Review Article
A crucial role of endoplasmic reticulum stress in cellular responses during pulmonary arterial hypertension

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Abstract: Pulmonary arterial hypertension (PAH), a chronic and progressive disease of the lung vascular system, is characterized by vasculopathy in the pulmonary arterioles, especially in endothelial cells and pulmonary vascular smooth cells. Several mechanisms are involved in PAH occurrence and development, and all are characterized by excessive pulmonary vasoconstriction and abnormal vascular remodeling, which leads to a progressive resistance to blood flow and an increase in pulmonary artery pressure. Recent studies have shown that endoplasmic reticulum (ER) stress is implicated in the pathophysiology of PAH. In this review, we highlight the effect of ER stress on the proliferation and apoptosis of endothelial cells and pulmonary vascular smooth muscle cells, and discuss the feasibility of targeting unfolded protein response components as a strategy to reverse or alleviate the progression of PAH.

Introduction

Pulmonary arterial hypertension (PAH) is a serious lung disease that results in right heart failure. The main symptoms of PAH are dyspnea, chest pain, dizziness and syncope; some patients may have hemoptysis. The World Health Organization (WHO) classifies PAH into four groups: primary PAH formerly encompassed idiopathic, familial, and anoxia-induced PAH as group I; other factors induce PAH as group II; PAH with left heart disease is classified as group III; PAH associated with lung diseases and/or hypoxemia comprises group IV; and PAH due to chronic thrombotic and/or embolic disease is classified as group V. Several mechanisms are involved in PAH occurrence and development, and all are characterized by excessive pulmonary vasoconstriction and abnormal vascular remodeling, which leads to progressive resistance to blood flow and an increase in pulmonary artery (PA) pressure [1]. Molecular biology techniques have revealed three signal transduction pathways involved in

PAH: the nitric oxide (NO) pathway [2, 3], the prostacyclin (PGI_2) pathway [4], and the endothelin-1 (ET-1) pathway [5]. The discovery of those key signaling pathways has deepened our understanding of PAH and promoted the treatment of PAH from the era of symptomatic supportive therapy to the era of targeted therapy. Pharmacological interventions, including NO, PGI_2, and ET-1 receptor agonists and phosphodiesterase-5 inhibitors, are available, but the therapeutic effect of these drugs is still limited. These diverse pathogenic mechanisms increase the difficulty of treatment and lead to an unsatisfactory prognosis. Studies have shown that the mortality of PAH is 15% within 1 year of modern therapy [6].

The endoplasmic reticulum (ER), an essential cellular organelle, produces diverse molecules ranging from hormones to signaling molecules and regulates systemic metabolic, inflammatory, and endocrine processes and other activities in the body [7]. The ER can be influenced by hypoxia, oxidative injury, toxins, aberrant Ca^{2+}
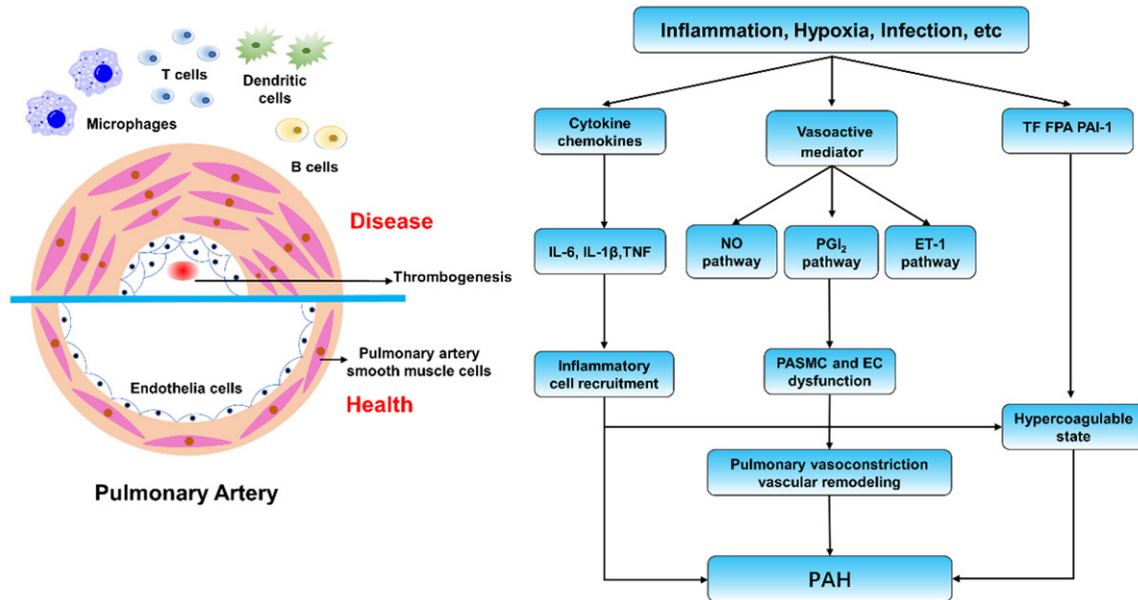
Figure 1. Overview of the mechanism of pulmonary hypertension. Multiple stimuli activate three signal transduction pathways: the nitric oxide (NO) pathway, the prostacyclin (PGI$_2$) pathway, and the endothelin-1 (ET-1) pathway and eventually cause various pathological processes. The imbalance in vasoactive mediators, dysfunction of endothelial and smooth muscle cells, vascular remodeling, and thrombogenesis mediate the pathogenesis of PAH.

regulation, viral infection and other physiological and pathological insults during protein folding processes [8], leading to an increase in unfolded proteins. Recent studies have suggested that ER stress is involved in the pathogenesis of PAH through unfolded and/or misfolded proteins [9, 10]. To help elucidate the pathomechanism of PAH, in this review, we focus on the relationship between ER stress and PAH at the cellular level and hope to provide a new strategy against PAH progression in clinical settings.

The occurrence and development of PAH

PAH is characterized by excessive pulmonary vasoconstriction and abnormal vascular remodeling [11]. Although the etiology and categories of PAH are diverse and complex, the pathological processes of various types of PAH are similar. Several studies have suggested that inflammation plays a vital role in the initiation and progression of PAH. Cytokines such as tumor necrosis factor, IL-6, and IL-1β were increased in IPAH [12-14], and animal models also support this view [14]. Some chemokines lead to inflammatory cell recruitment [15]. Previous research investigated the levels of vasoactive mediators among patients with PAH, and the

results suggested that some mediators (ET-1, thromboxane, 5-hydroxytryptamine) increase while others (PGI$_2$) decrease [16]. These changes may induce or inhibit signaling pathways that regulate the proliferation, apoptosis and remodeling of endothelial cells (ECs) and pulmonary artery smooth muscle cells (PASMCs). Recent studies have reported that the presence of perivascular inflammatory cells and factors might influence the microenvironment, leading to disordered angiogenesis [17, 18]. Moreover, increased plasma levels of tissue factor, fibrinopeptide A, and plasminogen activator inhibitor 1 result in a hypercoagulable state in patients [19, 20]. In summary, the imbalance in vasoactive mediators, dysfunction of ECs and PASMCs, vascular remodeling, and thrombogenesis mediate the pathogenesis of PAH [11] (**Figure 1**).

ER stress and signal transduction pathways

The ER is one of the most important organelles in eukaryotes and participates in the synthesis, folding, modification and transportation of proteins. Notably, protein folding is the most error-prone step in gene expression [21] (**Figure 2**). Under normal conditions, the ER chaperone BiP/GRP78 is combined with three canonical
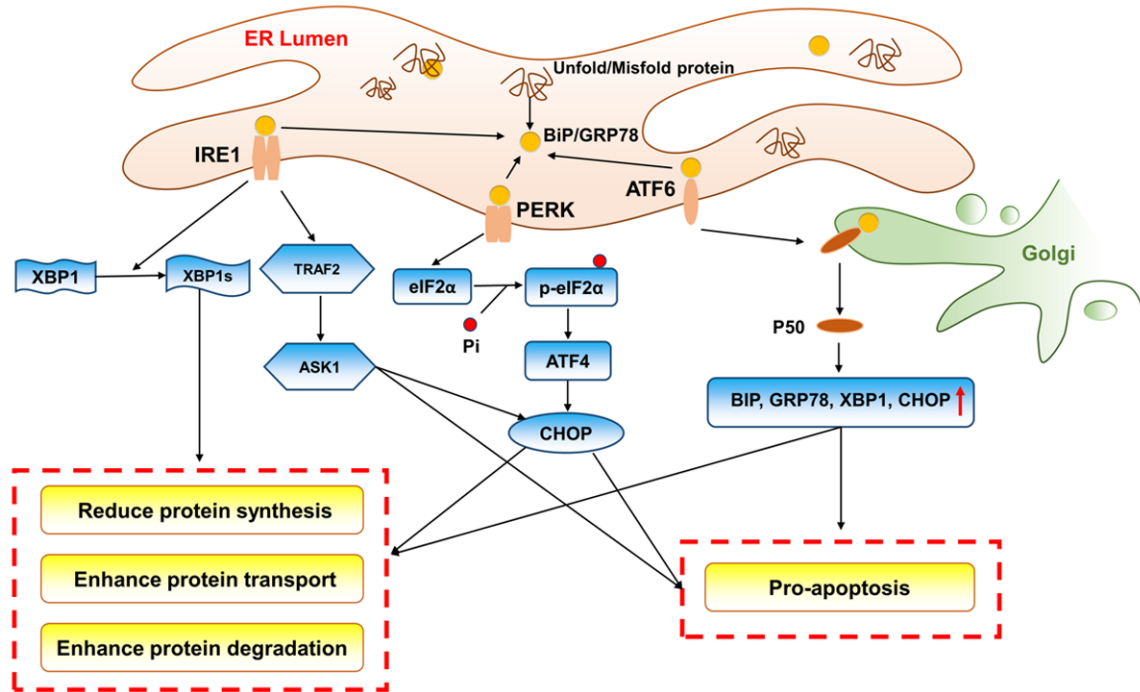
Figure 2. Schematic illustration of ER stress and the activation of the three UPR pathways. Under stressed conditions, the separation of BiP/GRP78 and UPR sensors activates the signal transduction pathways. PERK is activated to phosphorylate eIF2α at Ser51, and this process increases the ATF4 level. DDIT/CHOP is the target of ATF4 and can help to upregulate apoptosis genes and downregulate antiapoptosis genes. IRE1 cleaves a 26-nucleotide segment from *XBP1* mRNA to produce a transcriptionally active XBP1 (XBP1s). XBP1 can accelerate protein folding and transport as well as degeneration, and IRE1 can also react directly with TRAF2, leading to activation of the apoptosis signaling pathway and inhibition of the antiapoptosis signaling pathway. ATF6 transits to the Golgi and is processed by the proteases S1P and S2P to become an activated fragment called PSOATF6. PSOATF6 moves into the nucleus and subsequently activates the expression of BiP, ER protein 57, and Grp 94. These proteins enhance the capacity to alleviate protein loading and maintain ER homeostasis through accelerating protein folding, transduction and degradation.

unfolded protein response (UPR) sensors, PKR-like eukaryotic initiating factor α kinase (PERK), activating transcription factor-6 (ATF6), and inositol-requiring enzyme 1 (IRE1) [7], and the related downstream pathway is blocked. Oxidative stress, hypoxia, Ca+ disorder or other pathologic conditions will disturb protein folding, leading to an accumulation of unfolded proteins, which results in the separation of BiP/GRP78 and UPR sensors and finally activates the signal transduction pathways.

The PERK signaling pathway

PERK, a type-I transmembrane protein, phosphorylates eIF2α at Ser51. On the one hand, eIF2α-P can inhibit the formation of a translation initiation complex to reduce global protein synthesis [22]. On the other hand, this process increases the levels of ATF4, which can enhance protein transportation in the ER [23]. Addi-

tionally, DDIT/CHOP is a target of ATF4 and can help upregulate apoptosis genes and down-regulate anti-apoptosis genes [24]. When ER stress protein folding homeostasis is restored, ATF4 and CHOP can induce the upregulation of DNA-damage-inducible protein 34 (GADD34) and the dephosphorylation of eIF2α-P, restoring the protein synthesis [25].

The IRE1 signaling pathway

IRE1 is the most evolutionarily conserved bifunctional type-I transmembrane protein of the UPR [8]. This protein cleaves a 26-nucleotide segment from *XBP1* mRNA to produce a transcriptionally active XBP1 (XBP1s). XBP1 can accelerate protein folding and transport as well as degeneration, which alleviates protein loading in the ER and reverses the UPR [21]. Additionally, IRE1 can react directly with tumor necrosis-associated receptor 2 (TRAF2)

through a series of cascade reactions, leading to activation of the apoptosis signaling pathway and inhibition of the anti-apoptosis signaling pathway [26, 27].

The ATF6 signaling pathway

ATF6 is a type-II transmembrane-activating transcription factor. Under ER stress conditions, ATF6 transitions to the Golgi and is processed by the proteases S1P and S2P, yielding an activated fragment called p50. P50 moves to the nucleus and subsequently increases the expression of protein chaperones (binding immunoglobulin protein (BiP), ER protein 57), glucose-regulated proteins (glucose-regulated protein 94 (Grp 94)), and ER-associated proteins [21]. These proteins enhance the capacity to alleviate protein loading and maintain ER homeostasis by accelerating protein folding, transduction and degradation.

Pathological mechanisms of ER stress promoting PAH

PAH is related to excessive pulmonary vasoconstriction and abnormal vascular remodeling, and past research has identified a potential link between ER stress and PAH. Therefore, we hypothesized that ER stress causes vascular pathological changes and thus participates in the occurrence and development of PAH. In this review, we examined the relevant research and literature to explore how ER stress affects the function of vascular ECs and PASMCs.

ECs

Persistent ER stress and the UPR are important pathogenic mechanisms for many chronic diseases, such as neurodegenerative disease, atherosclerosis, type 2 diabetes, liver disease, and cancer [28, 29]. Prolonged perturbation of ER stress and UPR activation can cause increased oxidative stress and inflammation in ECs, which often leads to dysfunction and disease [30]. On the one hand, studies have shown that EC injury or dysfunction may be a pivotal pathophysiological mechanism that increases the susceptibility to PAH [31, 32]. On the other hand, various studies have proven that damage to ECs can result from the excessive activation of ER stress and the UPR [33-37]. On this basis, Guignabert [38] explored the correlation between PAH and ER stress in ECs

using a dasatinib-treated experimental group and a control group treated with imatinib. Dasatinib is a drug used to treat chronic myeloid leukemia and can also induce PAH in humans and rodents, while imatinib cannot. This study examined the levels of endothelial and vascular dysfunction and damage markers, both of which increased significantly in the experimental group compared with the control group. Furthermore, the author examined markers of ER stress and found an increase in the molecular chaperone GRP78, ATF6 and XBP1 in rat lung homogenates and a significant decrease in eIF2α in the dasatinib-treated group but not the control group. These results indicated that EC damage and ER stress caused by dasatinib treatment of chronic myeloid leukemia may be one of the pathological mechanisms. The results of other studies are similar. In another review, the author explored whether the UPR pathways are activated in ECs [39]. They examined the expression of UPR markers in the lung vasculature of patients with systemic sclerosis-associated PAH (SSc-PAH). The researchers found that the BiP and CHOP levels were markedly increased in the vasculature and microphages of SSc-PAH lungs compared with those of the control group. Notably, the increase in CHOP was primarily observed in ECs. These studies investigated the relationship between PAH and ECs through testing the expression of BiP and CHOP as well as other ER stress/UPR markers, and the results indicated that the pathogenesis of PAH is correlated with ER stress and the UPR in ECs.

Interestingly, ER stress does not only influence the function of ECs through these three signal transduction pathways. Past studies have shown that increased levels of ET-1 can be a risk factor in the development of SSc patients with iPHT [40-43]. Further research indicated that the gene HLA-B35 can induce ER stress in ECs, and consequently, the ER stress will increase the expression of ET-1. However, the molecular mechanism of the HLA-B35-ER stress-ET-1 axis requires further research [44].

PASMCs

Pulmonary vascular remodeling is a key component in the pathological process of PAH; therefore, analysis of the proliferation and apoptosis of PAMCs is needed. Under anoxic or other con-

ditions, PAMCs develop a synthetic phenotype, resulting in proliferation, hypertrophy, and migration; activating a series of signal transduction pathways; and eventually participating in the progression of PAH [45]. In the above section, we mainly discuss the role of ECs. In this section, we focus on the relationship between SMCs and PAH.

Therapeutic studies have explored whether drugs that can inhibit ER stress as chemical chaperones can attenuate PAH. In Wu's research [46], exogenous H_2S was verified to inhibit hypoxia-induced PASMC proliferation and reverse proliferation and migration, which correlated with the suppressed expression of UPR markers. These results suggested that using chemical factors to prevent proliferation and migration in PASMCs via inhibition of ER stress and the UPR is effective and significant. From another perspective, Mao. [47] established a PAH model in rats through hypoxia to examine the effect of intermedin (IMD) on hypoxic pulmonary vascular remodeling. IMD is a member of the calcitonin gene-related peptide family that can dilate systemic and pulmonary vessels [48-50]. In this study, the author found that IMD not only reduced hypoxic PAH and right ventricular hypertrophy but also alleviated pulmonary vascular remodeling through inhibiting PASMC proliferation and promoting apoptosis. Notably, the GRP78, GRP94 and caspase-12 protein levels increased in the IMD treatment group, indicating that IMD treatment exacerbates the ER stress apoptosis pathway in PASMCs. These results suggest that drugs that can improve PAMSC function can be used to treat PAH and that the activation of the ER stress apoptosis pathway in PASMCs can be used as a mechanism for antivascular remodeling. Other therapeutic studies yielded similar results; drugs that restrain ER stress could normalize proliferation and induce apoptosis [37, 51, 52]. During the few past years, our laboratory has focused on the effect of ER stress on PAH. In our research, we demonstrated that 4u8c, an inhibitor of the IRE1α/XBP1 pathway, could restrain hypoxia-induced cell proliferation and migration and reverse hypoxia-induced apoptosis arrest [53]. Thus, suppressing ER stress by altering UPR signaling pathways can normalize PASMC function, which is of therapeutic significance for PAH.

Since the above studies have shown that ER stress and the UPR signaling pathways are associated with the onset of PAH, an abnormal ER structure may also have a negative effect. Nogo, a member of the reticulon family of proteins that regulates the tubular structure of the ER, is implicated in vascular remodeling and in the tissue response to hypoxia [54-56]. Sutendra [57] found that the lack of Nogo-B in PASMCs from Nogo-A/B-deficient mice (missing both the Nogo-A and Nogo-B isoforms) prevented hypoxia-induced changes in vitro and in vivo, resulting in complete resistance to PAH. The researchers also found that the increased levels of Nogo-B during ER stress may lead to a restructuring of the ER and disruption of the mitochondria-ER unit, resulting in mitochondrial hyperpolarization, closure of the mitochondrial transition pore, and suppression of apoptosis. These findings imply that the absence of Nogo, especially Nogo-B, can disrupt PASMC hyperproliferation. The normal structure of the ER is the basis of its normal function. Structural abnormalities can not only affect the function of the ER itself but also cause dysfunction of mitochondria or other related organelles and eventually participate in the development of PAH. Remarkably, the rising phenomenon of Nogo-B does not appear similarly in carotid arteries, which suggested that this kind of artery soomth muscle cells response is vascular selective [58, 59].

In addition, multiple studies have identified genes associated with PAH, such as bone morphogenetic protein receptor 2 (BMPR2) [60-63], TGF-β superfamily genes [64], activin receptor-like kinase 1 (ALK1) [65], SMAD1/4/8 [66], and BMPR1B [67]. The guidelines of the European Cardiology Society recommend analyses for genetic alterations in BMPR2, BMPR1B, EIF2AK4, CAV1, KCNK3 and ENG, as BMPR2 has a key role in the etiology of the disease [68]. These genetic studies have substantially increased our understanding of the molecular mechanisms of pulmonary hypertension. Research has shown that PAH can be identified by overexpression of NOTCH3 in PASMCs [69]. On this basis, Chida analyzed two novel NOTCH3 mutants in PAH patients and subsequently established stable cell lines expressing wild-type and mutant NOTCH3. The researchers found that the majority of the protein-folding chaperone GRP78/BiP was translocat-
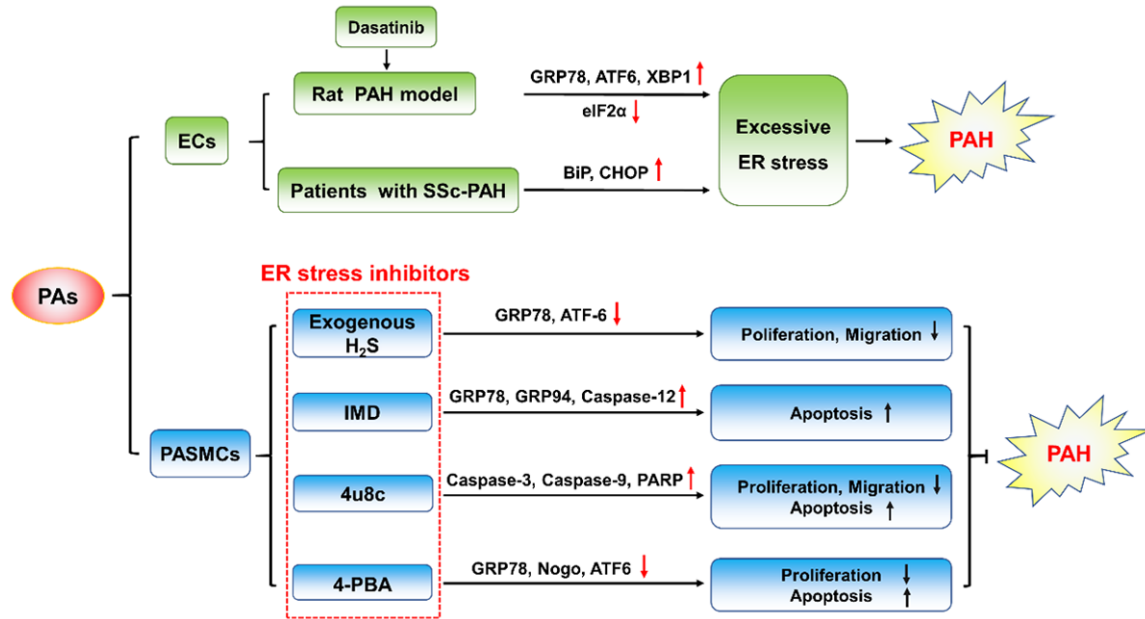
Figure 3. The relationship between ER stress and PAH. Excessive ER stress can cause dysfunction of endothelial cells and smooth muscle cells, leading to excessive pulmonary vasoconstriction and abnormal vascular remodeling and eventually participating in the onset of pulmonary hypertension.

ed into nuclei in the mutant group, while in the wild-type group, GRP78/BiP was located in the ER. These changes might influence unfolded protein degradation or transduction. NOTCH3 mutants impaired NOTCH3-HES5 signaling and affected the proliferation and viability of PASMCs. These results, which assessed the occurrence and development of PAH at a genetic level, may help to illuminate the pathogenesis of PAH. Currently, personalized treatment is the developmental direction of many refractory diseases, such as tumors. For PAH, research on the mechanism at the genetic level is forward-looking, and genes related to ER structure and function can be potential targets.

Summary and perspectives

In this review, we summarized studies of ER stress in PAH, focusing on how ER stress and the UPR interact with ECs and PASMCs (**Figure 3**). From these studies, we found that ER stress is part of the complex process of the onset and progression of PAH. Many triggers of PAH, including hypoxia, gene mutation, and NOTCH induction, can affect the degree of ER stress. Excessive ER stress and the UPR affect the state and function of ECs and PASMCs, thus influencing the formation and aggravation of pulmonary hypertension. Therefore, targeting

ER stress might be a broad-spectrum and effective treatment strategy in PAH. From these studies, we can conclude that the dysfunction of ER stress in ECs and PASMCs exists during the occurrence and development of PAH, but the specific pathway of how ER stress and PAH interact remains unclear. In the future, with the advancement of molecular mechanisms and signaling pathways, there will be an increasing number of new drugs or treatments targeting PAH.

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Disclosure of conflict of interest

None.

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