Endoplasmic reticulum stress and pulmonary hypertension

Yanan Hu1,*, Wenhao Yang2,3,4,*, Liang Xie3,4, Tao Liu1, Hanmin Liu2,3,4 and Bin Liu1

1Department of Pediatrics, The Affiliated Hospital of Southwest Medical University, Luzhou, China; 2Department of Pediatrics, West China Second University Hospital, Sichuan University, Chengdu, China; 3Key Laboratory of Birth Defects and Related Diseases of Women and Children (Sichuan University), Ministry of Education, Chengdu, China; 4The Vascular Remodeling and Developmental Defects Research Unit, West China Institute of Women and Children’s Health, West China Second University Hospital, Sichuan University, Chengdu, China

Abstract

Pulmonary hypertension is a fatal disease of which pulmonary vasculopathy is the main pathological feature resulting in the mean pulmonary arterial pressure higher than 25 mmHg. Moreover, pulmonary hypertension remains a tough problem with unclear molecular mechanisms. There have been dozens of studies about endoplasmic reticulum stress during the onset of pulmonary hypertension in patients, suggesting that endoplasmic reticulum stress may have a critical effect on the pathogenesis of pulmonary hypertension. The review aims to summarize the rationale to elucidate the role of endoplasmic reticulum stress in pulmonary hypertension. Started by reviewing the mechanisms responsible for the unfolded protein response following endoplasmic reticulum stress, the potential link between endoplasmic reticulum stress and pulmonary hypertension were introduced, and the contributions of endoplasmic reticulum stress to different vascular cells, mitochondria, and inflammation were described, and finally the potential therapies of attenuating endoplasmic reticulum stress for pulmonary hypertension were discussed.

Keywords

pulmonary hypertension, endoplasmic reticulum stress, unfolded protein response, vascular remodeling

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Introduction

Pulmonary hypertension (PH) is a highly hazardous disease, and gradually increasing pulmonary artery (PA) pressure is one of the main characteristics. The symptoms accompanied with high PA pressure include the remodeling of pulmonary vessels, enhanced vasoconstriction, and the compensatory hypertrophy of right ventricle (RV). In the final phase of this disease, heart failure and even death will occur. According to the current clinical classification system from World Health Organization, PH is classified into five categories based on presumed molecular etiologies, histopathology, and clinical associations.1 Group 1 includes a severe form of PH called pulmonary artery hypertension (PAH). Other groups affect a much larger global population and reflect a wide variety of conditions, such as congenital or acquired left heart disease, lung diseases and/or hypoxic, chronic thromboembolism, and unclear multifactorial mechanisms. In the past, landmark studies have shown similar changes of PASMCs (pulmonary artery smooth muscle cells)/ECs (endothelial cells) in the pathology of cancer and PH, which, to a certain extent, explains the pivotal mechanism of PH.2–4 That is to say, some cancer-related studies could provide some references for PH research.5

At present, endoplasmic reticulum (ER) stress is a hot topic in the researches of cancer pathogenesis.6 In recent years, several studies have shown that the glucose-regulated protein 78 kDa (GRP78), a molecular chaperone in ER stress, is involved in proliferation and survival of cancer cells and angiogenesis in tumor tissues.6,7 Angiogenic transformation with tumor angiogenesis was discovered as a downstream target of the unfolded protein response
The endoplasmic reticulum stress

Endoplasmic reticulum

ER is the central organelle for intracellular secretion. It is responsible for post-translational modification, folding and maturation, and secretion of transmembrane and secreted proteins. The proteins are then further transported to the Golgi and eventually secreted as the vesicles or displayed on the surface of the plasma. Moreover, ER is also crucial for other cellular functions like biosynthesis of lipids (including triglycerides, phospholipids, and cholesterol), Ca\(^{2+}\) buffering, and carbohydrate synthesis. However, the speed of proteins transportation and folding is affected by intracellular and extracellular factors, and changes among different cell types. Thus, by enlarging the entire size of ER preferentially and increasing the production of chaperone proteins, cells will adjust to the need for the entering of numerous nascent proteins into the lumen of ER for folding.

ER stress

Stress conditions of ER refer to situations such as the status of high glucose or lack of energy, hypoxia, Ca\(^{2+}\) overload, oxidative stress, and exposure to chemicals that will cause imbalances in the homeostasis. These stimulations activate the related signals to promote new proteins synthesis for dealing with stress, while these signals will reduce the general protein synthesis. When the amount of translating proteins exceeds that which ER folding can handle, it will cause misfolded proteins to accumulate in the ER. Due to heaping up of these misfolded proteins in ER, an evolutionarily conserved response of stress which is called unfolded protein response (UPR) will be activated. The adaptive response that takes place in the initial phase of UPR aims to rehabilitate protein folding homeostasis. When cells cannot recover from ER stress, UPR will terminate this adaptive response and trigger cell apoptosis. The function of UPR prevented the damaged and non-functional proteins heaping up in the ER by re-establishing body homeostasis or triggering cell death.

There are three types of UPR signal transducers in mammalian cells, all of which are ER-resident transmembrane proteins. The three proteins are inositol-requiring enzyme 1 α/β (IRE1α/β), protein kinase RNA-like ER kinase (PERK), and activating transcription factor 6 (ATF6) (Fig. 1). IRE1 is essential for UPR in plants and animals. As a multidomain protein of the ER transmembrane, IRE1 has the properties of both a kinase and an endoribonuclease. Under ER stress, IRE1 RNase is activated through autophosphorylation, conformational changes, and higher order oligomerization. The second sensor of UPR is PERK which primarily attenuates the translation of protein and modulates oxidative stress. This protein can phosphorylate and activate a transcription factor- nuclear factor erythroid-2-related factor 2 (NRF2) and eukaryotic initiation factor 2α (eIF2α) to alleviate unfolded proteins or misfolded proteins. ATF6, the last sensor of the three branches of UPR, is a transmembrane protein and it also functions as a transcription factor when it is cleaved. The direct target of the cleaved ATF6 is the UPR proteins, such as chaperones. At present, there is a more comprehensive understanding of the signaling pathways in ER stress, but the connections between ER stress and many diseases are still waiting for more researchers to explore.

The relationship between ER stress and PH

It is widely accepted that the vascular remodeling is a major feature in the pathogenesis of PH and may be present in a wide range of diseased tissues. The corresponding pathological process causing vascular remodeling in PH is very complicated and involves a variety of environmental factors and genes. This feature was associated with hyperplasia of PASMCs, excessive proliferation of PAECs, microthrombus formation, and persistent pulmonary vasoconstriction. And the uncontrolled over-proliferation of PASMCs and PAECs in complicated vascular lesions will eventually lead to occlusion of the PA, which further cause the adverse rise in pulmonary blood pressure. In addition, these individual and collective changes were related to excessive cell proliferation, apoptosis resistance, circulating inflammatory cells recruitment, and phenotypic switching.

Studies have shown that vascular remodeling is closely related to ER stress, especially the proliferation of PASMCs. Other aspects of PH, such as proliferation of PAECs and the subcellular connections, are also closely related to ER stress.

Pulmonary artery smooth muscle cells

As indicated by the above description, vascular remodeling is one of the significant features in PH, but the mechanisms are still under investigating. In several studies, it has been pointed out that the imbalance of proliferation and apoptosis in PASMCs contributes to medial thickening.
Pathological studies have also shown that some cells in the intraluminal occlusion express SMC markers. Therefore, some features such as over-proliferation and anti-apoptosis of PASMCs made the concept of carcinoid appearing. Meanwhile, there was increasing evidence that the hypertrophy, proliferation, and apoptosis resistance of PASMCs were critical components of abnormal vascular remodeling in PH (Fig. 2).

Recent studies on the role of ER stress in PH have focused on PASMC. It has been suggested that PASMCs proliferation and resistance to apoptosis are essential for vascular remodeling in PH. ER stress is a basic cellular response that promotes the proliferation and enhances inflammatory response of PASMCs. The abnormal proliferation of PASMCs is the most important cause of pulmonary vascular remodeling in PH. The abnormal proliferation of PASMCs is the most important cause of pulmonary vascular remodeling in PAH. Recently, some studies have shown that autophagy is involved in the development of PAH induced by MCT. And eIF2α can activate ER autophagy after ER stress. At the same time, eIF2α plays a key role in regulating cell proliferation and hypertrophy, and participated in the regulation of SMC proliferation and migration. According to Wang et al., they observed that after transfected with eIF2α siRNA in hypoxia-promoted PASMCs proliferation, it can significantly inhibit the proliferation of PASMCs. Moreover, Cao et al. found that an inhibitor of the IRE1α/XBP1 pathway, 4u8c, promoted apoptosis and repressed cell proliferation and migration of PASMCs. All of the above indicate that ER stress is involved in the abnormal proliferation of PASMC in PH.

There was evidence indicating that recruiting the inflammatory cells and continuous development of inflammation in PH are two crucial components of pathological vascular remodeling. Recently, it has been reported that ER stress may trigger smooth muscle cells to produce HA. In the rat PH model induced by MCT, the activity of hyaluronidase-1 (HYAL1) increased significantly at the beginning of the disease, while its degradation and synthesis increased in the late stage of PH. However, apart from the increase in the production of HA, there is no systematic description of the mechanism of HA clearance in the diseased lungs, and no complete description of the mechanism of the accumulation of HA in a balanced state. It is well known that the clearance of HA is critical to organ homeostasis. Previous studies have shown that PH was related to the reducing degradation and increasing synthesis of HA, especially isoforms of high molecular weight. Yeager et al. recently pointed out that the activation of UPR and the generation of pro-inflammatory biomolecule were attributed to ET-1 signaling in rat PASMCs. The occurrence of ER stress promotes the proliferation and inflammatory state of PASMCs, which will promote the onset of PH. These experimental
results further demonstrate the importance of PASMC in the inflammatory process of PH development, especially in recruiting and preserving the immune cells. The persistent inflammation produced by PASMCs is related to vascular remodeling and inhibition of this process is a potential method of treating PH.

Dromparis et al. found that the ATF6 pathway could be activated in the vasculature of both hypoxic models in vivo and cultured vascular SMCs. Surprisingly, they did not mention if the CHOP pathway was also activated. Meanwhile, the ATF6 signaling pathway has been shown to up-regulate Nogo to disrupt mitochondrial-ER units in PASMC, leading to the occurrence of PH. Although the function of ER stress in PASMCs have been extensively studied, the mechanism is still unknown. In summary, inhibiting ER stress that stimulates the above PASMCs responses is also an extremely appealing treatment option.

Pulmonary arterial endothelial cells

The endothelium secretes many mediators which are necessary for normal vascular functioning, including the mediators of regulating vascular tone and coagulation, modulating immune responses, and controlling vascular cell growth. Moreover, vascular ECs compose a barrier between the vessel lumen and the wall, through which liquid, gas, and macromolecular substances could be selectively transported. At the same time, it also maintains the homeostasis of blood vessels and mediates the passage of nutrients and white blood cells through the vessel wall. Vascular intima can maintain the balance between vasodilation and vasoconstriction in physiological conditions. ECs play an important role in the early stage of PH due to their apoptosis. Furthermore, in the studies of late-stage PH, it was confirmed that the over-proliferation endothelial cells, vasoconstriction, the formation of microthrombus, and eventual vascular remodeling are crucial for pathological changes of PH (Fig. 2).

Changes of endothelial function in the pulmonary vasculature contribute to the neointimal formation, thickening of the intima, and occlusion of the distal pulmonary artery, which is of great significance for PH. One feature in some patients with severe PH is a complex vascular lesion called a plexiform lesion. These lesions can be seen in the myofibroblastic stroma in which the monoclonal EC of the lesion is proliferating. The plexiform or complex vascular lesions are characterized by anti-apoptotic and phenotypic altered EC.

ECs produce the vasodilator/anti-mitotic agents and vasoconstrictors/mitosis, such as prostacyclin, NO, thromboxane A2 and ET-1, to regulate SMCs activity. Endothelial dysfunction is thought to be an imbalance that favors the production of vasoconstrictors and proliferative factors, including pro-inflammatory and thrombogenic effects, which has been clearly shown in PH. Under the normal conditions, the main players are prostacyclin, nitric
oxide (NO), and thrombomodulin. These participants inhibit SMC proliferation, platelet aggregation, and the expression of leukocyte adhesion molecule.98

Some dysfunctions are related to the reducing activity of endothelial NO synthase (eNOS), increasing endothelium-produced ROS, decreasing anticoagulant properties, adhesion molecules up-expression, and release of chemokines and cytokines. It has been confirmed in previous studies that the release of the vasoconstrictor thromboxane A2 increased in these patients, in contrast, the release of prostacyclin is depressed.89 90

At the same time, more and more evidence showed that the imbalance of signal transduction of ROS and NO was related with PH.90 Moreover, the decreasing expression of prostacyclin synthase in the pulmonary arteries could be detected in some patients with primary PH,91 and the expression of NO synthase decreased in lung ECs in some PH patients.92 Recently, the studies with independent PH model and muscles of CHOP−/− mice have suggested that in the ECs the expression of eNOS induced by ER stress has negative impacts on ECs function.93 94

There are also some patients with elevating plasma ET-1 levels.95 In addition, elevating plasma ET-1 levels have also been reported in experimental models of PH.96 Interestingly, ER stress also induces changes in ECs secretion.97 In a study to confirm whether the UPR pathway was activated in ECs, Lenna et al.98 examined the expression of pulmonary vascular UPR markers in the systemic sclerosis-associated PH (SSC-PH) patients. In this study, they observed that the expressions of BIP and CHOP as markers of ER stress in SSC-PH pulmonary vasculature and macrophages were significantly higher than those in healthy subjects. Interestingly, the elevating CHOP levels existed mainly in the ECs of SSC-PH lung.88 In SSC-PH model of the mouse, the elevating ER stress markers could also be found in Gata6-KO mice.99 The experimental result of Lenna et al.98 suggested that macrophages and ECs might be the reason for increasing ER stress/UPR in hypoxic mice. According to the study of Lenna et al., thapsigargin, the ER stress inducer, could up-regulate the expression of ET-1 by increasing the formation of the ATF4/c-Jun transcription complex in ECs.100 These findings have important implications for the study of the mechanism of ER stress in the endothelium of PH. Perhaps it is a good idea to focus on the ER stress in the treatment of PH.

Subcellular networks

Increasing evidence indicated that the ER stress/UPR pathway had a relationship with other subcellular networks.88 These networks included the ER-mitochondrial units, inflammatory response network and other subcellular factors.

Among all other organelles associated with ER, mitochondria are the most prominent organelle that regulates metabolism and cell survival. Previous studies have shown that mitochondrial dysfunction may lead to vascular remodeling, thus affecting the occurrence of PH.101–103 Mitochondria-associated membrane (MAM) is a special contact formed by the interaction of ER and mitochondrion in physical structure.104 Membrane and luminal components can mix and exchange in the MAM, and the composition of MAM is adapted in response to a variety of internal and external stimuli.104–106 The structure of MAM is complicated and involves a large number of proteins with widely various functions.107 IP3R (inositol 1,4,5-triphosphate receptor) and VDAC (voltage-dependent anion channel) are the major ER-mitochondrial calcium (Ca2+) transfer channels (Fig. 3). They are located on both the ER side and mitochondria side of the MAM and physically connect the two organelles by forming a complex with the chaperone GRP75, respectively.108 Ca2+ has been identified as an important regulator of cell proliferation and apoptosis, and it also plays an important role in PASMC proliferation and vasoconstriction of PH.109–113 And the ER is the main intracellular reservoir of Ca2+, while many ER chaperone proteins are dependent on Ca2+, thus the regulation of Ca2+ homeostasis in ER is very important.114 115 Alterations of calcium manipulate by the ER/mitochondrial couple providing a pathway to activate apoptosis.116 A key process linking apoptosis to ER-mitochondrial interactions is the change of the Ca2+ homeostasis, which results in massive and/or prolonged mitochondrial Ca2+ overload.107 Moreover, Mitochondria are accepted as indispensable oxygen sensor for hypoxic pulmonary vasoconstriction in cells.117 And mitochondrial signals will promote apoptosis-resistance and proliferative diathesis of vascular remodeling in PH.118

Recently, several studies have shown that ER stress have involved in the development of inflammation during the PH, but all these are preliminary studies.12 74 98 However, the roles of inflammation both in ER stress and PH are highly correlated. Proinflammatory cytokines and chemokines have been shown to be involved in the animal model of monocrotaline-induced PH.119–122 Most patients with primary PH have evidence of autoimmune and/or active inflammation. In these patients, circulating antimicrobial antibodies and increasing serum levels of interleukin (IL)-1 and IL-6 can be detected.123 124 And several studies have demonstrated that in ER stress IRE1 and PERK signaling pathways also participate in the inflammatory response.125 126 The UPR pathway also can trigger the activation of NF-kB, another major inflammatory mediator.127 The specific relationship among ER stress, inflammation and PH could contribute to reveals the pathogenesis of PH, which requires further research.

Among the other various factors affecting PH, the PDGF-β receptor is one of the most concerned focuses in PH researches and it is responsible for proliferation of PASMCs and neointimal hyperplasia of the PA.128–130 Other experiments of cultured PASMCs have indicated that using platelet-derived growth factor (PDGF)-BB to
treat cells will activate the UPR pathway. These results suggest that PDGF-BB may be one of the contributors to activate the UPR pathway in PH. There has been increasing evidence showing that the ER stress and UPR pathways have a critical effect on regulating the expression of proangiogenic factors. They also serve as novel mediators of angiogenesis. Up-regulation of angiogenic factors such as VEGF-A, FGF, IL-8, and ET-1 has been reported to occur during the UPR. Moreover, according to reports, as a regulatory ER structural protein, Nogo-B is involved in vascular remodeling and plays a role in PAH. At the same time, it has also been demonstrated that PH-related loss of function mutations of BMPRII and the resultant protein transporting dysfunction can also induce ER stress. Furthermore, recent researches on metabolic abnormalities of the pulmonary vascular system and the right ventricle have also attracted more and more attention.

Potential therapies

Recently, several studies have suggested that the chemical chaperones of ER stress, like 4-PBA or tauroursodeoxycholic acid (TUDCA), could reduce the mPAP significantly in PH animal model. Chemical chaperones are often defined as low molecular weight compounds. So far, the exact mechanism of chemical chaperones in ER stress has not been elucidated. Most likely, these two molecules exert their effects by stabilizing the structure of incorrectly folded proteins, stimulating molecular chaperones to obtain more efficient protein trafficking, reducing protein aggregation, and preventing the interactions of non-specific proteins and other proteins. 4-PBA, One of the chemical chaperones which most commonly mentioned, is a low molecular weight fatty acid and a non-toxic pharmacological compound. Due to its ammonia scavenger properties, it has been approved by the FDA for clinical use in pathological diseases of the urea cycle. At present, it has been widely studied as a small chemical chaperone to modulate restoration of ER homeostasis and multiple concerning pathological conditions. Furthermore, using 4-PBA can down-regulate several key proteins in ER stress, thereby improving PA and RV remodeling. Another chemical chaperone, TUDCA, a safe hydrophilic bile acid. Although the exact mechanism of TUDCA as a chemical chaperone is still unclear, it has been shown to prevent UPR dysfunction and reduce ER stress in various cell types. Recently, there has been shown that TUDCA binded with the hydrophobic regions of proteins to prevent subsequent protein aggregation and unfolded protein accumulation, thus attenuating ER stress. And it has been reported that TUDCA also exerts these effects partially by assisting in the transfer of mutant proteins and partially by improving protein folding capacity through the activation of ATF6.
These above results showed that attenuating ER stress could be an effective treatment strategy to protect the PA from damage. If the association between ER stress and PH was figured out, the use of these drugs which have non-toxic and does not burden other organs would be a nice choice for clinical treatment of PH.

Summary

A review of all the literature mentioned in the text makes it evident that ER stress was involved in the PH, and may play an important role. These above content in this article show that attenuating ER stress may be an effective treatment to protect the PA. It is also clear that the exact molecular mechanisms of ER stress in the pathology of PH remains unclear. More studies are needed in future.

Authors’ contribution

YH and WY contributed in original draft preparation, LX, BL and HL in review and editing, YH and TL in literature correction, and BL and HL in supervision.

Guarantor

Yanan Hu, Bin Liu, Hanmin Liu.

Ethical approval

No ethical statement will be required for this study because there is no direct involvement of human.

Conflict of interest

The author(s) declare that there is no conflict of interest.

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