmonary vascular constriction.^{6,28} Additionally, Rathore and colleagues showed that ROS production through the ROS-Protein Kinase C Epsilon (PKCε)-Nox axis played a pivotal role in transmembrane Ca²⁺ influx and the constriction of PASMCs.²⁹ N-acetyl-cysteine, an antioxidant could effectively inhibit peroxides from increasing, and prevent vascular pathological changes during the development of HPH.³⁰ However, another team showed that as a key endothelium-derived hyperpolarizing factor, H₂O₂ mediates flow-induced dilation of human coronary arterioles through activating BK(Ca) channels in smooth muscle cells.^{31,32} To summarize those aforesaid studies, different concentrations of ROS affect the pathophysiological processes of vascular constriction/dilation and cell proliferation/apoptosis.

On the other hand, HIFs play a key role in response to hypoxia, and modulate genes transcription during various pathophysiological processes. The battery of genes regulated by HIF-1 and HIF-2 are overlapping but distinct, and are dependent on cell types. For example, HIF-2α is present but does not activate the transcription in mouse embryonic stem cells.³³ HIF-1α modulates various genes expression such as endothelin-1, platelet derived growth factor, heme oxygenase-1, and inducible nitric oxide synthase-1, all of which play important roles during hypoxia-induced vasoconstriction and arterial

remodeling.^{34,35} Moreover, HIF-2 α plays a significant role in tumorigenesis.^{36,37}

HIF-1 is a heterodimer composed with HIF-1 α and HIF-1 β , which is regulated by Fe and oxygen dependent PHD. The main viewpoint on the increase of HIF-1 α under hypoxia is that this is due to the decrease of oxygen-dependent degradation, and the increase of oxygen-independent protein synthesis.^{38,39} A recent study exhibited that, besides hypoxia, NO and ROS can also activate HIF-1 α .²³ Chronic intermittent hypoxia leads to increased synthesis and stability of HIF-1 α , and the calpain-dependent degradation of HIF-2 α . Thus, the loss of redox homeostasis due to the imbalance of HIF-1 α -dependent and pro-oxidant/HIF-2 α -dependent anti-oxidant activity is considered as the main mechanism underlying the pathogenesis of autonomic morbidities associated with chronic intermittent hypoxia.²⁴ Mechanisms responsible for increased ROS generation may involve transcriptional dysregulation of genes encoding pro- and anti-oxidant enzymes by HIF-1 and HIF-2, respectively.⁴⁰ Therefore, we consider that the interaction of ROS and HIF mutually promotes each other, forming a positive feedback loop, which disturbs the constriction/dilation of pulmonary vessels and cell proliferation/apoptosis during the pathophysiological progression of HPH.

Hypoxia induced pulmonary vasoconstriction mainly occurs in resistant arterioles (the forth division, diameter < 200 μ m), not in conduit arteries.⁴¹ The anatomical (proximal-distal) and functional (conduit-resistance) differences also exhibit at the cellular and molecular level, such as the difference of K⁺ channel distribution in different arterial segments.^{42,43} In conduit PSMCs, the whole cell potassium currents (I_K) exhibit contributions from both voltagegated (K_V) and large-conductance, calcium-sensitive (BKCa) channels. However, I_K in resistance PSMCs mainly manifests K_V current.⁴⁴ The reason for these differences may lie in the different origins of conduit and resistance arteries in the embryological vascular beds: the conduit arteries originate from the 6th aortic arch, whereas the resistance arterioles originate from the mesenchymal lung bud by capillary plexus expansion.⁴⁵ Hypoxia-induced pulmonary vasoconstriction is dependent on the inhibition of K_V, for 4-AP pretreatment could completely inhibit the activity of K_V. Along with the longitude of pulmonary arteries, the mRNA levels of K_V 1.2, K_V 1.5, K_V 3.1, K_V 4.3, and K_V 9.3 all increase. However, only the protein level of K_V 1.5 increases in resistance arterioles.²⁵ Study showed that K⁺ is transported to mitochondria more quickly in

hypoxia-resistant rats than in hypoxia-sensitive rats; meanwhile, the concentration of K⁺ in hypoxia-resistant rats is higher than that in hypoxia-sensitive rats.⁴⁶ The study also showed that adaptation to hypoxia manifests not only as a faster transportation of K⁺, but also as the exchange of K⁺/H⁺. When K_{ATP} channels are blocked, the production of H₂O₂ in mitochondria of hypoxia-resistant rats is faster than that of hypoxia-sensitive rats. Extralife, a flavonoid extract, was shown to increase the transportation of ATP-dependent K⁺, and elevate the tolerance ability by 5 times in hypoxia-sensitive rats.⁴⁶ Brown et al found that hypoxia that lasts several seconds to minutes inhibits K_V through decreasing ROS production in mitochondria and directly stimulating catecholamine secretion in adrenal medulla chromatocytes; meanwhile, chronic hypoxia leads to the production of HIF-2 α by a ROS-independent pathway in fetus-derived chromatocytes, and intermittent chronic hypoxia results in anti-oxidative response modulated by Nrf-2 that is associated with increases of ROS and HIF-1 α .⁴⁷ Dong *et al* found that, with 18-hour hypoxia exposure, HIF-1 α increased hypoxia-induced expression of K_V channels in cultured PSMCs through erythropoietin (EPO) enhancer effects.⁴⁸ According to the research above, HIF-1 α may have protective effect *in vitro* during early hypoxic phase. Thus, the role of HIF-1 α during hypoxia process should be fully explored. Besides, Shin and colleagues found that HIF-1 α upregulated a two-pore domain K⁺ channel in mouse B cells, which may be due to humoral immune responses and B cell differentiation.⁴⁹ Taken together, there are different types of potassium channels existing in vascular endothelial cells and smooth muscle cells, whose activation or inhibition play important roles in regulating vascular constriction/dilation. The ROS production, diversity of K⁺ channels expression, and HIF axis activation may be closely related with hypoxia-induced vasoconstriction and vascular remodeling during chronic hypoxia.

To summarize, the "ROS/K_V/HIF axis" partakes and greatly contributes in the process of hypoxia-induced pulmonary vasoconstriction and vascular remodeling. The schematic diagram below elucidates the "ROS/K_V/HIF axis" during HPH (Fig. 1). Hypoxia leads to the production of ROS, and ROS affects the balance of K⁺, followed by stabilizing of HIF system. Those aforesaid factors further cause the imbalances of pulmonary vascular constriction/dilation and proliferation/apoptosis, which promote the development of HPH. Further investigation in the "ROS/K_V/HIF axis" may offer new theoretical bases and potential targets for treating HPH.

Figure 1. Schematic flowchart of the pathway of ROS/ Kv/HIF axis in hypoxia-induced HPH.

Hypoxia causes stabilization of HIF-1 α , and then leads to the expression of various genes such as VEGF, PDGF and ET-1, all of which participate in HPV and PVR during HPH. Hypoxia induced ROS production affects the balance of K⁺ and stabilizes the HIF system. VEGF also promotes ROS through the NADPH pathway. ROS increases HIF-1 α by inhibiting its degradation. Moreover, NADPH oxidase-derived ROS can inhibit Kv channel current. The imbalance of K⁺ participates in vasoconstriction and remodeling of pulmonary vessels under hypoxia condition. On the other hand, ROS and HIF promote each other, and form a positive feedback loop, which further disturbs the constriction/dilation of pulmonary vessels and cell proliferation/apoptosis during the pathophysiological progression of HPH.

HIF, hypoxia inducible factor; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; ET, endothelin; HPV, hypoxic pulmonary vasoconstriction; PVR, pulmonary vascular remodeling; ROS, reactive oxygen species; VHL, von Hippel-Lindau; PHD, prolyl hydroxylase domain; HREs, hypoxia response elements; CBP, c-AMP responsive element binding protein; HPH, hypoxic pulmonary hypertension.

Conflict of interest statement

The authors have no conflict of interest to disclose.

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