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To decipher the hypoxic pulmonary hypertension: Vascular heterogeneity and the hypothesis of hypoxic responsive threshold

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Abstract Pulmonary hypertension (PH) is a complex and multi-factorial chronic disease characterized by progressively increased pulmonary vascular resistance and vascular remodeling, and it has been recognized as ‘the cancer of cardiovascular diseases’ because of its high morbidity and mortality. Pathophysiological changes of pulmonary arteries, which implicate endothelial dysfunction, smooth muscle cell proliferation, and increased vasoconstriction, decrease the lumen area of the pulmonary microvasculature, optimizing the pulmonary ventilation/perfusion ratio as well as causing fixed elevation of pulmonary resistance. Among various types of PH, hypoxic pulmonary hypertension (HPH) which occurs in patients with cardiopulmonary disease or in residents at high altitude has aroused great interest in researchers. Intriguingly, synchronously exposed to the hypoxic circumstances, the peripheral vessels make responses different from pulmonary arteries, which, besides the effects exerted by nervus and the microenvironment (involving the inflammatory mediators, angiotensin II and other ingredients), has always been expounded as the vascular heterogeneity. Nevertheless, nobody has articulated such heterogeneity and its mechanism to date. Based on our prior experiments, we propound the hypothesis of hypoxic responsive threshold (HRT) for the first time, which means that once the partial pressure of oxygen diminishes to certain degree, vessel in different tissues reacts via the reactive oxygen species (ROS)–potassium channels (Kv)–hypoxia inducible factors (HIF) triangle, resulting in hypoxic vasoconstriction and vascular remodeling.

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Introduction

Pulmonary hypertension (PH) is a haemodynamic and pathophysiological condition that can be found in multiple clinical conditions [1]. It is defined as a sustained elevation of resting mean pulmonary arterial pressure greater than 25 mmHg, as well as a pulmonary capillary wedge pressure or left ventricular end diastolic pressure less than 15 mmHg. There are five clinical groups of PH with specific characteristics, among which hypoxic pulmonary hypertension (HPH) is commonly and arouses great interest in researchers [2]. Totally, the etiologies of HPH are classified into seven subtypes according, including chronic obstructive pulmonary disease (COPD), developmental abnormalities and so on. Patients with severe HPH may develop obstructive plexiform lesions in the distal pulmonary circulation. These occlusive lesions are associated with decreased lumen cross-sectional area and progressive increases in pulmonary vascular resistance, which leads to the development of right ventricular hypertrophy (RVH) and aggravates PH. To date, there has been no magic cure for pulmonary hypertension, and the goal of treatment is to delay or prevent the progression of this disease.

Pathogenic vascular alterations in HPH are characterized by abnormal muscularization of small pulmonary arteries and progressive intimal hyperplasia [3]. At the meantime, hypoxic vasoconstriction known as the von Euler–Liljestrand mechanism is one important physiological responses in hypoxia for maintaining proper ventilation/perfusion ratio [4]. Though exact mechanisms underlying the pulmonary vascular remodeling and vasoconstriction during HPH still remain obscure, it has been widely accepted that HPH undergoes the alteration in molecular and cellular aspect [5]. The former implies the functional changes (i.e. production of matrix protein and ROS, activity of K+ channel and HIF) and growth factors, segregation of cytokines like endothelin and thromboxane) of vascular cells (i.e. endothelial cells, smooth muscles, adventitial fibroblasts) [6–8], while the later tend to focus on structural transformation such as the endothelial lesion, smooth muscle and adventitial fibroblast proliferation which is consistent with former modulation. Besides, the innervation and microenvironment also contribute significantly to the structural remodeling and persistent vasoconstriction of the pulmonary circulation [5–9].

Notably, our prior study implicates reactive oxygen species (ROS) play an important role in HPH [10]. Recent studies also confirm the over production of ROS in pulmonary artery smooth cells [11–13]. On one hand, ROS induce the oxidative stress injury, causing the dysfunction and apoptosis of endothelia in pulmonary arterioles and the pathological changes in right ventricle [14–16]. On the other hand, ROS affect cellular migration, proliferation, and matrix protein deposition, which are involved in the vasoconstriction and vascular remodeling [14–17]. Thus, ROS are becoming a new target for treating PH, which has been partly proved valid [18].

Taken together, we harbor the idea that ROS are the initiating factor inducing vascular response to hypoxia exposure (Fig. 1).

In particular, the hypoxia-inducible factor 1 (HIF-1), a highly conserved transcription factor in almost all cell types, has been identified as a key mediator of adaptation to hypoxia, playing a key role in development of organs and progression of diseases [7]. It exists as a heterodimer, consisting of HIF-1α and HIF-1β subunits, tightly regulated by O2 availability and regulating the expression of hundreds of genes. HIF-1β is ubiquitously expressed, whereas under normoxic conditions, HIF-1α protein is ubiquitinated and subjected to proteasomal degradation; thus, HIF-1α confers sensitivity and specificity for hypoxic induction of HIF-1 transcriptional activity [19]. HIF-1 leads to the downregulation of voltagegated K+ channel family member 1.5 (Kv 1.5) and as well as upregulation of Na+/H+ exchange isoform 1 (NHE1) and transient receptor potential canonical family member 1 (TRPC1). Such alterations lead to depolarization as well as increased intracellular K+ concentration, an alkaline shift in intracellular pH, and elevated intracellular calcium concentration, resulting in a cell phenotype that is more contractile, proliferative, and/or migratory, contributing to the development of HPH [20] (Fig. 1).

There are different types of potassium channels exist in vascular endothelial cells and smooth muscle cells, whose activation or inhibition plays important roles in regulating vascular constriction/dilation [14]. Recent research showed that oxygen-sensitive voltage-gated K+ (Kv) channels are predominantly expressed in resistant pulmonary arterioles, responsible for local pulmonary vasoconstriction spurred by hypoxia [21]. In early phase of hypoxia, hydrogen carriers decrease along with decrease of oxygen radical, which will result in elevation of GSH/GSSG and NADPH/NADP. Next, intracellular deoxidation tendency increases, and the calcium-dependent potassium channels will be inhibited, transmembrane influx of Ca2+ increases, followed by pulmonary vascular constriction [22,23]. Besides, inhibition of K+ channel activity is also implicated in stimulating pulmonary vascular smooth cells (PASMCs) proliferation by increasing intracellular Ca2+ [24] and in attenuating PASMC apoptosis by decelerating apoptotic volume decrease and decreasing cytoplasmic caspase activity [25]. All these contribute to vascular remodeling (Fig. 1).

Interestingly, in the patients diagnosed as HPH with COPD or residing in plateau, simultaneously undergoing the hypoxic circumstances, peripheral vessels seem to be more reserved and consolidated. There scarcely exist the progressively increased peripheral vascular resistance and vascular remodeling; instead, such vessels will dilate to supply ample blood perfusion in anoxic organs [26,27]. Complicated elements participate the nascent and development of HPH, while, based on our preliminary experiment, we are prone to account pulmonary vascular heterogeneity as the primary pathogenesis, which has been expressed in another way before [5]. Further, we propose the hypothesis of hypoxic responsive threshold...
For the first time, which consists of three major sections. Firstly, the interaction of the oxygenic atmosphere and vessels can be divided into three phases, namely physiological normoxic accommodation stage (I), pathological hypoxic responsive stage (II), and ultimate super-hypoxic exhaustion stage (III). All the three phases implicate the reactive oxygen species (ROS)–potassium channels (K_v)–hypoxia inducible factors (HIF) triangle. In the middle this figure shows the relationship among ROS, K_v and HIF: ROS directly suppress the activity of K^+ channels, while decreased K_v currents result in membrane depolarization, promoting Ca^{2+} influx through voltage-dependent Ca^{2+} channels (VDCC) opening, inducing vascular constriction; The increased ROS could activate and stabilize HIF-1α, and HIF-1α could also increase ROS production; Active HIF-1α may downregulate K_v 1.5 expression through its downstream signaling. Left displays the vascular modeling in stage I, contributing to the higher hypoxic responsive threshold of pulmonary arterioles. Right exhibits the pathogenesis of HPH based on the ROS–K_v–HIF triangle.

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oxygen diminishes to certain degree which is called the HRT, there would be vascular vasoconstriction and remodeling (Fig. 4). This theory may lay a solid foundation for the study about vascular physiogenesis in healthy individual, and for the further research about the vascular alteration in HPH, which in turn probably offers new targets to treat such intractable malady.

The hypothesis of hypoxic responsive threshold (HRT)

There does exist the vascular heterogeneity

The idea that vascular heterogeneity plays a vital role in causing HPH and significant structural remodeling of pulmonary arteries in humans is supported by observations that, there is chronic elevation of pulmonary artery pressure in persons who are exposed to a low oxygen condition, and the residents in high altitude experience a far greater increase in pulmonary artery pressure in response to exercise than in sea-level dwellers [28]. In the lungs of these persons, increased expression of α-smooth muscle actin is observed in the walls of pulmonary arterioles, which normally have little if any smooth muscle, and larger, more proximal vessels exhibit the thickened media and adventitia. Such findings are considered as hallmarks of hypoxia-induced pulmonary vascular remodeling and hypertension. In further support of the idea are the results of a simulated climb of Mount Everest, in which study, pulmonary artery pressures were significantly high in volunteers who were exposed to decreasing levels of hypobaric hypoxia over a 6-week period [29,30]. In addition, our study also found that 10% oxygen spurs the pulmonary artery smooth muscles
rather than that of aorta ascendens (Fig. 4). In these studies, hypoxia is the single interference factor to which both the pulmonary artery and peripheral vessels are exposed to, while the former seems to be more active than the latter. Consequently, we presume that there does exist the vascular heterogeneity, and it initiates the vascular vasoconstriction and remodeling in HPH.

*The hypothesis of hypoxic responsive threshold (HRT): syllogism of vascular reaction*

Since the first piercing cry of the newborns, pulmonary alveoli gradually open and the organisms initiate the gas ventilation and gas exchange in lungs. Thus, the pulmonary vessels begin to interact with oxygen of high concentration. Here goes the syllogism. We divide the interaction of the oxygenic atmosphere and vessels into three phases, namely physiological normoxic accommodation stage (I), pathological hypoxic responsive stage (II), and ultimate super-hypoxic exhaustion stage (III). All these stages have close relationship with the ROS–K⁺–HIF triangle.

**Stage I: the basis for vascular heterogeneity and HRT**

Oxygen dissolves in blood physically and forms oxyhemoglobin under normal circumstances, flowing in the vasculum and remodeling the vessels. In normoxic condition, the partial pressure around pulmonary vessels can be as high as 105 mmHg, while that around peripheral vessels generally below 40 mmHg. Hence, the physiological surroundings of affluent oxygen contrast that of the peripheral visavis hypoxia determine that the pulmonary vessels are more sensitive to hypoxic condition than the peripheral ones, which signify that the former maintain a higher HRT than the later. Such vascular heterogeneity is referred to the oxygen-related vascular shaping and development (Fig. 1).

To begin with, the dense oxygen atmosphere may help elevate the expression of oxygen-sensitive channels such as Kᵥ, 1.5 in PASMCs, making these vessels impressionable to hypoxia. Hypoxia induced pulmonary vasoconstriction mainly occurs in resistant arterioles (the forth division, diameter <200 µm), not in conduit arteries [31]. The anatomical (proximal-distal) and functional (conduit–resistance) difference also exhibits in cellular and molecular level, such as the K⁺ channels distribution difference in different segments [32,33]. Hypoxia-induced pulmonary vasoconstriction is mainly attributed to the activity of Kᵥ, for 4-AP pretreatment could completely inhibit the activity of Kᵥ and hamper the hypoxic vasoconstriction [21]. In conduit PASMCs, the whole cell potassium currents (I_K) exhibit contributions from both voltage-gated (Kᵥ) and large-conductance calcium-sensitive channels; however, I_K in resistance PASMCs mainly manifests Kᵥ current [34]. Along with the longitude of pulmonary arteries, the mRNA levels of Kᵥ, 1.2, Kᵥ, 1.5, Kᵥ, 3.1, Kᵥ, 4.3, and Kᵥ, 9.3 are all increased. However, only the protein level of Kᵥ, 1.5 increases in resistance arterioles [21]. On one hand, the endopathic reason of these differences may lie in different origins of conduit and resistance arteries in the embryological vascular beds, in which the main conduit arteries originate from the 6th aortic arch, whereas the resistance arterioles originate from the mesenchymal lung bud by capillary plexus expansion [35]. On the other hand, considering that hypoxia could directly repress Kᵥ, channel expression [20], we hypothesize that, in stage I, prolonged dense oxygen promotes the Kᵥ expression in resistance arteries as an exogenous variable. Oxygen collaborates with such O₂-sensitive Kᵥ channels of PASMCs, keeping their activity to increase Kᵥ currents. This will result in membrane hyperpolarization, inhibiting voltage-dependent Ca²⁺ channels (VDCC) activity and causing pulmonary vasodilation [36]. Furthermore, activation of K⁺ channels is also involved in mediating apoptotic volume decrease, an early hallmark of apoptosis [37], and facilitating apoptosis [38].

In addition, sufficient oxygen contributes to the balance of ROS and HIF in pulmonary arterioles, laying the first tone for creating higher HRT. Studies showed that intracellular redox reaction can affect cellular signaling transduction and genes expression, which exerts important function during cells proliferation, growth inhibition, and apoptosis pathophysiological processes. ROS are produced during intracellular redox reaction, in which ROS are traditionally regarded as byproducts of mitochondrial electron transport chain in aerobic metabolism and NADPH oxidase (Nox) is the source of ROS in PASMCs under hypoxia condition [32]. According to the Pasteur Effect [39], when the oxygen concentration grows, pyruvate is converted to acetyl CoA that can be used in the citric acid cycle, thus suppressing the glycolytic pathway and promoting the aerobic oxidation of glucose. As a consequence, the activity of mitochondria of PASMCs has been elevated and the function has been enhanced, thus making the mitochondria have the potential ability to produce more ROS compared to the peripheral vessels. Along with the reduction of oxygen density, the overproduction of ROS is easier to occur in PASMCs, thus we conclude that ROS are the key mediators in forming HRT.

Emerging evidence indicates the activation of HIF-1α plays a central regulatory role in HPH, while under normoxic conditions, the HIF-1α protein is ubiquitinlated and subjected to proteasomal degradation. To note, besides oxygen, some researches imply that iron ion also participates in the degradation of HIF-1α [40,41]. Here we assume that the functional proportion of oxygen and other factors like iron ion, which are both aimed at the degradation of HIF-1α, varies in pulmonary arterioles and peripheral vessels. Considering physiological perennual situation with high oxygenic pressure, the oxygen may account for a larger part in decomposing HIF-1α in pulmonary arterioles, which means that slight oxygen reduction could have an evident influence on enhancing the HIF-1α expression.

**Stage II: the crucial course of pulmonary vasoconstriction and vascular remodeling**

Based on forehead hypothesis, we conclude that there is the HRT difference between the vessel in lungs (HRTₚ) and peripheral tissues (HRTₚₑ). Obviously, HRTₚ is higher than HRTₚₑ, and our previous study shows that HRTₚₑ is between normoxic 20% [42] and 10% while HRTₚₑ between 10% and 3% (Fig. 4), which may explain why pulmonary arterioles are subject to hypoxia than the peripheral one [2]. Refined from tremendous literature and our years of basic study, we propose that, in stage II, the hypoxia-induced vascular reaction is closely related to the ROS–Kᵥ–HIF triangle (Fig. 1). On one hand, these three ingredients operate independently as we displayed in
the introduction. On the other hand, we note that there is sub-
tle interplay among ROS, HIF-1α, and K⁺ channels. At first,
hypoxia results in exaggerated ROS in pulmonary arterioles.
The increased ROS could activate and stabilize HIF-1α, and
HIF-1α could in turn increase ROS production through its
downstream signaling involved the imbalance of the pro-
oxidation and anti-oxidation [20,43,44]; meanwhile, ROS,
especially H₂O₂, directly suppress the activity of K⁺ channels,
and HIF-1α could also downregulate Kᵥ 1.5 expression, which
both decreases K⁺ currents, and results in membrane depolar-
ization, promoting Ca²⁺ influx through VDCC opening,
inducing vascular constriction [20,36,45]. Moreover, the
ROS–K⁺ signaling pathway modulates NO, ET-1, and VEGF
secretion in human pulmonary arterial endothelium under
oxidative stress, further affecting the initiation of HPH [6].
Besides, our previous study found in the PH induced by
monocrotaline, the activity of telomerase reverse transcriptase
(TERT) was increased and TERT promoted the PASMCs pro-
liferation. We hypothesize that ROS may also stabilize the
HIFs, thus motivating TERT and extending the lifespan of
PASMCs. Such ROS–Kᵥ–HIF triangle is important in pro-
moting pulmonary vasoconstriction and vascular remodeling
in HPH.

Stage III: the breakdown of pulmonary arterioles
This phase rarely exist in clinical experience for that most
patients lose their precious lives because of asphyxia with the
super-hypoxic atmosphere within minutes. In theory, the
extremely low oxygen condition will suppress the growth of
PASMCs (as confirmed in our study, Fig. 4) in the early phase,
and ultimately cause severe cellular injury considering the ATP
depletion, lactate accumulation, the overproduction of free
radicals, and the overload of calcium during hypoxia [46-48].
During this vascular functional decompensatory period, both
endothelial cells and smooth muscles undergo the irreversible
lesions, decreasing the lumen area of the pulmonary microvas-
culature and aggravating the hypoxic condition of whole body.

Evaluation of hypothesis
The hypothesis will be tested in holistic, cellular and molecular
aspects. Firstly, the accurate value of HRT in pulmonary arte-
rioles should be measured. In terms of the animal experiments,
the mice should be exposed to conditions of a diminishing oxy-
gen pressure gradient. Then test which group will engender
HPH. Based on our early researches, HRT淡定 is between nor-
moxic 20% and 10% while HRT₀ between 10% and 3%, so
space the concentration gradient could be set as 1%. To the
cellular field, it’s necessary to observe the responses of
endothelial and smooth cells, which are detached from pul-
monary arterioles, to different oxygen pressure (set according
to the holistic results). Secondly, the ROS–Kᵥ–HIF triangle
related molecular pathogenesis need to be further discussed.
To test whether the Kᵥ or HIF plays an important role in
HPH, the gene knockdown animal model could be applied.
To test whether ROS is the initial part of HPH and correlated
other factors, the expression of Kᵥ and HIF, as well as the
activity of TERT in PASMCs, should be analyzed after using
antioxidants in HPH model. Thirdly, if the above results are
satisfactory, then some clinical studies could be performed.

Discussion
As a special organ, the lung plays an essential role in main-
taining the viral activities. The pulmonary vasculature has
to accommodate the entire cardiac output with the arterial
pressure nearly 10 times lower than the systemic circulation
[20]. The pulmonary vasculature is also unique in its response
to hypoxia. In contrast to the systemic circulation, which
dilates with hypoxia attempting to promote circulation and
increase oxygen delivery, the pulmonary arterioles constrict
after oxygen tension falls. The first demonstration of such
phenomenon can be attributed to Beutner [49]. In 1946, the
first detailed study characterizing this response in the intact
cat was published by von Euler and Liljestrand [50].
Although the precise teleology of hypoxic pulmonary vaso-
constriction remains hot of debate, it is widely held that when
the hypoxic challenge is transient and localized, the vasocon-
striction serves to optimize ventilation/perfusion matching.
Nevertheless, when the hypoxic stimulus is prolonged, wide-
spread vasoconstriction results in rapidly elevating pul-
monary vascular resistance and remodeling, initiating the
HPH. Although many of the structural and functional
changes that occur in the lung with exposure to prolonged
hypoxia have been documented, the mechanisms underlying
the pathogenesis of HPH or the pulmonary vascular hetero-
geneity remain incompletely understood.

To tackle the theoretical dilemma, we propound the con-
cept of hypoxic responsive threshold (HRT) based on our
prior experiments for the first time. The vascular HRT varies
in different parts of the body, originates from normoxic con-
dition, and is the nucleus of heterogeneity. Physiological
milieu with higher oxygen pressure in lungs (usually
105 mmHg contrast peripheral 40 mmHg) promotes the high-
er HRT, which explains why the pulmonary arterioles are
more susceptible to hypoxia. To articulate the vascular
heterogeneity, we put up with the syllogism of vascular reac-
tion, namely physiological normoxic accommodation stage,
pathological hypoxic responsive stage, and ultimate super-hy-
poxic exhaustion stage. This procedure implicates reactive
oxygen species (ROS)-potassium channels (Kᵥ)–hypoxia
inducible factors (HIF) triangle, and may be the core part
in HPH. In stage I, the dense oxygen atmosphere may help
elevate the expression of oxygen-sensitive channels such like
Kᵥ 1.5 in PASMCs, make the mitochondrial more active
and have latent ability to produce more ROS, and block
the downstream of HIF by degenerating HIF-1α, thus build-
ing a higher HRT in lungs. In stage II, when exposed to cer-
tain degree of hypoxia, which usually occurs as in interstitial
lung disease and sleep-disordered breathing, the pulmonary
arterioles undergo the overproduction of ROS, inhibition of
Kᵥ channel, and the activation of HIF-1α. These factors
function independently or interact mutually, ultimately caus-
ing the vasoconstriction and vascular remodeling in HPH.
At last, in stage III, the vessels break down in an extremely
hypoxic environment.

In fact, between stage I and stage II, there exists one
compensatory phase caused by the hypoxic-stress-motivated
cellular intrinsic protection. This process may implicate the
increased activity of antioxidant enzymes such as superoxide
dismutase (SOD) and glutathione peroxidase (GSH-Px)
against free radical injury, up-regulation of inducible nitric
Vascular heterogeneity and hypoxic responsive threshold

oxide synthase (iNOS) and \( K_v \) channel against vasoconstriction, etc. However, when it comes to the decompensatory phase, stage II begins. This may explain several eccentric or paradox phenomena in the experiments. To exemplify, some studies prove the high expression of iNOS mRNA and protein in pulmonary vascular walls exposed to hypoxia [51,52], while most researches imply that the NO generation is generally decreased in endothelial cells [53,54]. In addition, Dong et al. reported that hypoxia induces \( K_v \) channel expression in pulmonary arterial smooth muscle cells through HIF-1 [55], while most study proved the contrast result [20]. All these are the consequence of intrinsic protective procedure, which has close relationship with experimental methods (i.e. the exposure time, oxygen density of hypoxia).

Among the ROS–\( K_v \)–HIF triangle, ROS is believed as the initiating factor inducing vascular response under hypoxia exposure, while, a controversy about whether ROS participates in HIF-1\( \alpha \) modulating has arisen recently [56]. The ‘ROS’ hypothesis considered that hypoxia induces superoxide production in compound III of mitochondrial electron transport chain, and the activity of prolyl hydroxylase (PHD) will be inhibited by oxidation of the nonheme Fe (II), stabilizing HIF-1\( \alpha \) [57]. On the contrary, another opinion of ‘\( \text{O}_2^\cdot \)’ considered that the activity decrease of mitochondrial electron transport chain will result in elevation of oxygen concentration in cytoplasm, reactivating PHD and degrading HIF-1\( \alpha \) [58]. Nevertheless, most researches are inclined to the former one. Calvani et al. reported that hypoxia induces increase of ROS in mitochondria, and inhibition of PHD will stabilize HIF-1\( \alpha \) [44]. As a consequence, VEGF expression will be increased, bonding with its VEGF receptor 2. Then the activate oxidase of NADPH results in a second increase of ROS [44]. HIF-1\( \alpha \) will be further stabilized and render cells acclimatize to oxidative stress. So there may be a reciprocal positive feedback between ROS and HIF.

One more intriguing phenomenon exists. In the residents inhabiting on plateau, there still exists the progressive HPH. Among these cases, the lungs are exposed to the hypoxic condition initially and consistently, which may contradict our HRT hypothesis. Actually, this is involved in the gap between normoxic pressure and HRT, and the hypoxic situation of daily routines. In the resident at high altitude, such gap is narrow, so individual’s everyday activities, even the jogging, may reach the HRT and induce vascular reaction. Accumulation of such process finally leads to HPH. Prior study pointed that in high altitude residents, a far greater increase in pressure of pulmonary arterioles will be further stabilized and render cells acclimatize to oxidative stress. So there may be a reciprocal positive feedback between ROS and HIF.

Though the hypothesis of HRT explains some contradictions about HPH in theory, there still need more testimony to identify and confirm the presence of HRT. The ROS–HIF triangle may initiate and promote the HPH, which renders new treatment targets to relieve pain and delay the progression of HPH. We genuinely hope that one day the mechanism of HPH is no more opaque, and ‘the cancer of cardiovascular diseases’ would be tamed.

Overview box

First question: What do we already know about the subject?

1. There exists the vascular heterogeneity between the pulmonary and peripheral vessels, for that they respond differently to the hypoxic condition.
2. Hypoxic pulmonary hypertension (HPH) is characterized with the pulmonary vasoconstriction and vascular remodeling. The pathogenic changes involve the abnormal muscularization and progressive intimal hyperplasia, while the specific mechanism still remains obscure.
3. Reactive oxygen species (ROS), potassium channels (\( K_v \)), and hypoxia inducible factors (HIF) have been proved to participate in the foresaid physiopathologic process in HPH, respectively.

Second question: What does your proposed theory add to the current knowledge available, and what benefit does it have?

1. The hypothesis of hypoxic responsive threshold (HRT) evolved out of our interesting observations in experiments. It means that once the partial pressure of oxygen diminishes to certain degree, vessel in different tissues reacts differently, which may result in hypoxic vasoconstriction and vascular remodeling. Furthermore, we firstly correlated the ROS, \( K_v \), and HIF to construe the molecular mechanism of HPH.
2. Our conjecture of HRT maintains two palpable meanings. On one hand, such hypothesis replenishes the empty fields about oxygen-induced vascular heterogeneity. As to the pulmonary vascular smooth cells (PASMCs), the dense oxygen condition activates the mitochondria, which probably produce more ROS stimulated by hypoxia, elevates the expression of oxygen-sensitive channels like \( K_v \), and mainly controlling the degradation of HIF. Such ROS–\( K_v \)–HIF triangle determines a higher pulmonary vascular HRT, which explains why the pulmonary arterioles are more susceptible to hypoxia. On the other hand, the ROS–\( K_v \)–HIF triangle provides new targets to prevent or treat HPH. In clinical practice, the HPH may be alleviated by applying antioxidant, \( K_v \) activating or HIF-1\( \alpha \) degradation related drugs.

Third question: Among numerous available studies, what special further study is proposed for testing the idea?

The exact figure of HRT in pulmonary arterioles should be measured in the holistic and cellular aspects. Then ROS–\( K_v \)–HIF triangle will be tested to further explain the specific mechanism of HPH. Through the gene knockdown technology, the relationship of \( K_v \) or HIF with HPH will be proved. By using reagents reducing ROS, the expression of \( K_v \) and HIF, as well as the activity of TERT in PASMCs, should be analyzed.
Conflict of interest
None declared.

References