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Hypoxia and the integrated stress response promote pulmonary hypertension and preeclampsia: implications in drug development

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Abstract

Chronic hypoxia is a common cause of pulmonary hypertension, preeclampsia, and intrauterine growth restriction (IUGR). The molecular mechanisms underlying these diseases are not completely understood. Chronic hypoxia may induce the generation of reactive oxygen species (ROS) in mitochondria, promote endoplasmic reticulum (ER) stress, and result in the integrated stress response (ISR) in the pulmonary artery and uteroplacental tissues. Numerous studies have implicated hypoxia-inducible factors (HIFs), oxidative stress, and ER stress/unfolded protein response (UPR) in the development of pulmonary hypertension, preeclampsia and IUGR. This review highlights the roles of HIFs, mitochondria-derived ROS and UPR, as well as their interplay, in the pathogenesis of pulmonary hypertension and preeclampsia, and their implications in drug development.

Teaser:—

The integrated stress response (ISR) is a major contributor to pulmonary hypertension and preeclampsia, and a potential therapeutic target for these two diseases.

Keywords

hypoxia; pulmonary hypertension; preeclampsia; reactive oxygen species; mitochondria; endoplasmic reticulum; unfolded protein response; integrated stress response; vascular remodeling

Introduction

Oxygen (O_2) is essential to sustain mammalian life. O_2 is primarily utilized for energy generation and for biomolecule synthesis via oxidation-reduction reactions. Many biological processes in mammalian cells are dependent on the ATP produced by the electron transport

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chain (ETC) in mitochondria, with O_2 being the terminal electron acceptor in this chain. O_2 is also a pivotal substrate in protein synthesis and protein folding in the endoplasmic reticulum (ER). Both oxidative phosphorylation and oxidative protein folding are coupled to the generation of reactive oxygen species (ROS) [1, 2]. A reduction of O_2 supply (i.e. hypoxia) disrupts mitochondrial and ER functions. Hypoxia has been shown to alter the homeostasis of mitochondrial ROS (mitoROS) and to induce ER stress [3]. To respond to hypoxic stress, cells undergo adaptive responses that are primarily mediated by the activation of hypoxia-inducible factors (HIFs), reprogramming of mitochondrial metabolism, increased ROS flux, an unfolded protein response (UPR) in the ER, and the subsequent integrated stress response (ISR). HIFs are transcriptional activators that function as master regulators of hypoxia-activated gene expression, whereas the UPR/ISR attempts to restore proteostasis. Interestingly, HIFs, ROS and UPR/ISR are mutually connected [3, 4]. When the production of HIF and ROS and the UPR/ISR become sustained, they are deleterious and disrupt cell functions.

In mammals, the lung extracts oxygen from the air and transfers it into the bloodstream, and discharges carbon dioxide from the bloodstream into the air. In utero, fetal respiratory function is executed by the placenta where gas exchange occurs between maternal and fetal blood. The pulmonary and uteroplacental circulatory systems share some similarities. Both are low-pressure, high-flow systems [5, 6]. Upon exposure to acute hypoxia, systemic arteries and arterioles dilate, whereas pulmonary and uteroplacental arteries and arterioles constrict, leading to increased vascular resistance and reduced blood flow [7, 8].

Pulmonary hypertension is a disorder of pulmonary arteries, characterized by a mean pulmonary arterial pressure ≥20 mm Hg [9]. The disorder is classified into five groups based on the proceedings of the 6th World Symposium on Pulmonary Hypertension (WSPH), held in 2019 [9]. Among these categories, Group III involves cases of pulmonary hypertension that are caused by lung diseases and/or hypoxia, including obstructive lung disease, restrictive lung disease, other lung disease with mixed restrictive/obstructive pattern, hypoxia without lung disease and developmental lung disorders. In pulmonary hypertension, the pulmonary arteries are constricted and/or obstructed due to vasoconstriction and vascular remodeling, leading to increased pulmonary vascular resistance and pulmonary pressure. The remodeling of pulmonary arteries (a pathological process) primarily involves the proliferation of vascular smooth muscle cells (VSMCs) [10]. Pulmonary hypertension often leads to right ventricular overload, and heart failure is the most common cause of mortality in patients who have pulmonary hypertension[11]. The incidence, prevalence, and mortality of patients who have pulmonary hypertension are increasing[12].

Adequate uteroplacental blood supply to the placenta is pivotal for the development and growth of both the placenta and the fetus. The increase in uteroplacental blood flow during pregnancy is mainly achieved by structural and functional adaptation of uterine arteries[13, 14]. One critical process in uteroplacental vascular adaptation is the remodeling of the uterine radial arteries (a physiological process). During this process, invading extravillous trophoblasts (EVTs) replace endothelial cells and VSMCs from the arterial walls, transforming the arteries into widened, low-resistance vascular channels. Preeclampsia is a common pregnancy complication originated in the placenta, in which there

is new onset of hypertension after 20 weeks of pregnancy. It affects ~5% of pregnant women worldwide and is associated with high maternal and fetal morbidity and mortality[15]. This disorder is currently defined as elevated systolic and diastolic blood pressure (≥140 and ≥90 mmHg, respectively) with one or more of following criteria: proteinuria, other maternal organ dysfunction, and/or uteroplacental dysfunction[16]. In preeclampsia, uterine vascular remodeling is incomplete and uterine vascular adaptation is compromised, resulting in increased vascular resistance and reduced blood flow in the uteroplacental circulation[17]. Uteroplacental blood flow is a critical determinant of fetal growth and health [18], and preeclampsia is frequently complicated by intrauterine growth restriction (IUGR) [19].

Preeclampsia presents in heterogeneous forms and is commonly classified into early-onset (delivery before 34 weeks' gestation) and late-onset (delivery after 34 weeks' gestation) according to the time of delivery. Early-onset preeclampsia accounts for ≤20% of all preeclampsia cases, and is typically associated with placental dysfunction (i.e., insufficient trophoblast invasion, reduced spiral artery remodeling and placental malperfusion), hypoxia/ischemia-reperfusion injury, and IUGR. By contrast, late-onset preeclampsia, comprising ≥80% preeclampsia cases, is believed to result from maternal factors and is often associated with normal placental and fetal growth [20]. Late-onset preeclampsia frequently occurs in women with pregestational obesity and diabetes who may have existing chronic systematic inflammation [21, 22]. Intriguingly, early-onset preeclamptic placentas exhibit reduced antioxidant capacity, whereas placentas of late-onset preeclampsia display mitochondria-related adaptions and compensatory antioxidant responses [23]. Moreover, placentas of late-onset preeclampsia are unable to respond to hypoxia and display no apparent activation of the UPR when compared to normotensive placentas [24, 25]. Early-onset preeclampsia is the most clinically important form because it contributes to most of the maternal and perinatal morbidity and mortality [26–28]. Given the scope of this review, we primarily discuss early-onset preeclampsia herein.

Pulmonary hypertension and preeclampsia are two distinct diseases. Their phenotypes and biochemistries are compared in Table 1. Nevertheless, hypoxia is a common contributor to the pathogenesis of both diseases. Circulating endothelin-1 and its receptors in VSMCs are increased in pulmonary hypertension, in preeclampsia and in animal models of these diseases [29, 30]. Moreover, prolonged hypoxia impairs pulmonary and uteroplacental function, resulting in pulmonary hypertension and preeclampsia, respectively [14, 31]. HIFs, ROS and UPR are all induced or activated in these two disorders [32–35]. This review summarizes our knowledge of the roles of HIFs, ROS, UPR and their interplay in the ISR and in the pathogenesis of hypoxia-related pulmonary hypertension and preeclampsia. Potential therapeutic approaches for pulmonary hypertension and preeclampsia are also discussed.

Roles of oxidative stress and stress responses in the mitochondria and ER

ROS and oxidative stress

ROS are products of O_2 metabolism in cells. Common ROS include the superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($HO^{\bullet}$). In general, $O_2^{\bullet -}$, which is

the precursor of most other ROS, is produced by the reduction of molecular O_2. The major sources of ROS in mammalian cells are mitochondria, ER, and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase s (NOXs). Redox hemostasis is maintained by a delicate balance of the generation and destruction of ROS. Oxidative stress occurs when ROS generation overwhelms the antioxidant defense. ROS can be detrimental or beneficial to cellular functions depending on their levels in the cells. ROS may modulate cellular homeostasis through direct oxidative damage and by altering signal transduction pathways. Owing to their extreme reactivity, $O_2^{\bullet -}$ and $HO^{\bullet}$ often cause lipid, protein, and DNA damage when present at high levels. H_2O_2 can alter the conformation and/or activity of target proteins, such as enzymes and transcription factors, by oxidizing cysteine thiols (Cys-SH) to cysteine sulfenic acids (Cys-SOH) [36].

ER stress and the UPR

Only properly folded proteins are allowed to exit the ER. The accumulation of unfolded or misfolded proteins in the ER lumen promotes ER stress, and activates the UPR to mitigate this stress and to restore protein-folding capacity. The UPR can be activated in various cellular compartments or organelles. To date, a UPR has been identified in the ER, mitochondria, and cytoplasm [37, 38], but only the ER UPR is discussed in this review. In the ER, the UPR is mediated by three types of ER transmembrane receptor: protein kinase RNA-like ER kinase (PERK), inositol-requiring enzyme 1α(IRE1α) and activating transcription factor 6 (ATF6) [39]. The luminal domains of these proteins sense the protein folding status of the ER. Under normal conditions, these sensors are kept in the inactive state by forming a complex with the ER chaperone BiP/Grp78 (binding immunoglobulin protein/78-kDa glucose-regulated protein). However, the accumulation of unfolded and/or misfolded proteins in the ER lumen causes the dissociation of BiP/Grp78 from the sensors, leading to changes in the oligomerization state of the sensors and to downstream signaling activities. PERK activation promotes the global inhibition of translation through the phosphorylation and subsequent inactivation of eukaryotic translation initiation factor 2α(eIF2α), which results in reduced protein synthesis and misfolded protein load. Paradoxically, eIF2α phosphorylation also triggers the translation of ATF4, which in turn stimulates the transcription of a variety of genes, including those that boost antioxidant defense mechanisms [40]. During ER stress, the phosphorylation of nuclear factor 2 (Nrf2), mediated by PERK, promotes the dissociation of Nrf2 from Kelch-like ECH-associated protein 1 (KEAP1) in the cytosol, enabling Nrf2 to enter the nucleus where it regulates genes encoding antioxidant proteins [41]. ATF4 also activates the transcription of C/EBP homologous protein (CHOP) during chronic ER stress. The dissociation of BiP/Grp78 promotes IRE1α trans-autophosphorylation and its endoribonuclease activity, generating the stable transcription factor X-box binding protein 1 (XBP1) and thus upregulating UPR-targeted genes including BiP/Grp78 and components of ER-associated degradation (ERAD). Following the release of BiP/Grp78 from its luminal domain, ATF6 transits to the Golgi apparatus where it is cleaved by site-specific proteases S1P and S2P. The released cytosolic fragment, ATF6f, migrates to the nucleus where it activates the transcription of genes encoding various ER chaperones.

ISR

The ISR is a signaling pathway in mammalian cells that regulates gene expression by reprogramming mRNA translation. A variety of extrinsic or intrinsic stresses, such as hypoxia, amino acid deprivation, heme deprivation, oxidative stress, and viral infection, are sensed by eIF2 kinases, which include double-stranded RNA-dependent protein kinase (PKR), PERK, general control nonderepressible 2 (GCN2) and heme-regulated inhibitor (HRI) [42]. PKR is activated primarily by double-stranded RNA during viral infection. The accumulation of unfolded protein in ER triggers PERK activation. GCN2 is activated by amino acid deprivation and by UV light. HRI, which is mainly expressed in erythroid cells, is activated in response to heme deficiency and oxidative stress [42, 43].

The activation of these eIF2 kinases converges on the phosphorylation of serine 51 in eIF2α, leading to the ISR. eIF2 is a heterotrimer composed of an α-, a β-, and a γ-subunit. eIF2 forms a ternary complex (TC) with GTP and Met-tRNA, and the binding of the TC to the 40S ribosomal subunit results in the formation of the 43S preinitiation complex. The recognition of the start codon AUG triggers the hydrolysis of GTP and the subsequent dissociation of eIF2-GDP from the preinitiation complex, initiating the elongation phase of translation [44]. To reactivate eIF2 for the next round of translation initiation, the guanine nucleotide exchange factor eIF2B catalyzes the exchange of GDP with GTP. eIF2α phosphorylation converts eIF2-GDP into a competitive inhibitor of eIF2B, thus limiting the availability of the TC to form the preinitiation complex and inhibiting global protein synthesis, while selectively triggering the translation of a subset of genes with special 5' untranslated regions that encode transcription factors and other proteins including ATF4 [45].

Both pro-survival and pro-apoptotic pathways are activated in the ISR and the cell fate is determined by the balance of these two pathways, which is largely determined by the magnitude and duration of the ISR. The ISR is terminated by eIF2α dephosphorylation mediated by protein phosphatase 1 (PP1) with the participation of one of the regulatory subunits: PPP1R15A (also known as growth arrest and DNA damage-inducible protein, GADD34) or PPP1R15B (also known as constitutive repressor of eIF2α phosphorylation, CReP) [46, 47]. Where PPP1R15A is induced by the ISR, PPP1R15B is constitutively expressed [43]. Lines of evidence have implicated the ISR in the pathogenesis of various human diseases, including cardiovascular and lung diseases [48, 49].

ROS biogenesis and metabolism in mitochondria and ER

The ETC, composed of complexes I to IV in the inner mitochondrial membrane, transfers electrons from NADH (the reduced form of nicotinamide adenine dinucleotide) or FADH2 (a reduced form of flavin adenine dinucleotide (FAD)) to O_2 via a series of oxidation-reduction reactions, leading to the generation of H_2O (Figure 1A). During the electron transferring process, protons (H^+) are pumped from the mitochondrial matrix into the intermembrane space by complexes I, II and IV. The resultant H^+ gradient is then used by the ATP synthase complex (complex V) for the phosphorylation of ADP to ATP. The transfer of electrons by the ETC is not perfect. It is estimated that up to 2% of electrons escape from the chain and interact with O_2 to produce superoxide ($O_2^{\bullet-}$) [50]. Complexes

I and III are the major sites of ROS production in mitochondria (Figure 1A) [50, 51]. Complex I generates $O_2^{\bullet-}$ exclusively in the mitochondrial matrix, whereas Complex III releases $O_2^{\bullet-}$ into both the matrix and the intermembrane space. Dismutation of $O_2^{\bullet-}$ to H_2O_2 in mitochondria is fulfilled by superoxide dismutase 1 (SOD1, Cu, Zn-SOD) in the intermembrane space and by SOD2 (Mn-SOD) in the matrix [1] In an antioxidant mechanism that occurs in mitochondria, H_2O_2 is decomposed into H_2O primarily via the glutathione (GSH) redox system, which involves GSH peroxidases 1 and 4 (GPX1 and GPX4), GSH reductase (GR), peroxiredoxins 3 and 5 (PRX3 and PRX5), thioredoxin-2 (TRX2), and TRX reductase 2 (TRXR2) [1, 52].

Oxidative protein folding in the ER is also driven by redox reactions [53]. Protein disulfide isomerase (PDI) catalyzes the formation of disulfide in the nascent unfolded proteins via oxidation of two adjacent cysteines. To be reactivated, PDI is re-oxidized by ER oxidoreductin 1 (ERO1). ERO1 is then re-oxidized by transferring electrons to molecular O_2 in the presence of FAD. The activity of ERO1 produces stoichiometric amounts of H_2O_2 for every disulfide bond generated. H_2O_2 is eliminated by PRX4 and GPX7/8 to prevent its build-up inside the ER (Figure 1B) [54]. In addition, ER transmembrane NOX4 also contributes to the ER ROS pool by constitutively producing H_2O_2 [55].

Interplay between oxidative stress and ER stress

Oxidative stress and ER stress are interconnecting processes and often form a vicious cycle. As mentioned earlier, the activation of the PERK pathway is associated with the increased transcription of genes that are involved in antioxidant defense, implicating ER stress in redox regulation. PERK-induced activation of Nrf2 contributes to the maintenance of intracellular GSH levels following ER stress [56]. PERK deficiency increases ROS generation in mouse embryonic fibroblasts following ER stress [57]. ATF4 deletion impairs the expression of genes that are involved in glutathione biosynthesis [57]. Mitochondria and the ER appear to be important sites of ROS generation during ER stress and the UPR. Tunicamycin-induced oxidative stress is attenuated in ρ^0 fibroblasts devoid of endogenous mitochondrial DNA (mitoDNA) and a functional ETC. [56]. Activation of CHOP in the PERK pathway upregulates ERO1α, promoting H_2O_2 generation in the ER [58].

Alternatively, ER stress and the UPR could be the downstream of oxidative stress. The PERK pathway appears to be the major UPR component targeted by oxidative stress. Exogenous H_2O_2 increased both PERK-dependent and -independent phosphorylation of eIF2α [59] Interestingly, NOX4-derived ER H_2O_2 apparently activates the PERK pathway as knockdown of NOX4 or expression of ER-targeted catalase prevented tunicamycin-induced activation of this pathway [60]. Overexpression of ERO1β also induces ER stress, as evidenced by increased expression of BiP/Grp78 and CHOP [61]. MitoROS appear to play a critical role in ER stress and the UPR. Hypoxia-induced eIF2α phosphorylation and ATF4 accumulation, as well as the induction of BiP/Grp78 and CHOP, were attenuated by the expression of catalase and in cytochrome-c null cells [59], implicating a role for mitoROS in promoting ER stress.

ER–mitochondria coupling and ROS generation

Electron microscopy reveals a physical connection between mitochondri a and ER [62]. The contact sites are termed mitochondria-associated endoplasmic reticulum membranes (MAMs). This coupling has functional importance, allowing lipid and Ca^{2+} transfer [63]. ER inositol trisphosphate receptor (IP_3R) and mitochondrial voltage-dependent anion-selective channel protein 1 (VDAC1), which are proteins that occur on the outer membrane of the mitochondria (OMM), are enriched in MAMs. IP_3R and VDAC1 form a conduit for Ca^{2+} transfer, and Grp75 structurally links these two channels [64]. Ca^{2+} that is released from the ER lumen through IP_3Rs is funneled via VDAC1 into the intermembrane space of mitochondria, and is subsequently transported into the mitochondrial matrix via mitochondrial Ca^{2+} uniporter (MCU) in the inner mitochondrial membrane (IMM) [65–67]. The increased Ca^{2+} load in the matrix stimulates the activity of various enzymes of the tricarboxylic acid (TCA) cycle, ATP synthesis, and ROS generation [66, 68]. ER stress increases ER–mitochondria coupling, resulting in increased ATP levels and mitochondrial Ca^{2+} uptake [69]. Moreover, abundant ERO1α is also found in MAMs and enhances Ca^{2+} shuttling from the ER to the mitochondria by stimulating IP_3Rs [70].

Impacts of hypoxia on mitochondrial and ER function

Hypoxia and HIFs

To cope with hypoxia, mammals have evolved adaptive mechanisms. Central to this adaptation are HIFs that reprogram the expression of a broad range of genes, including those involved in energetic metabolism, angiogenesis, and proliferation [3, 71]. HIFs are heterodimers consisting of an oxygen-regulated α-subunit (HIF-1α, HIF-2α or HIF-3α) and a constitutively expressed nuclear β-subunit (HIF-1β). Under normoxic conditions, the HIF-α subunit is hydroxylated at conserved proline residue(s) by prolyl hydroxylase domain proteins (PHDs), which utilize O_2 and α-ketoglutarate (also known as 2-oxoglutarate) as substrates. The hydroxylated HIF-α subunit is recognized and targeted for proteasomal degradation by the von Hippel-Lindau (VHL) E3 ubiquitin ligase complex. In hypoxic conditions, PHD activity is inhibited. The HIF-α subunit is then stabilized and translocated to the nucleus, where the α and β subunits form a heterodimer that binds to hypoxia response elements (HREs) in the promoter regions of HIF-regulated genes, triggering their transcription.

Hypoxia, HIFs and mitochondrial function

Mitochondrial oxidative phosphorylation depends on substrate availability. Hypoxia has been shown to disrupt substrate supply to the TCA and oxidative phosphorylation. A variety of glycolytic enzymes and glucose transporters are induced or activated by hypoxia through HIF-1. Among them are glucose transporter 1 (GLUT1), hexokinase 2 (HK2), phosphoglycerate kinase 1 (PGK1), pyruvate dehydrogenase kinase 1 (PDK1), and lactate dehydrogenase A (LDH-A) [72]. These adaptive changes that occur in response to hypoxia shift ATP generation from oxidative phosphorylation towards glycolysis. A similar phenomenon of glycolytic shift can also occur even under an adequate supply of O_2, and is termed ‘the Warburg effect’.

ETC activity is also altered during hypoxia, in part due to the remodeling of ETC components exerted by HIFs [3]. HIF-1-mediated downregulation of NADH dehydrogenase (ubiquinone) 1α subcomplex, 4-like 2 (NDUFA4L2) is found to inhibit Complex I activity [73]. In addition, HIF-1 upregulates the expression of cytochrome c oxidase 4–2 (COX4–2) of Complex IV and the mitochondrial protease LON [74]. LON degrades COX4–1 and facilitates the swapping of COX4–1 for COX4–2, thereby enhancing the efficiency of mitochondrial respiration. The overall impacts of these changes are reduced ETC activity and ROS generation [74]. Moreover, hypoxia also impacts the ETC via HIF-1-responsive microRNA-210 (miR-210). MiR-210 downregulates the iron sulfur cluster protein (ISCU), succinate dehydrogenase complex subunit D (SDHD), and the cytochrome c oxidase (COX) assembly protein COX10, promoting ROS generation [75, 76]. Therefore, HIF-1 causes a paradoxical regulation of ROS production in mitochondria during hypoxia.

Both decrease and increase in ROS generation in mitochondria during hypoxia have been reported. Hypoxia reduces ROS generation in isolated liver mitochondria [77]. However, many studies have demonstrated an increase in mitoROS generation in response to hypoxia [78, 79]. Complexes I and III are the major sites of hypoxia-stimulated ROS production [79, 80]. Thus, the effect of hypoxia on ROS production is likely to be context-dependent and is regulated by the levels and durations of hypoxia.

Hypoxia, HIFs and ER function

The accumulation of unfolded or misfolded proteins in the ER that is induced by hypoxia triggers ER stress and the UPR [81]. Prolyl 4-hydroxylase subunit beta (P4HB), a PDI and a target of miR-210, is downregulated by miR-210 [82]. Hence, hypoxia may disrupt protein folding via the HIF-1α–miR-210 axis. ERO1α expression is increased by hypoxia in an HIF-1α-dependent way, thereby elevating H_2O_2 and triggering ER stress [83]. Among the three branches of the UPR, the PERK pathway appears to be the branch that is predominantly affected by hypoxia. Hypoxia activates the PERK pathway by causing PERK autophosphorylation and subsequent eIF2α phosphorylation [84]. Interestingly, the PERK pathway is a major component of the ISR [42]. Activation of the PERK–eIF2α–ATF4 axis appears to be a major mechanism to inhibit the translation of mRNAs and protein synthesis in hypoxia [85–87]. Hypoxia-induced eIF2α phosphorylation is reduced by catalase and cytochrome c deficiency and imitated by exogenous H_2O_2 [59], thus implicating a role of mitochondrial oxidative stress–ER stress coupling in regulating the UPR during hypoxia. The IRE1–XBP1 and ATF6 pathways are also targeted by hypoxia. Hypoxia induces HIF-1α-dependent XBP1 mRNA expression and splicing [88]. The ATF6α/ATF6 ratio is increased during hypoxia [89].

Regulation of HIFs by ROS and the UPR

Intriguingly, HIF expression and/or stabilization is also regulated by mitochondrial ROS and the UPR. Studies showing that exogenous H_2O_2 stimulated the accumulation of HIFs under normoxia and that scavenging of ROS prevents hypoxic HIF induction suggest a critical role for ROS in regulating HIF signaling [79, 90]. The loss of HIF-1α stabilization in hypoxia in ρ^0 cells implicates the mitochondrial ETC as the HIF-inducing source of ROS [79, 90]. ROS produced at Complex III are found to be the primary contributors

to HIF-1α stabilization [79, 90]. Inhibition of the hypoxic stabilization of HIF-1α by the overexpression of catalase, but not of SOD1 or SOD2, suggests that mitochondria-derived H_2O_2, but not $O_2^{\bullet-}$, is required for hypoxic stabilization of HIFα [79] Inhibition of ER stress with tauroursodeoxycholic acid (TUDCA) also abolishes the increase in HIF-1 expression and activity induced by hypoxia, implicating ER stress as a HIF-1 activator during hypoxia [91]. XBP1 enhances the HIF-1α-activated gene expression in hypoxia by assembling a transcriptional complex with HIF-1α and recruiting RNA polymerase II [88]. These observations suggest that HIFs and ROS or ER stress are interdependent. Depending on the severity and/or duration of hypoxia, their integration may lead to beneficial or deleterious outcomes.

Roles of HIFs, mitoROS and UPR in the pathogenesis of pulmonary hypertension and preeclampsia

Hypoxia is central to the pathogenesis of hypoxia-related pulmonary hypertension [92, 93] and preeclampsia [14, 94]. In humans, prolonged hypoxia due to chronic obstructive pulmonary disease and cystic fibrosis contributes to the development of pulmonary hypertension [92]. Placental hypoxia is a principal factor initiating preeclampsia [95]. Further evidence comes from observations in human and animal models under both hypobaric and normobaric hypoxia. Residing at high altitude is associated with elevated pulmonary artery pressure and pulmonary vascular remodeling in humans [96, 97]. Similarly, the incidence of preeclampsia is ~ 3-fold higher in pregnant women living at high altitude than in those living at low altitude [98, 99]. Rodent models that are exposed to normobaric hypoxia recapitulate many of the features of pulmonary hypertension and preeclampsia seen in humans [100–106].

Roles of HIFs in pulmonary hypertension and preeclampsia

HIFs in pulmonary hypertension—HIFs are major regulators of cellular responses to hypoxia. HIF-1α plays a major role in the proliferation of pulmonary artery smooth muscle cells (PASMCs), whereas HIF-2α primarily promotes endothelial growth pertinent to pulmonary vascular remodeling [107]. Hypoxia induces HIF-1α in human PASMCs and HIF-2α in human lung vascular endothelial cells, as well as HIF-1α in pulmonary arteries or PASMCs in animal models of hypoxia-induced pulmonary hypertension [103, 108–110]. Genetic manipulation has demonstrated that HIFs are mediators of hypoxia-induced pulmonary hypertension. HIF-1α knockdown suppresses the proliferation of PASMCs [103, 111–113]. Mice with a partial HIF-1α deletion (Hif-$1\alpha^{+/-}$) and a smooth muscle-specific HIF-1α deletion have attenuated hypoxia-induced pulmonary vascular remodeling and pulmonary hypertension [114–116]. Endothelial HIF-2α also contributes to the development of pulmonary hypertension in hypoxia. Hif-$2\alpha^{+/-}$ mice are protected from pulmonary vascular remodeling and pulmonary hypertension [117–119]. Moreover, mice expressing a human HIF-2α gain-of-function mutation (G537W) develop pulmonary hypertension [120].

HIF-1α-induced hypoxia inhibits the expression and activities of voltage-gated potassium channels Kv1.5 and Kv2.1 [108, 115] and upregulates the expression of transient receptor potential channels TRPC1 and TRPC6 [121] in PASMCs. Downregulation of the Kv channel

and upregulation of the TRPCs leads to an increase in intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$), which contributes to increased PASMC proliferation and pulmonary vascular tone in pulmonary hypertension [115, 122, 123]. Treatment with HIF-1α short interfering RNA (siRNA) prevents PASMC proliferation by suppressing dynamin-related protein 1 (Drp1) expression and phosphorylation [103, 109], suggesting that HIF-1α also promotes PASMC proliferation by regulating mitochondrial dynamics.

HIFs in preeclampsia—Adequate blood supply to the placenta during pregnancy depends in part on the successful completion of physiological remodeling in the uteroplacental spiral arteries. Failure to transform these vessels is a common feature of preeclampsia and IUGR [17, 124]. High altitude pregnancies also display a decrease in remodeling of the uteroplacental spiral arteries [125]. Following implantation, cytotrophoblasts (CTB) are differentiated to EVT s within anchoring villi. EVTs at the tips of the anchoring villi then invade the spiral arteries, forming plugs that occlude the lumen of these vessels [126]. Thus, placental development in the first trimester of pregnancy occurs in a low O_2 environment, resulting from a lack of uteroplacental blood flow caused by trophoblastic plugs, which is physiological at this stage. At the beginning of second trimester, the trophoblast plugs dislodge from spiral arteries, enabling the onset of uteroplacental blood flow and allowing high flow, low resistance, low velocity maternal blood to enter the intervillous space [127, 128]. Trophoblast invasion and spiral artery remodeling continue until the middle of the second trimester. The regulation of CTB biology by oxygen in the first trimester remains controversial. Some studies have found that hypoxia promotes trophoblast proliferation and inhibits trophoblast differentiation [129, 130], others demonstrate that low oxygen promotes the differentiation of trophoblasts into cells that have an invasive phenotype [131, 132]. It has been proposed that hypoxia promotes the formation of immature EVTs, and that the maturation of these cells into EVT that have invasive potential requires rising oxygen tension [133].

The expression of HIF-1α and HIF-2α in the placenta is consistently high in the first trimester and declines thereafter [129, 134]. This pattern of HIF expression is important for uterine spiral artery remodeling. Antisense-induced inhibition of HIF-1α expression in placental explants at 5–8 weeks of gestation results in inhibition of EVT proliferation and triggers a switch from a proliferative to an invasive trophoblast phenotype [129]. However, Cindrova-Davies and colleagues [135] report undetectable HIF-1α and HIF-2α in the first trimester placenta, a discrepancy that probably results from tissues being collected by different modes of delivery. Placental HIF-1α and HIF-2α are overexpressed in preeclampsia [136–138] and in trophoblast cell lines under hypoxic conditions [139, 140]. High-altitude pregnancy also exhibits high levels of HIF-1α in the placental and uterine arteries [141, 142]. Similarly, elevated placental HIF-1α is also observed in a rodent hypoxic model of preeclampsia [104]. The upregulation of HIFs in uteroplacental tissues plays a crucial role in the pathogenesis of preeclampsia. In an *in vitro* study, constitutive expression of an O_2-insensitive form of HIF-1α (CA-HIF-1α) suppresses the differentiation of rat trophoblast giant cells, which are analogous to human EVTs [143]. Pregnant mice with global overexpression of HIF-1α are hypertensive with proteinuria [144]. Prolonged expression of CA-HIF-1α in the mouse, specifically in trophoblasts using

lentiviral blastocyst transduction and non-surgical embryo transfer, also leads to failure to remodel spiral arteries, maternal hypertension, proteinuria, and fetal growth restriction [145]. Hypoxia-induced HIF-2α upregulates the expression of soluble fms-like tyrosine kinase-1 (sFlt-1) in trophoblasts [139]. Overexpression of sFlt-1 in animal models causes endothelial dysfunction, maternal hypertension and proteinuria, recapitulating preeclampsia phenotypes [146]. Elevated mitoROS induced by sFlT-1 probably contributes to endothelial dysfunction in preeclampsia [147].

HIF-sensitive miR-210, pulmonary hypertension and preeclampsia—miR-210 expression is upregulated in the pulmonary arteries of patients with pulmonary hypertension and is induced in the pulmonary arteries of mice by chronic hypoxia [148, 149]. miR-210 targets ISCU in pulmonary artery endothelial cells (PAECs) to disrupt mitochondria function, contributing to the development of pulmonary hypertension. [149]. In addition, miR-210 exhibits an antiapoptotic role in PASMCs [148]. HIF-1α may also indirectly impair uteroplacental functions by inducing miR-210 expression [142, 150, 151]. Overexpression of miR-210 is observed in preeclamptic placenta and in the uterine arteries in high-altitude pregnancies [150, 152, 153]. miR-210 inhibits trophoblast invasion, in part by promoting mitochondrial dysfunction [150, 154]. Moreover, miR-210 suppresses the expression and function of the large-conductance Ca^{2+}-activated K^+ channel (BK_{Ca}) in uterine arteries [153, 155], leading to increased uterine arterial myogenic tone and uterine vascular resistance.

mitoROS in the pathogenesis of pulmonary hypertension and preeclampsia

mito ROS and pulmonary hypertension—Disruption of mitochondrial ETC is a major contributor to ROS dysregulation. PAECs from patients with pulmonary arterial hypertension (PAH) exhibit elevated levels of mitochondrial $O_2^{\bullet -}$ and H_2O_2 [156]. Mice overexpressing mitochondria-targeted catalase (MCAT) have an attenuated hypoxia-induced increase in pulmonary vessel muscularization, whereas mice overexpressing SOD2 (Tg^{hSOD2}) have exacerbated hypoxia-induced pulmonary hypertension [157]. These findings suggest that H_2O_2 is an important mediator of pulmonary hypertension. Fe-S clusters are important cofactors in Complexes I, II, and III that participate in electron transfer. The NFU1 Fe-S cluster scaffold protein is involved in the biogenesis of Fe-S clusters. NFU1 mutations impair ETC function and are associated with pulmonary hypertension [158, 159]; for example, introduction of the NFU1G206C point mutation in the rat, the equivalent of human G208C, replicates the human pulmonary hypertension phenotype [160]. Like NFU1, ISCU facilitates the assembly of Fe-S clusters. miR-210 is upregulated and its target ISCU is downregulated both in the pulmonary arteries of humans with pulmonary hypertension and in mouse models of pulmonary hypertension induced by chronic hypoxia, SU5416/chronic hypoxia and VHL deficiency ($VHL^{-/-}$) [149]. In human PAECs, miR-210 is induced by prolonged hypoxia exposure [161]. ISCU suppression reduces the activity of mitochondrial Complex I, resulting in increased mitoROS generation in PASMCs and PAECs under hypoxic conditions [75, 161, 162]. Hypoxia also boosts the generation of mitoROS by activating the mitochondrial ATP-dependent K^+ channel ($mitoK_{ATP}$) in PASMCs [163], which subsequently stimulates the HIF–miR-210–ISCU axis to produce more mitoROS [164].

The expression levels and activities of the ion channels and enzymes that regulate Ca^{2+} movement in pulmonary arteries are impacted by chronic hypoxia. Reduced expression of Kv1.5 in pulmonary arteries and in PASMCs has been documented in human patients and in animal models of pulmonary hypertension [108, 165, 166], as well as in PASMCs exposed to chronic hypoxia [167]. By contrast, the expression of TRPC1, TRPC6, transient receptor potential cation channel subfamily V 4 (TRPV4), and ryanodine receptor 2 (RyR2) in PASMCs is increased by chronic hypoxia [121, 168–170]. Moreover, PASMCs from mice exposed to chronic hypoxia display higher phospholipase Cγ 1 (PLCγ 1) expression and activity [171]. ROS, including mitoROS, apparently contribute to the altered expression and activity of ion channels and phospholipase C. Studies from Archer's group show that a reduction in mitoROS causes downregulation of Kv1.5 by activating HIF-1α and nuclear factor of activated T cells cytoplasmic 2 (NFATc2) in PASMCs [108, 166]. Other studies suggest that chronic hypoxia activates mitoK_{ATP} to produce H_2O_2 in mitochondria, resulting in Kv1.5 downregulation in PASMCs [163, 172]. H_2O_2 also appears to regulate the expression of TRPC1 and TRPC6 in PASMCs. The abundance of TRPC1 protein in PASMCs is increased by polyethylene glycol (PEG)-conjugated superoxide dismutase (PEG-SOD) under normoxia and is decreased by PEG-catalase under hypoxia [173]. Chronic hypoxia-promoted dissociation of FKBP12.6 (FK506 binding protein, 12.6 kDa molecular weight) from RyR2 in PASMCs from a chronic-hypoxia-induced pulmonary hypertension mouse model is mediated by ROS generated in Complex III, as evidenced by the suppression of this dissociation by deletion of the Rieske iron-sulfur protein (RISP) [174]. The increased activity of phospholipase C in PASMCs in hypoxia is mediated by H_2O_2 [171]. Overall, the altered expression levels or activities of these channels and enzymes result in increased basal $[Ca^{2+}]_i$ in PASMCs and in increased vascular tone [169, 171, 175, 176].

In response to chronic hypoxia, PASMCs and PAECs display increased proliferation and/or reduced apoptosis [103, 157]. Pulmonary vascular remodeling is exacerbated and mitigated in mice overexpressing SOD2 and mitochondria-targeted catalase, respectively [157]. TEMPOL and mitochondrial-targeted coenzyme Q (MitoQ) reduce the migration and proliferation of Sugen 5416/hypoxia-induced microvascular endothelial cells (MVECs) [177]. Hypoxia promotes PASMC proliferation and suppresses apoptosis by activating mitoK_{ATP} and increasing the production of mitoROS and H_2O_2 [162, 164]. Hypoxia also increases the migration and proliferation of Sugen 5416/hypoxia-induced MVECs through mitoROS-stimulated TRPV4 activation [177]. However, Archer *et al.* [178] demonstrated that the reduction of mitochondrial H_2O_2 due to SOD2 downregulation promotes the proliferation and suppresses the apoptosis of PASMCs in fawn-hooded rats.

Ca^{2+} plays a pivotal role in cell proliferation [179]. Nuclear factor kappa B (NF-κB) and NFAT are Ca^{2+}-regulated transcription factors. NF-κB promotes cell proliferation through transcriptional regulation of cyclin D1, whereas NFAT regulates genes involved in the cell cycle and apoptosis. NF-κB activation is elevated in the PASMCs and PAECs of patients with pulmonary hypertension [180]. Inhibition of NF-κB decreases pulmonary artery hypertrophy *in vivo*, suppresses proliferation, and promotes the apoptosis of PASMCs *in vitro*, establishing a role for NF-κB in pulmonary vascular remodeling [181]. Hypoxia increases the nuclear translocation of NF-κB in rodent lungs and in cultured PAECs [182,

183]. Similarly, the expression and nuclear translocation of NFATc, which is activated in the cytoplasm by the Ca^{2+}-dependent phosphatase calcineurin, is increased in the PASMCs of patients with pulmonary hypertension and of monocrotaline-induced pulmonary artery hypertensive rats, as well as in PASMCs that are exposed to hypoxia [166, 184, 185]. Increased $[Ca^{2+}]_i$ promotes the proliferation and migration, and reduces the apoptosis, of PASMCs by activating NFATc and increasing the nuclear translocation of NFATc [166, 184, 185].

MitoROS and preeclampsia—The placenta, being metabolically active in order to meet the requirements of placental and fetal development and growth, consumes significant amounts of O_2, nutrients, and energy. Perturbations in mitochondrial function in the placenta may lead to excessive generation of ROS, contributing to the pathogenesis of preeclampsia and IUGR [186, 187]. Oxidative stress is commonly detected in placentas from pregnant women with preeclampsia [34], and women with mitochondrial dysfunction have a high incidence of pre-eclampsia [188]. Preeclamptic placentas display damaged mitochondria, as evidenced by swollen mitochondria and broken cristae [150, 189]. Proteomic analysis reveals that a number of proteins that are involved in fatty acid oxidation, TCA, ETC, and ROS homeostasis are altered in preeclamptic placentas [189. 190]. Among them, 2-oxoglutarate dehydrogenase (OGDH), LDH-A, PRXs, NADH dehydrogenase (ubiquinone) iron-sulfur protein 3 (NDUFS3), ubiquinol-cytochrome c reductase core protein 2 (UQCRC2), and ATP synthase are downregulated, whereas NDUFS7, NUDFB8, and NDUFB7 are upregulated.

Preeclampsia reduces both ETC components and activity in the placenta [38, 150, 191, 192]. Similar findings are also seen in placentas from high-altitude pregnancies, in the reduced uterine perfusion pressure (RUPP) rat model of preeclampsia, and in trophoblasts exposed to chronic hypoxia [191, 193]. As expected, mitochondrial $O_2^{\bullet -}$ and H_2O_2 are increased in preeclamptic placentas and in placentas of the hypoxic pregnancy model of preeclampsia [194–197]. Decreases in the expression and activities of SOD, TRXR and GPX are also observed in preeclamptic and high-altitude placentas [197–199]. Preeclampsia is thus associated with heightened oxidative stress in the placenta. Hypoxia plays an important role in mitochondrial dysfunction in preeclampsia [200, 201]. mitoROS production in the placenta could be regulated by HIF-dependent pathways [23, 201, 202]. Furthermore, overexpression of sFlt-1 in the placenta in preeclampsia [146] is secondary to hypoxia and is mediated by HIF-1 [203]. Exposure to serum from preeclamptic women, which is rich in sFlt-1, dissipates the mitochondrial membrane potential (Ψm) and increases mitoROS production in first-trimester trophoblast HTR-8/SVneo cells [147]. Mammalian target of rapamycin (mTOR) activity is also regulated by hypoxia [204]. Hypoxia-induced inhibition of the protein kinase B (AKT)/mTOR pathway has been shown to contribute to the development of preeclampsia [205]. Moreover, placental mTOR is downregulated in IUGR [206]. Inhibition of mTORC1 reduces both the protein expression of Complexes I–IV and mitochondrial respiration, mimicking the phenotype of IUGR placenta [207]. An *in vitro* study revealed that HIF-1α stabilization inhibits first-trimester primary trophoblast invasion by upregulating miR-210 [151]. Thus, if overexpression of HIF occurs in the first trimester (as in early-onset preeclampsia, its inhibitory effect on trophoblast invasion

could be executed in part by miR-210. HIF-1α-dependent miR-210 expression is increased in preeclamptic and high-altitude placentas as well as in hypoxic trophoblasts [150, 191, 208]. MiR-210 downregulates ETC components such as ISCU, COX10, NDUFA4, and SDHD and reduces mitochondrial respiration in trophoblasts [150, 151, 209]. Mitochondrial dysfunction and overproduction of mitoROS impair trophoblast invasion [154, 209] and induce trophoblast apoptosis [210, 211], thereby impairing spiral artery remodeling. The increase in mitoROS production can be imitated by exposing placental explants and trophoblasts to chronic hypoxia [23, 202]. The damaged mitochondria, impaired ETC activity and increased mitoROS in preeclamptic placentas are also replicated in rodent models of preeclampsia (RUPP and hypoxia models) [193, 197].

The BK_{Ca} channel β1 subunit is upregulated in the placenta and the BK_{Ca} channel plays an essential role in reducing uterine vascular resistance and in increasing uteroplacental blood flow in pregnancy [69, 153, 212]. This adaptation was disrupted by upregulated miR-210 in ovine uterine arteries of high-altitude pregnancy [153, 155]. As the expression and activity levels of BK_{Ca} channels are regulated by ROS in the uterine arteries [142, 213, 214], miR-210-induced mitochondrial dysfunction and mitoROS may contribute to the uterine vascular maladaptation seen at high altitude.

ER stress and the UPR in the pathogenesis of pulmonary hypertension and preeclampsia

ER stress and the UPR in pulmonary hypertension—The expression of ATF6 and/or CHOP in pulmonary arteries is increased in patients with PAH, idiopathic pulmonary artery hypertension (IPAH) and systemic sclerosis-associated PAH [215, 216]. In the monocrotaline rat model and in the chronic hypoxic mouse model of pulmonary hypertension, all three UPR pathways are activated in the lung [102, 216, 217]. Chronic hypoxia also activates the UPR *in vitro*, as evidenced by the increased expression of p-eIF2α, IRE1α and ATF6 in cultured PASMCs [215, 218, 219]. Both the PERK and IRE1α pathways are important mediators of hypoxia-induced proliferation of PASMCs. Knocking down eIF2α with siRNA ablates the proliferation of PASMCs under hypoxia [218]. Inhibiting the IRE1 pathway decreases the migration and proliferation of PASMCs and promotes their apoptosis [219].

ER stress and the UPR in preeclampsia and IUGR—Lines of evidence suggest the presence of misfolded proteins in the preeclamptic placenta [220], and a ctivation of the UPR is a common feature of early-onset preeclampsia and IUGR. Significantly elevated ER stress markers, such as BiP/GPR78, pPERK, p-eIF2α, ATF4, IRE1α, ATF6, and CHOP, are observed in preeclamptic and/or IUGR placentas [25, 138, 221–223]. In addition, placentas from high-altitude pregnancy also exhibit ER stress and activation of the PERK pathway [224], which are recapitulated by gestational hypoxia in a rat model of preeclampsia [225]. Hypoxic treatment of placental explants, primary trophoblasts and trophoblast cell lines similarly induces ER stress and activates the UPR [25, 223, 224, 226].

The concurrent increase in HIF-1α and CHOP in preeclamptic placentas suggests that HIF-1α could be the link between hypoxia and the UPR [138]. Induction of ER stress with tunicamycin in pregnant mice reduces both placental and fetal weights [227]. Increased

phosphorylation of eIF2α in preeclamptic, IUGR, and high-altitude placentas suppresses the AKT/mTOR pathway, contributing to fetal growth restriction [186, 221, 224]. Thus, it is not surprising that early-onset preeclampsia and high-altitude pregnancy are associated with increased incidence of IUGR. Placental growth factor (PlGF) is important in regulating placental angiogenesis and trophoblast invasion. In preeclampsia, the circulating level of PlGF is decreased [146]. PlGF expression is negatively regulated by ATF4 and ATF6b in trophoblasts [222], impairing the development and maturation of the placental vascular system. Activation of the PERK pathway is also observed in uterine arteries of pregnant sheep at high altitudes and contributes to an increase in uterine vascular tone [214].

ER–mitochondria interplay in pulmonary hypertension and preeclampsia—The ER and mitochondria are two cellular organelles that are structurally and functionally interconnected. Ca^{2+} flux from the ER to the mitochondria is the most important function of ER-mitochondria crosstalk [228]. Perturbations in ER-mitochondria connections may result in the progression of pulmonary hypertension and preeclampsia. Nogo-B, a member of the reticulon protein family that is primarily localized in the ER, participates in maintaining ER morphology [229]. Patients with pulmonary hypertension and the rat hypoxic model of pulmonary hypertension both have high levels of Nogo-B in their pulmonary arteries [215]. Hypoxia increases the distance between the ER and the mitochondria, reduces mitochondrial Ca^{2+} and mitoROS, and inhibits Ca^{2+}-dependent mitochondrial enzymes in PASMCs through ATF6-mediated upregulation of Nogo-B expression [215]. Furthermore, Nogo-B knockout mice are resistant to chronic hypoxia-induced pulmonary hypertension [215]. These findings suggest that Nogo-B functions as a link between ER stress and mitochondrial stress under hypoxia that promotes the development of pulmonary hypertension.

Uncoupling protein 2 (UCP2), which functions as a Ca^{2+} channel, also participates in translocating Ca^{2+} from the ER to mitochondria [230]. The expression of UCP2 is reduced in the pulmonary microvessels of patients with IPAH and of chronically hypoxic mice [231, 232]. Two independent studies have revealed that UCP2 deletion promotes PASMC proliferation and that UCP2-knockout mice undergo pulmonary vascular remodeling and develop pulmonary hypertension [231, 233]. However, contrasting data on mitoROS generation have been reported from PASMCs of UCP2-knockout mice. Pak *et al.* [231] observed an increase in mitoROS, whereas Dromparis *et al.* [233] detected a decrease in mitoROS. The disruption of Ca^{2+} transport between the ER and mitochondria probably contributes to mitochondrial dysfunction [233]. Placental expression of UCP2 is positively correlated with fetal weight and is reduced in IUGR pregnancies [234]. The downregulation of placental UCP2 is also observed in mice that are exposed to chronic hypoxia [234]. It remains to be determined whether and how ER–mitochondria crosstalk is impacted by reduced UCP2 expression in preeclamptic and IUGR placentas. In rodent models of IUGR, both chronic hypoxia and cadmium promote ER and/or mitochondrial stress in the placenta [225, 235]. Intriguingly, activation of the PERK pathway is prevented by the antioxidants MitoQ and α-phenyl-N-t-butylnitrone, suggesting that communication between the mitochondria and the ER plays an important role in impairing fetal growth in IUGR.

Caution is needed when interpreting data on placental HIFs, mitoROS, ER stress and the UPR because the mode of delivery can greatly impact the generation and activation of stress

[236]. During vaginal delivery, unlike caesarean section, the placenta is exposed to uterine contractions and the accompanying intermittent reduction in maternal blood supply, which are potent inducers of these cellular stress [236–238]. Therefore, comparisons of cellular stresses should be made using the samples from the same mode of delivery. The placenta is highly metabolic active, and therefore rapidly becomes hypoxic soon after disconnecting from the maternal blood supply. Ideally, placental tissues that are suitable for research on cellular stress should be collected from non-labored elective caesarean sections, and snap-frozen in liquid nitrogen or fixed within 10–20 min of detachment from the uterus [236]. Yung *et al.* [221] used samples from elective caesarean sections to demonstrate that ER stress is increased in IUGR placentas, supporting the notion that pregnancy complications heighten cellular stress in the placenta.

Targeting cellular stress as a potential therapy for pulmonary hypertension and preeclampsia

Despite significant progress in elucidating the mechanisms that underlie pulmonary hypertension and preeclampsia, there is still no cure for either disease. The current therapies mainly aim to improve symptoms. Given the high morbidity and mortality of these two diseases, novel therapies are urgently needed. As already discussed, it is apparent that hypoxia, which induces mitochondrial ROS, ER stress and the ISR, is the primary contributor to the pathogenesis of pulmonary hypertension and preeclampsia. Therefore, HIFs, mitochondrial ROS, ER stress, the ISR, and their constituent signaling elements could be promising targets for the development of therapeutics to treat both diseases (Figure 2). Various compounds have been developed to inhibit or lessen HIF signaling, mitochondrial oxidative stress and ER stress or the UPR, and some of them have been evaluated in animal models for the treatment of pulmonary hypertension and preeclampsia (Table 2).

Given the important role of HIFs in the pathogenesis of pulmonary hypertension and preeclampsia, the suppression of HIF signaling is of great therapeutic interest. A variety of pharmacological HIF inhibitors, including 2-methoxyestradiol, apigenin, baicalin, caffeic acid phenethyl ester, celastramycin, cyclosporin a, digoxin, emetine, melatonin, topotecan, and 3-(5’-hydroxymethyl-2’-furyl)-1-benzylindazole (YC-1), have been found to have beneficial effects in rodent models of pulmonary hypertension. In the hypoxic rodent models of pulmonary hypertension, HIF1α/HIF2α inhibitors prevent or reverse hypoxia-induced pulmonary vascular remodeling, right ventricle hypertrophy, and elevated right ventricular systolic pressure (RVSP) [239–250]. Comparable findings were seen with HIF1α/HIF2α inhibition in $Egln1^{Tie2Cre}$ mice and with monocrotaline-induced PAH rats [251, 252]. R59949, a PHD2 activator that inhibits HIF, also ameliorates pulmonary hypertension in hypoxic mice by reversing hypoxia-induced pulmonary vascular remodeling, right ventricle hypertrophy, and elevated RVSP [252]. Similarly, other therapeutic approaches, such as delivery of anti-CD146 to disrupt the CD146–HIF-1α axis, delivery of small hairpin RNA (shRNA) to silence HIF1α, and delivery of anti-miR-210 oligonucleotide to inhibit HIF-1-responsive miR-210, have been shown to attenuate experimental pulmonary hypertension in hypoxic rodents [112, 149, 253]. 2-Methoxyestradiol is a metabolite of the endogenous 17β-estradiol, formed through the enzymatic actions of cytochrome P-450 and

catechol-O-methyltransferase (COMT). The expression of COMT in the placenta and levels of circulating 2-methoxyestradiol are low in pregnant women with preeclampsia [254]. Furthermore, the administration of 2-methoxyestradiol amends hypertension and proteinuria in a $COMT^{-/-}$ mouse model of preeclampsia [254].

The reduction of mitoROS is also an attractive approach in the treatment of pulmonary hypertension and preeclampsia. Mice overexpressing mitochondria-targeted catalase exhibit attenuated chronic-hypoxia-induced increases in RVSP and pulmonary vascular remodeling [157]. Melatonin can be transported into mitochondria via peptide transporters PEPT1 and PEPT2 [255]. and protects mitochondria from stress by scavenging ROS [256]. When administered, melatonin reduced the mean arterial pressure in RUPP rats [257]. MitoQ, which harbors the antioxidant quinone moiety covalently attached to a lipophilic triphenylphosphonium cation, has been used in preclinical studies in rats and mice and in human trials [258], but it fails to prevent pulmonary hypertension in mice exposed to chronic hypoxia [259], probably due to insignificant changes in the lung mitoROS of this animal model. In a rat model of IUGR produced by prenatal hypoxia, maternal injection of MitoQ-loaded nanoparticles prevented placental oxidative stress and rescued fetal growth [260, 261]. MitoQ has also been seen to restore fetal growth in hypoxic sheep [262]. Similarly, the mitochondria-specific antioxidants MitoQ and MitoTEMPO reduced maternal mean arterial pressure and improved fetal growth in the RUPP rat model of preeclampsia [263]. It appears that timing is a critical factor for the actions of MitoQ. MitoQ treatment in early pregnancy exacerbated maternal blood pressure, proteinuria, and fetal growth restriction, whereas MitoQ administration in late gestation alleviated the preeclampsia phenotype in RUPP rats [197].

ER stress and the UPR may also be targeted in the development of treatments for pulmonary hypertension and preeclampsia. Two chemical chaperones, 4-phenylbutyric acid (4-PBA) and TUDCA, have been approved by the US Food and Drug Administration (FDA) for the treatment of urea-cycle disorders and primary biliary cirrhosis, respectively [264, 265]. Interestingly, 4-PBA effectively prevents and reverses the elevat ed pulmonary artery pressure and pulmonary vascular remodeling seen in chronic-hypoxia-exposed mice and/or monocrotaline-induced PAH rats [101, 102, 217, 266]. Pharmacological inhibition of the PERK pathway with GSK2606414 reduced pulmonary vascular remodeling, right ventricle hypertrophy, and elevated RVSP in Sugen 5416/hypoxia PAH mice [267]. H_2S also alleviates pulmonary hypertension by inhibiting ER stress in hypoxic rats [268]. Furthermore, TUDCA and GSK2606414 inhibit the effect of gestational hypoxia, reduce uterine arterial myogenic tone and decrease placental protein levels of endothelin-1 and sFlt-1 [213, 214, 269]. A recently developed drug, ISRIB (integrated stress response inhibitor), potently inhibits the ISR and is bioavailable *in vivo* with no overt toxicity, owing to its bi-mode of action and bell-shaped response to the ISR [42, 265]. It has been found to improve inflammation and cognitive disorders in preclinical studies in rodents [270], and its therapeutic potential in pulmonary hypertension and preeclampsia is yet to be determined.

Although promising preclinical results have been obtained in animal models, the translation of these findings into clinical applications is challenging. As components of HIF, ROS, and the UPR and ISR signaling pathways are broadly expressed, the specific delivery

of the therapeutic agent to the target site(s), such as organ, tissue, cell, or organelle undergoing cellular stress, is preferable in order to avoid unintended effects or toxicity. Some progress have been made; for example, mitochondria-targeted antioxidants that conjugate the lipophilic triphenylphosphonium cation to an antioxidant moiety allow these compounds to pass through biological membranes easily and thus to accumulate to levels that are increased several-hundred-fold within mitochondria [271]. The selective delivery of drugs to the placenta has been reviewed recently [201]. Therapeutic molecules conjugated with specific peptide sequences that are designed to recognize cell surface macromolecules in uteroplacental cells have been successfully and selectively delivered to the placenta [201]. It is also important to note that HIFs, mitochondrial ROS and the UPR are also pivotal for physiological signaling. We anticipate the development of therapeutic approaches that can selectively inhibit only the disease-relevant HIF, ROS, UPR or ISR with minimal impact on stress-mediated physiological signaling. Greater effort in future research and drug development will be needed to meet this expectation.

Conclusions

Oxygen is vital sustaining the activities of mitochondria and the ER, and this for cellular functions. Intriguingly, the activities of these two organelles are closely interlinked. Hypoxia has been shown to reprogram or perturb functions performed in the mitochondria and ER, resulting in the activation of HIFs, change in mitoROS production, and induction of the UPR or ISR. Substantial evidence suggests the involvement of HIFs, mitoROS and the UPR in the development and progression of pulmonary hypertension and preeclampsia. HIFs, mitoROS and the UPR or ISR could form a complex signaling network, and could act independently or interdependently to promote these two disorders. Significant progress has been made in revealing the mechanistic links between hypoxia and pulmonary hypertension or preeclampsia. Nevertheless, we are still far away from a comprehensive understanding of the molecular mechanisms underlying these two diseases. F urther research is undoubtedly needed so that we can comprehend the pathogenesis of pulmonary hypertension and preeclampsia and can develop more specific and effective therapeutic interventions.

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Abbreviations:

AKT	protein kinase B
ATF6	activating transcription factor 6
BiP	binding immunoglobulin protein
BK_{Ca}	large-conductance Ca^{2+}-activated K^+ channel
CHOP	C/EBP homologous protein
COMT	catechol-O-methyltransferase
COX4–2	cytochrome c oxidase 4–2
CTB	cytotrophoblasts
eIF2α	eukaryotic translation initiation factor 2α
ER	endoplasmic reticulum
ERAD	ER-associated degradation
ERO1	ER oxidoreductin 1
ETC	electron transport chain
EVT	extravillous trophoblast
FAD	flavin adenine dinucleotide
GCN2	general control nonderepressible protein 2
GPX1	glutathione peroxidase 1

Grp78	78-kDa glucose-regulated protein
GSH	glutathione
HIF	hypoxia inducible factor
$HO^{\bullet}$	hydroxyl radical
HRI	heme-regulated inhibitor
IP_3R	inositol trisphosphate receptor
IPAH	idiopathic pulmonary artery hypertension
IRE1α	inositol-requiring enzyme 1α
ISCU	iron sulfur cluster protein
ISR	integrated stress response
IUGR	intrauterine growth restriction
LDH-A	lactate dehydrogenase A
mitoK$_{ATP}$	mitochondrial ATP-dependent K^+ channel
mitoQ	mitochondrial-targeted coenzyme Q
mitoROS	mitochondrial ROS
MAM	mitochondria-associated endoplasmic reticulum membrane
mTOR	mammalian target of rapamycin
NADH	reduced form of nicotinamide adenine dinucleotide
NDUFS3	NADH dehydrogenase (ubiquinone) iron-sulfur protein 3
NFATc2	nuclear factor of activated T cells cytoplasmic 2
NF-κB	nuclear factor kappa B
NOX	nicotinamide adenine dinucleotide phosphate (NADPH) oxidase
Nrf2	nuclear factor2
$O_2^{\bullet-}$	superoxide
PAEC	pulmonary artery endothelial cell
PAH	pulmonary arterial hypertension
PASMC	pulmonary artery smooth muscle cell
PDI	protein disulfide isomerase
PEPT1	peptide transporter 1

PERK	protein kinase RNA-like ER kinase
PHD	prolyl hydroxylase domain protein
PKR	double-stranded RNA-dependent protein kinase
PlGF	placental growth factor
PRX3	peroxiredoxin 3
ROS	reactive oxygen species
RUPP	reduced uterine perfusion pressure
RVSP	right ventricular systolic pressure
RyR2	ryanodine receptor 2
SDHD	succinate dehydrogenase complex subunit D
sFlt	soluble fms-like tyrosine kinase
siRNA	short interfering RNA
SOD1	superoxide dismutase 1
TC	ternary complex
TCA	tricarboxylic acid
TRPC1	transient receptor potential channel 1
TRPV4	transient receptor potential cation channel subfamily V 4
TRX2	thioredoxin 2
TRXR2	thioredoxin reductase 2
TUDCA	tauroursodeoxycholic acid
UCP2	uncoupling protein 2
UPR	unfolded protein response
VDAC1	voltage-dependent anion-selective channel protein 1
VHL	von Hippel-Lindau complex
VSMCs	vascular smooth muscle cells
XBP1	X-box binding protein 1

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Highlights

- Hypoxia is a common cause of pulmonary hypertension and preeclampsia.
- Mitochondria and endoplasmic reticulum (ER) are the main targets of the hypoxic response.
- Oxidative and ER stress converge in the ISR.
- The ISR contributes to pulmonary hypertension and preeclampsia.
- Potential drug development may target the ISR.

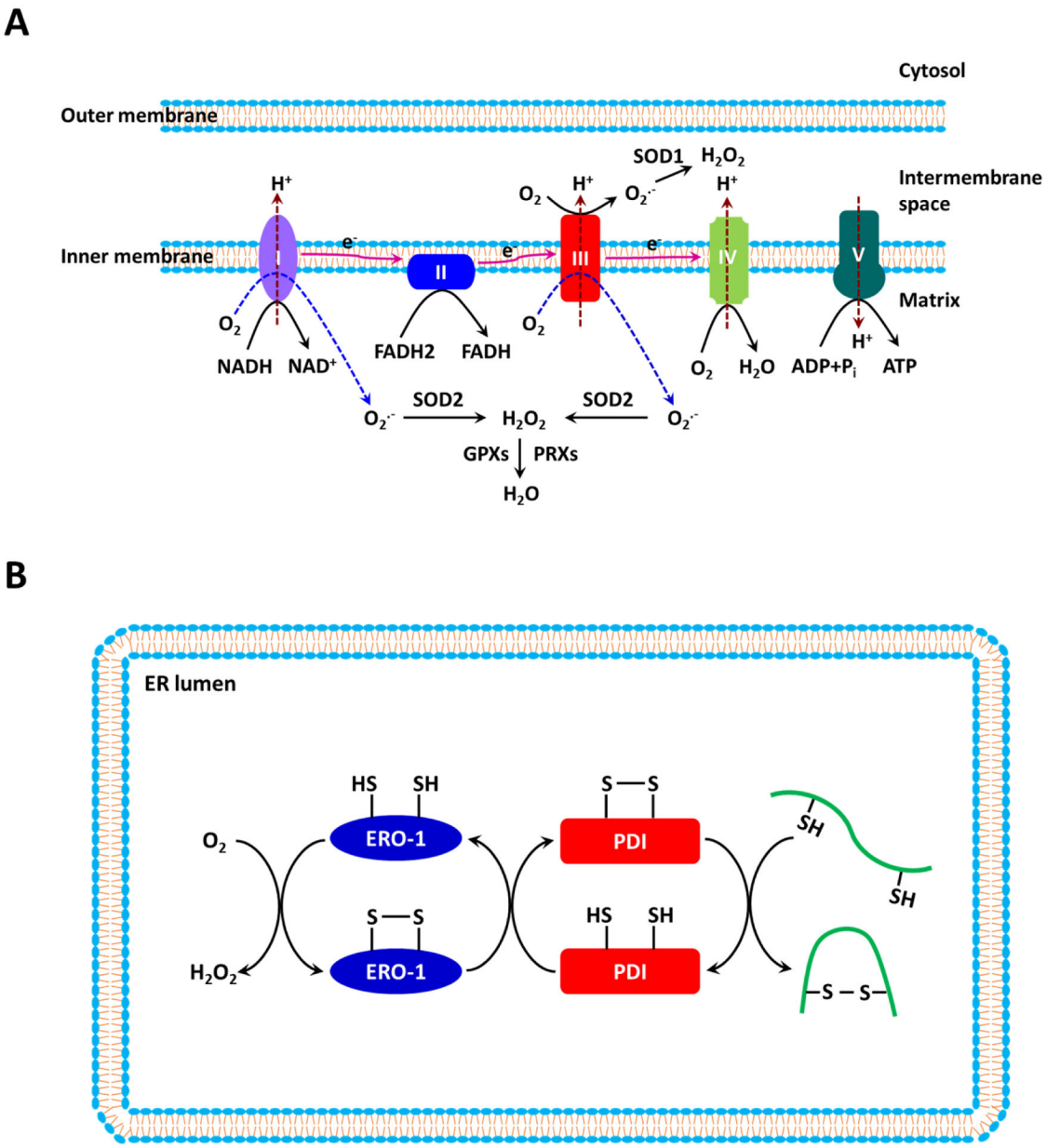

Figure 1. Reactive oxygen species (ROS) biogenesis and metabolism in mitochondria and the endoplasmic reticulum (ER).

(a) The mitochondrial electron transport chain (ETC), which is composed of four complexes (I–IV) in the inner mitochondrial membrane, transfers electrons from electron donors to electron acceptors. Electrons from reduced nicotinamide adenine dinucleotide (NADH) enter the ETC at Complex I, whereas electrons from reduced flavin adenine dinucleotide (FADH2) enter the ETC at Complex II. Molecular O_2 serves as the final electron acceptor. The chief function of the ETC is to synthesize ATP by coupling oxidative phosphorylation with the ATP synthase. Superoxide ($O_2^{\bullet-}$) is produced primarily at Complexes I and III as a result of the incomplete reduction of O_2. At Complex I, $O_2^{\bullet-}$ is produced within the matrix, whereas at Complex III, $O_2^{\bullet-}$ is released into both the matrix and the intermembrane space. $O_2^{\bullet-}$ is dismutated to H_2O_2 by superoxide dismutase 1 (SOD1) in the intermembrane

space and by SOD2 in the matrix. H_2O_2 is subsequently reduced to H_2O by glutathione peroxidases (GPXs) and peroxiredoxins (PRXs). **(b)** The formation of disulfide bonds in nascent proteins in the ER is driven by protein disulfide isomerase (PDI) and endoplasmic reticulum oxidoreductin-1 (ERO-1). H_2O_2 is generated as the result of electron transfer between PDI and ERO-1 during the oxidative protein folding process.

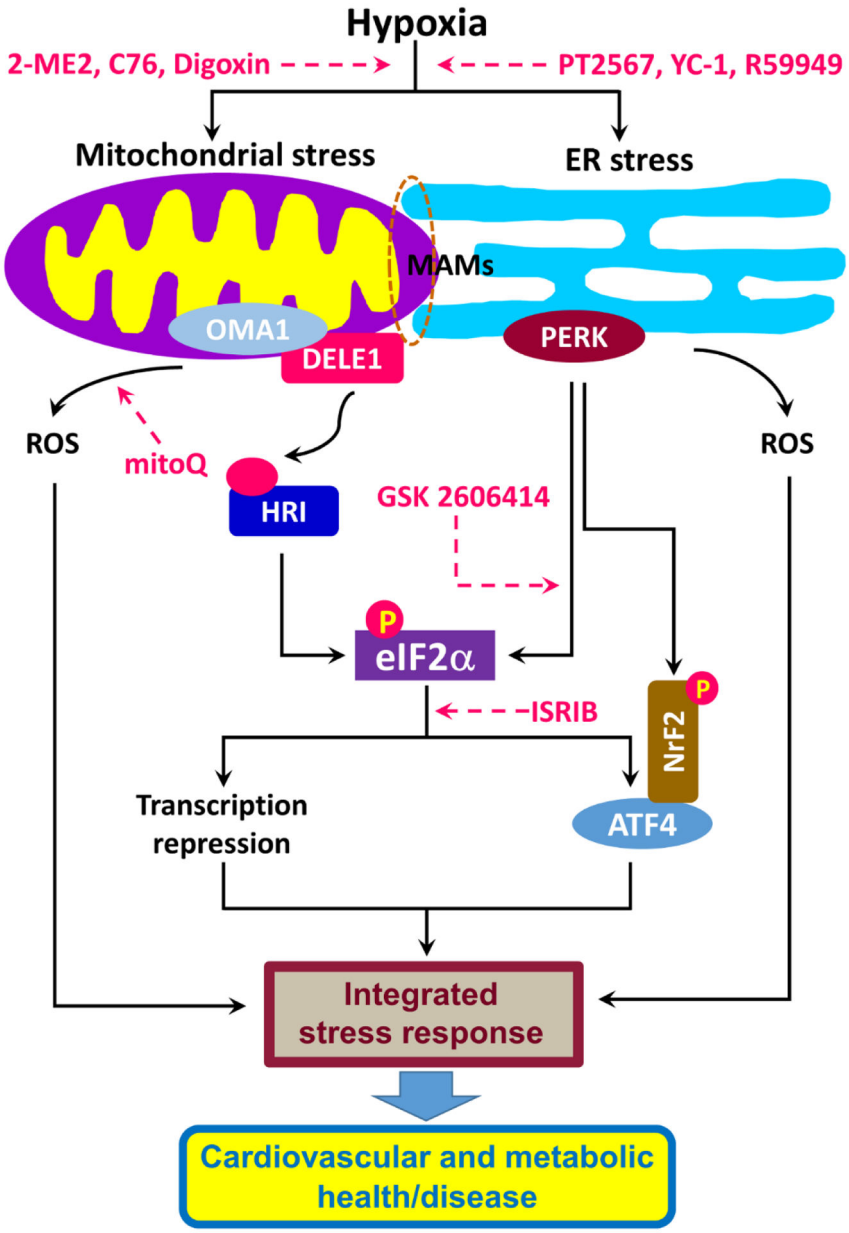

Figure 2. Hypoxia and the integrated stress response (ISR): potential drug targets.

Hypoxia induces mitochondrial stress and endoplasmic reticulum (ER) stress. Mitochondrial stress produced via the OMA1-DELE1-HRI pathway and ER stress resulting from the PERK pathway converge at the phosphorylation of eIF2α, leading to changes in the transcription of genes and subsequent cardiovascular and metabolic (mal)adaptations. The ISR can also be activated by oxidative stress resulting from the accumulation of reactive oxygen species (ROS). Potential targets for drug development are indicated by dashed pink arrows.

Abbreviations: ATF4, activating transcription factor 4; C76, compound 76; DELE1, DAP3-binding cell death enhancer 1; eIF2α, eukaryotic initiation factor 2α; HRI, heme-regulated inhibitor; ISRIB, integrated stress response inhibitor; MAM, mitochondria-associated

membrane; ME, 2-methoxyestradiol; mitoQ, mitochondrial-targeted coenzyme Q; NrF2, Nuclear factor erythroid 2-related factor 2; OMA1, a mitochondrial metallopeptidase encoded by the OMA1 gene; PERK, PKR-like ER kinase 2; YC-1: 3-(5'-hydroxymethyl-2'-furyl)-1-benzylindazole.

Table 1.

Comparison of phenotype and biochemistry between pulmonary hypertension and preeclampsia.

	Pulmonary hypertension	Preeclampsia
Local vascular resistance	• Increased pulmonary vascular resistance • Increased mean pulmonary arterial pressure (>20 mm Hg)	• Increased uteroplacental vascular resistance • Reduced uteroplacental blood flow
Systemic blood pressure		• Elevated systolic and diastolic blood pressure (>140 and >90 mmHg, respectively
Vascular remodeling	• Increased vascular remodeling due to intimal and medial hypertrophy, fibrosis, inflammation, etc.	• Reduced vascular remodeling due to impaired endovascular trophoblast invasion and subsequent failure to replace vascular smooth muscle cells (VSMCs) and endothelial cells
Complications	• Right ventricular overload and heart failure	• Intrauterine growth restriction • Proteinuria • Uteroplacental and other maternal organ dysfunction
Endothelin-1	• Increased circulating endothelin-1	• Increased circulating endothelin-1
Hypoxia, hypoxia inducible factor (HIFs) and microRNA-210 (miR-210)	• Hypoxia is a major contributor to Group III pulmonary hypertension resulting from lung disease and/or hypoxia • Increased incidence at high altitude • Increased expression of HIFs in pulmonary arteries • Increased expression of miR-210 in pulmonary arteries	• Hypoxia is a major contributor to preeclampsia • Increased incidence at high altitude • Increased expression of HIFs in uteroplacental tissues • Increased expression of miR-210 in uteroplacental tissues
Reactive oxygen species (ROS) and oxidative stress	• Increased ROS and oxidative stress in pulmonary arteries (some studies suggest decreased ROS and oxidative stress)	• Increased ROS and oxidative stress in uteroplacental tissues
ER stress and the unfolded protein response (UPR)	• Increased ER stress and activation of the UPR in pulmonary arteries	• Increased ER stress and activation of the UPR in uteroplacental tissues

Table 2.

Promising compounds targeting hypoxia and the mitochondrial ROS and ER stress signaling pathways in animal models of pulmonary hypertension and preeclampsia.

Compound	Mode of action	Animal model	Pulmonary hypertension			Preeclampsia			Reference
			Pulmonary artery pressure (PAP)/right ventricular systolic pressure (RVSP)	Right ventricle hypertrophy	Pulmonary artery remodeling	Mean arterial pressure	Proteinuria	Fetal growth	
Hypoxia inducible factor (HIF) pathway									
2-methoxyestradiol	HIF-1α inhibition	Hypoxic rats	↓	↓	↓				[239]
2-methoxyestradiol	HIF-1α inhibition	$COMT^{-/-}$ mice				↓	↓		[254]
Apigenin	HIF-1α inhibition	Hypoxic rats	↓	↓	↓				[240]
Baicalin	HIF-1α inhibition	Hypoxic rats	↓	↓	↓				[241]
C76	HIF-2α inhibition	$Egln1^{Tie2Cre}$ mice, monocrotaline (MCT) rats, Sugen/hypoxic rats	↓	↓	↓				[242]
Caffeic acid phenethyl ester	HIF-1α inhibition	MCT rats	↓	↓	↓				[251]
Celastramycin	HIF-1α inhibition	Hypoxic mice, MCT rats, Sugen/hypoxic rats	↓	↓	↓				[243]
Cyclosporin A	HIF-1α inhibition	Hypoxic rats	↓	↓	↓				[244]
Digoxin	HIF-1α inhibition	Hypoxic mice	↓	↓	↓				[245]
Emetine	HIF-1α and HIF-2α inhibition	MCT rats, Sugen/ hypoxic rats	↓	↓	↓				[246]
Melatonin	HIF-1α inhibition	Hypoxic rats	↓	↓	↓				[247]
PT2567	HIF-2α inhibition	Hypoxic rats		↓	↓				[248]
Topotecan	HIF-1α inhibition	Hypoxic rats	↓	↓	↓				[249]
YC-1	HIF-1α inhibition	Hypoxic mice	↓	↓	↓				[250]
R59949	PHD2 activation	Hypoxic mice	↓	↓	↓				[252]
Anti-CD146 antibody	HIF-1α inhibition	Hypoxic mice	↓	↓	↓				[253]
HIF-1α small hairpin RNA (shRNA)	HIF-1α silencing	Hypoxic rats	↓		↓				[112]
Anti-miR-210 oligonucleotide	microRNA-210 (miR-210) inhibition	Sugen/hypoxic mice	↓		↓				[149]

Compound	Mode of action	Animal model	Pulmonary hypertension				Preeclampsia		Reference
			Pulmonary artery pressure (PAP)/right ventricular systolic pressure (RVSP)	Right ventricle hypertrophy	Pulmonary artery remodeling	Mean arterial pressure	Proteinuria	Fetal growth	
mitoROS									
Melatonin	mitoROS scavenger	RUPP rats				↓			[257]
MitoQ	Mitochondria-targeted antioxidant	Hypoxic mice	↔	↓	↔				[259]
MitoQ	Mitochondria-targeted antioxidant	RUPP rats				↓		↑	[263]
MitoQ	Mitochondria-targeted antioxidant	RUPP mice				↓		↑	[197]
MitoQ	Mitochondria-targeted antioxidant	Hypoxic sheep						↑	[262]
MitoQ-nanoparticles (NPs)	Mitochondria-targeted antioxidant	Hypoxic rats						↑	[260, 261]
Mito-Tempo	Mitochondria-targeted antioxidant	RUPP rats				↓		↑	[263]
ER stress and the unfolded protein response (UPR)									
4-PBA	ER stress inhibition	Hypoxic mice MCT rats	↓	↓	↓	↓			[101]
4-PBA	ER stress inhibition	Hypoxic mice	↓	↓	↓	↓			[102]
4-PBA	ER stress inhibition	MCT rats	↓	↓	↓	↓			[217, 266]
GSK2606414	PERK inhibition	Sugen/hypoxic rats	↓	↓	↓	↓			[267]
H_2S	ER stress inhibition	Hypoxic rats	↓	↓	↓				[268]

Abbreviations: 4-PBA, 4-phenylbutyric acid; C76, compound 76; COMT, catechol-O-methyltransferase; H2S, hydrogen sulfide; MitoQ, Mitochondrial-targeted coenzyme Q; RUPP, reduced uterine perfusion pressure; YC-1, 3-(5'-hydroxymethyl-2'-furyl)-1-benzylindazole.