Knockdown of ERp57 increases BiP/GRP78 induction and protects against hyperoxia and tunicamycin-induced apoptosis

Dong Xu,1 Ricardo E. Perez,1 Mohammad H. Rezaiekhaligh,1 Mohammed Bourdi,2 and William E. Truog1

1Neonatology Research Laboratory, Section of Neonatal-Perinatal Medicine, Department of Pediatrics, Children’s Mercy Hospitals and Clinics, University of Missouri-Kansas City School of Medicine, Kansas City, Missouri; and 2Molecular and Cellular Toxicology Section, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

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Xu D, Perez RE, Rezaiekhaligh MH, Bourdi M, Truog WE. Knockdown of ERp57 increases BiP/GRP78 induction and protects against hyperoxia and tunicamycin-induced apoptosis. Am J Physiol Lung Cell Mol Physiol 297: L44–L51, 2009. First published May 1, 2009; doi:10.1152/ajplung.90626.2008.—Supplemental oxygen therapy (hyperoxia) in preterm babies with respiratory stress is associated with lung injury and the development of bronchopulmonary dysplasia. Endoplasmic reticulum (ER) homeostasis plays critical roles in maintaining cellular functions such as protein synthesis, folding, and secretion. Interruption of ER homeostasis causes ER stress and triggers the unfolded protein response, which can lead to apoptosis in persistently stressed cells. ERp57 is an ER protein and is associated with calreticulin and calnexin in protein glycosylation. In this study, we found hyperoxia downregulated ERp57 in neonatal rat lungs and cultured human endothelial cells. Transient transfection of ERp57 small interfering RNA significantly knocked down ERp57 expression and reduced hyperoxia- or tunicamycin-induced apoptosis in human endothelial cells. Apoptosis was decreased from 26.8 to 9.9% in hyperoxia-exposed cells and from 37.8 to 5.0% in tunicamycin-treated cells. The activation of caspase-3 induced by hyperoxia or tunicamycin was diminished and immunoglobulin heavy chain-binding protein/glucose-regulated protein 78-kDa (BiP/GRP78) induction was increased in ERp57 knockdown cells. Overexpression of ERp57 exacerbated hyperoxia- or tunicamycin-induced apoptosis in human endothelial cells. Apoptosis was increased from 10.1 to 14.3% in hyperoxia-exposed cells and from 14.0 to 21.2% in tunicamycin-treated cells. Overexpression of ERp57 also augmented tunicamycin-induced caspase-3 activation and reduced BiP/GRP78 induction. Our results demonstrate that ERp57 can regulate apoptosis in human endothelial cells. It appears that knockdown of ERp57 confers cellular protection against hyperoxia- or tunicamycin-induced apoptosis by inhibition of caspase-3 activation and stimulation of BiP/GRP78 induction.

Apoptotic cell death plays an important role in hyperoxia-induced lung injury and therefore potentially in the development of BPD because ROS can induce apoptotic cell death through either intrinsic or extrinsic apoptosis pathways (34, 44). Accumulating evidence has indicated that endoplasmic reticulum (ER) stress is also implicated in apoptosis (31, 32, 33, 52). However, the importance of ER stress and the role of ER stress-induced apoptotic cell death in the pathogenesis of neonatal lung injury and BPD are unknown. ER is an important organelle in mammalian cells and has functions of maintaining intracellular calcium homeostasis, protein secretion, lipid synthesis, protein glycosylation, and folding. Conditions interfering with the homeostasis of ER are collectively called ER stress (51). Cells undergoing ER stress demonstrate activation of signal transduction cascades that are collectively named the unfolded protein response (UPR). The initial result of UPR can reduce proteins to translocate into the ER and maintain ER homeostasis. If the overload of unfolded or misfolded proteins in the ER is not resolved, the persistent activation of UPR will trigger the cell death pathway because of prolonged ER dysfunction (14, 42). Emerging data have shown that oxidative stress-induced ER stress may be crucial in the regulation of apoptotic cell death (23).

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Immunoglobulin heavy chain-binding protein/glucose-regulated protein 78-kDa (BiP/GRP78) is an ER chaperone protein and plays a key role in ER homeostasis (20). BiP/GRP78 binds to ER stress sensor proteins, protein kinase R-like ER kinase (PERK), inositol-requiring enzyme 1α (IRE1α), and activating transcription factor 6 (ATF6), and prevents activation of UPR. Additionally, BiP/GRP78 functions as a chaperone and helps protein proper folding and prevents aggregation of proteins;
BiP/GRP78 also binds to Ca\(^{2+}\) in the ER and maintains ER Ca\(^{2+}\) homeostasis. It has been shown that reduction of BiP/GRP78 expression could activate UPR and lead to apoptosis (30, 50), and overexpression of BiP/GRP78 protects against ER stress-induced apoptosis (12, 38).

ER protein 57 (ERp57) is a member of protein disulfide isomerase (PDI) family and is also a glucose-regulated protein (2, 6). It is mainly present in the ER and can also be found in the nucleus, extracellular space, cytosol, and cell surface (4, 47). ERp57 forms complexes with calreticulin and calnexin in the ER and functions as part of the glycoprotein-specific quality control machinery operating in the lumen of the ER (9, 26). However, the role of ERp57 in hyperoxia-induced lung injury is unknown, especially in rapidly growing lungs. In the present study, we found that ERp57 was significantly decreased in the neonatal rat lung tissue after a prolonged hyperoxic exposure, suggesting ERp57 might be associated with hyperoxia-induced neonatal lung injury. We demonstrated that ERp57 helps to modulate ER stress-induced apoptosis.

**EXPERIMENTAL PROCEDURES**

**Oxygen exposure.** The animal use was approved by the Institutional Animal Care and Use Committee (IACUC), University of Missouri-Kansas City. The newborn rats at 4 days of age were randomly divided into two exposure groups, room air (normoxia) and oxygen (hyperoxia), according to our previously published procedure (53). The animals were housed in regular rat cages that were placed into Lucite chambers. The newborn rats in the chambers breathed either room air or humidified 95\% oxygen. Oxygen concentration was monitored continuously with an oxygen analyzer. Dams were given food and water ad libitum, kept on a 12:12-h on-off light cycle, and fostered by rotating in and out of the chamber every 24 h to avoid oxygen toxicity. At the designated exposure time points, the animals from both treatment groups were killed by exsanguination after receiving intraperitoneal pentobarbital for anesthesia. Lung tissue from each group was collected, minced, and stored in liquid nitrogen for protein extraction.

**Two-dimensional gel electrophoresis and protein identification.** Protein was extracted from the neonatal rat lungs treated with room air or 95\% O\(_2\). Lungs were homogenized in RIPA buffer containing PBS, 0.1\% SDS, 1% Igepal CA-630 (Sigma, St. Louis, MO), and 0.5\% sodium deoxycholate. At the time of use, the following inhibitors were added in per gram of tissue: 100 \(\mu\)g/ml PMSF, 30 \(\mu\)M aprotinin (Sigma), and 1 mM sodium orthovanadate. The homogenate was centrifuged at 15,000 \(g\) for 10 min. The supernatants were saved for analysis. Protein concentration was determined by BCA protein assay kit (Sigma). Samples were subject to SDS-PAGE gels and transferred to nitrocellulose membranes. The blots were then probed with various antibodies such as anti-BiP/GRP78 (cat. no. 3183; 1:1,000; Cell Signaling Technology), anti-Cleaved Caspase-3 (Asp175) (cat. no. 9661; 1:1,000; Cell Signaling Technology), anti-BiP/GRP78 (cat. no. 3183; 1:1,000; Cell Signaling Technology), and anti-\(\alpha\)-tubulin (Clone B-5-1-2; 1:10,000; Sigma). Statistical analysis. The results are expressed as the means \(\pm\) SE of data obtained or, where appropriate, as means \(\pm\) SD. Statistical analysis was performed using Student's \(t\)-test for paired comparisons. A value of \(P < 0.05\) was considered significant.

**RESULTS**

Hyperoxia downregulated ERp57 expression in neonatal rat lungs and cultured human endothelial cells and lung epithelial cells. We exposed newborn rats at 4 days of age to normoxia (room air) or hyperoxia (95\% O\(_2\)) for 10 days and identified several candidate proteins that were altered by hyperoxia using two-dimensional gel electrophoresis and mass spectrometry. One of the candidate proteins identified by mass spectrometry (Fig. 1C) was ERp57, an ER protein. This protein showed several spots with different isoelectric points (pI) in the newborn rat lung under normoxic condition (spots 1-5 in Fig. 1, A and B). The ERp57 spots with a higher pI disappeared after

**Plasmid construction and transfection.** For human ERp57 plasmid construction, the full-length human ERp57 cDNA was cut out from pSVL-ERp57 plasmid (2). The resulting human ERp57 cDNA fragment was subcloned into retroviral plasmid vector pQCXIP (Clontech, Carlsbad, CA). pQCXIP-ERp57 and pQCXIP-empty plasmids were transfected into PT67 cells using Lipofectamine (Invitrogen, Carlsbad, CA). The supernatants from transfected PT67 cells were used to infect EA.hy926 endothelial cells, and pooled clones were selected by 0.3 \(\mu\)g/ml puromycin for 5 days.

**Cell culture and cell treatment.** The human endothelial cell line EA.hy926 (a gift from Dr. C.-J. S. Edgell, University of North Carolina at Chapel Hill; Ref. 8) was grown in DMEM containing 10\% fetal bovine serum, 50 \(\mu\)g/ml penicillin, and 50 \(\mu\)g/ml streptomycin in 5\% CO\(_2\) at 37\°C. Normoxic exposure of cultured cells was conducted under room air and 5\% CO\(_2\) in a humidified cell culture incubator at 37\°C. Hypoxic exposure of cultured cells was conducted in a humidified chamber (Billups-Rothenberg, Del Mar, CA), and the chamber was flushed with 95\% O\(_2\)-5\% CO\(_2\) at a flow rate of 10 l/min for 15 min before incubation at 37\°C. In some experiments, EA.hy926 cells were treated with tunicamycin (5 \(\mu\)g/ml) for 0, 6, 16, and 24 h.

**Measurement of apoptotic cell death.** Apoptosis detection kit was from R&D Systems (Minneapolis, MN). Treated cells were trypsinized and collected by centrifugation at 500 \(g\) for 5 min. Cells were washed with cold PBS once and resuspended in 100 \(\mu\)l of binding buffer containing 10 mM HEPES, pH 7.4, 150 mM NaCl, 5 mM KCl, 1 mM MgCl\(_2\), and 1.8 mM CaCl\(_2\). Cells were stained with propidium iodide/annexin V-FITC for 15 min according to manufacturer’s instructions. The stained cells were then subjected to flow cytometry analysis for apoptosis.

Western blotting analysis. Cultured cells were washed with cold PBS three times and then lysed in buffer containing 20 mM HEPES, pH 7.5, 150 mM NaCl, 1 mM EDTA, 10\% glycerol, 1\% Triton X-100, and Complete, Mini, EDTA-free Protease Inhibitor Cocktail Tablets (Roche Diagnostics). Cell lysates were centrifuged at 13,000 rpm for 10 min. The supernatants were saved for analysis. Protein concentration was determined by BCA protein assay kit (Sigma). Samples were subject to SDS-PAGE gels and transferred to nitrocellulose membranes. The blots were then probed with various antibodies such as anti-BiP/GRP78 (cat. no. 3183; 1:1,000; Cell Signaling Technology), anti-Cleaved Caspase-3 (Asp175) (cat. no. 9661; 1:1,000; Cell Signaling Technology), anti-ERp57 (cat. no. SPA-585; 1:1,000; Stress-Gen Bioreagents), anti-actin (cat. no. SC-1616; 1:5,000; Santa Cruz Biotechnology), and anti-\(\alpha\)-tubulin (Clone B-5-1-2; 1:10,000; Sigma).
hyperoxic exposure for 10 days (spots 1 and 2 in Fig. 1, A and B), which suggest that hyperoxic exposure modified ERp57 protein and reduced total ERp57 levels in the newborn rat lungs. Next, we measured ERp57 protein expression levels in human endothelial cell line (EA.hy926) and type II lung epithelial cell line (A549) under either normoxic or hyperoxic condition. We found that hyperoxic exposure for 24, 48, and 72 h significantly reduced ERp57 expression in cultured human endothelial cells and type II lung epithelial cells (Fig. 2, A and B), which indicated that ERp57 reduction may be implicated in hyperoxia-induced lung injury.

Knockdown of ERp57 by siRNA reduced hyperoxia- or tunicamycin-induced apoptosis in human endothelial cells. Next, we knocked down ERp57 expression using ERp57 siRNA and tested the effects of hyperoxia or tunicamycin on endothelial cells. Transient transfection of ERp57 siRNA knocked down ~80% of ERp57 protein expression in EA.hy926 human endothelial cells (Fig. 3). Surprisingly, ERp57 knockdown conferred cell protection and significantly diminished hyperoxia- or tunicamycin-induced apoptosis in EA.hy926 human endothelial cells (Fig. 4, A and C; n = 3; P < 0.01). Hyperoxia (95% O₂ for 48 h) or tunicamycin (5 μg/ml for 24 h) treatment increased apoptotic cell death in EA.hy926 human endothelial cells. The apoptotic cell death was increased from 6.6 to 26.8% (Fig. 4A) in hyperoxic exposure for 10 days (spots 1 and 2 in Fig. 1, A and B), which suggest that hyperoxic exposure modified ERp57 protein and reduced total ERp57 levels in the newborn rat lungs. Next, we measured ERp57 protein expression levels in human endothelial cell line (EA.hy926) and type II lung epithelial cell line (A549) under either normoxic or hyperoxic condition. We found that hyperoxic exposure for 24, 48, and 72 h significantly reduced ERp57 expression in cultured human endothelial cells and type II lung epithelial cells (Fig. 2, A and B), which indicated that ERp57 reduction may be implicated in hyperoxia-induced lung injury. 

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Knockdown of ERp57 decreases hyperoxia or tunicamycin-induced apoptotic cell death in human endothelial cells. EA.hy926 human endothelial cells were transiently transfected with control or ERp57 siRNA for 48 h and then treated with 95% O₂ or tunicamycin. A: transfected cells treated with room air or 95% O₂ for 48 h were stained with annexin V and analyzed by flow cytometry for apoptosis. Open bar, normoxia; closed bar, hyperoxia. n = 3; **P < 0.001. B: transfected cells were treated with room air or 95% O₂ for 24 and 48 h. Caspase-3 activation was determined by Western blotting analysis with a specific antibody against cleaved caspase-3. α-Tubulin was used as a loading control. N, normoxia; H, hyperoxia. C: transfected cells were treated with tunicamycin (5 μg/ml) for 24 h, stained with annexin V, and analyzed by flow cytometry for apoptosis. Open bar, DMSO control; closed bar, tunicamycin. n = 3; **P < 0.001. D: transfected cells were treated with tunicamycin (5 μg/ml) for 0, 6, 16, and 24 h. Caspase-3 activation was determined by Western blotting analysis with a specific antibody against cleaved caspase-3. α-Tubulin was used as a loading control.

Knockdown of ERp57 protects ER stress-induced apoptosis in human endothelial cells. To further investigate the role of ERp57 in human endothelial cells, we cloned ERp57 gene and established an EA.hy926 human endothelial cell line that overexpressed ERp57. Western blotting analysis showed that ERp57 protein was significantly increased by 8.4-fold in ERp57-EA.hy926 endothelial cell line compared with control pQCXIP-EA.hy926 endothelial cells (Fig. 6A).

Knockdown of ERp57 by siRNA increases immunoglobulin heavy chain-binding protein/glucose-regulated protein 78-kDa induction in human endothelial cells. EA.hy926 human endothelial cells were transiently transfected with control or ERp57 siRNA, followed by treatment with DMSO (D) or tunicamycin (TM; 5 μg/ml) for 6, 16, and 24 h. BiP/GRP78 induction was determined by Western blotting analysis with a BiP/GRP78 antibody. Western blotting analysis showed BiP/GRP78 induction in EA.hy926 human endothelial cells after tunicamycin treatment. The fold changes of BiP/GRP78 expression were from 1.0 to 1.4, 6.3, 9.6, and 11.5 in ERp57 knockdown cells compared with control cells.

Overexpression of ERp57 exacerbated hyperoxia- or tunicamycin-induced apoptosis in human endothelial cells. To further investigate the role of ERp57 in human endothelial cells, we cloned ERp57 gene and established an EA.hy926 human endothelial cell line that overexpressed ERp57. Western blotting analysis showed that ERp57 protein was significantly increased by 8.4-fold in ERp57-EA.hy926 endothelial cell line compared with control pQCXIP-EA.hy926 endothelial cells (Fig. 6A).
Hyperoxia decreased ERp57 expression by approximately 50–80% in ERp57-EA.hy926 endothelial cells (Fig. 6B). Hyperoxic exposure for 48 h and tunicamycin treatment for 24 h significantly induced apoptotic cell death in EA.hy926 human endothelial cells (Fig. 7, A and B). The apoptotic cell death was increased from 0.7 to 10.1% (Fig. 7A) in hyperoxia-exposed cells and from 0.9 to 14.0% (Fig. 7B) in tunicamycin-treated cells. Overexpression of ERp57 significantly augmented hyperoxia- or tunicamycin-induced apoptotic cell death, which was increased from 10.1 to 14.3% in hyperoxia-exposed cells (Fig. 7A; n = 3; P < 0.01) and from 14.0 to 21.2% in tunicamycin-treated cells (Fig. 7B; n = 3; P < 0.01).

Overexpression of ERp57 decreased BiP/GRP78 expression and increased caspase-3 activation induced by tunicamycin in human endothelial cells. We next tested whether overexpression of ERp57 could affect tunicamycin-induced BiP/GRP78 induction and caspase-3 activation in human endothelial cells. When we treated pQCXIP-EA.hy926 or ERp57-EA.hy926 cells with tunicamycin for 0, 6, 16, and 24 h, we found that tunicamycin increased BiP/GRP78 induction after 6, 16, and 24 h in both pQCXIP-EA.hy926 and ERp57-EA.hy926 cells. The increased BiP/GRP78 induction was significantly reduced in ERp57-EA.hy926 cells compared with pQCXIP-EA.hy926 cells after 6-, 16-, and 24-h treatment (Fig. 8A). Western blotting analysis showed that tunicamycin treatment for 24 h increased caspase-3 activation in pQCXIP-EA.hy926 endothelial cells and ERp57-EA.hy926 endothelial cells. However, more caspase-3 activation induced by tunicamycin was observed in ERp57-EA.hy926 cells compared with pQCXIP-EA.hy926 cells (Fig. 8B).

**DISCUSSION**

In the present study, we demonstrate that hyperoxia decreased ERp57 expression in a rat model of neonatal lung injury and cultured human endothelial cells. Knockdown of ERp57 conferred cellular protection against hyperoxia and tunicamycin-induced apoptosis, which may be due to increased BiP/GRP78 levels and reduced caspase-3 activation in human endothelial cells. Additionally, overexpression of ERp57 attenuated tunicamycin-induced BiP/GRP78, increased caspase-3 activation, and exacerbated hyperoxia or tunicamycin-induced apoptosis in human endothelial cells.

In our neonatal rat model of hyperoxia-induced lung injury and cultured cells, hyperoxia decreased ERp57 expression, suggesting that reduced ERp57 might be implicated in ER stress since ERp57 is an ER protein and is associated with increased BiP/GRP78 expression and decreased caspase-3 activation in human endothelial cells. Knockdown of ERp57 could affect tunicamycin-induced BiP/GRP78 induction and caspase-3 activation in human endothelial cells. Overexpression of ERp57 attenuated tunicamycin-induced BiP/GRP78, increased caspase-3 activation, and exacerbated hyperoxia or tunicamycin-induced apoptosis in human endothelial cells. Further studies are needed to elucidate the mechanisms by which ERp57 regulates ER stress responses in endothelial cells.
Changes are denoted based on the ratios of BiP/GRP78 to H9251 with mitochondrial, and nucleus (4, 47). ERp57 forms complexes with ERp57 in the ER, but also in the cytoplasm, nucleus, and cell death largely depends on quantity of calreticulin on cell membrane surface (25, 29). Calreticulin has been known to interact with ERp57 in the ER, and the interaction of calreticulin and ERp57 is required for their cotranslocation to cell membrane. Knockdown and knockout of ERp57 can decrease translocation of calreticulin to the cell membrane and reduce immunogenicity-induced apoptotic cell death (25, 29).

It is generally believed that ER stress is an adaptive mechanism to preserve cell function and survival. However, persistent ER stress can initiate apoptosis and plays a critical role in pathogenesis of multiple diseases, such as diabetes, atherosclerosis, and neurodegenerative diseases (15, 24, 35). ROS produced during supplemental oxygen therapy are critical in hyperoxia-induced apoptosis in lung injury and the development of BPD in premature babies. Lung microvascular endothelial apoptosis and reduced vascularization are major pathological changes in lung pathology of BPD (40). Recent studies have shown that ER stress can induce endothelial cell apoptosis. For example, peroxynitrite generated by the reaction of nitric oxide and superoxide can induce ER stress in human vascular endothelium (7), and homocysteine-induced apoptotic cell death due to oxidative stress produced by homocysteine can cause apoptotic cell death through activation of the ER stress in human vascular endothelial cells (54). In ER stress-mediated apoptosis, one of the major regulators is BiP/GRP78 (14, 33, 51, 52). Unfolded/misfolded proteins or cell stress can trigger UPR through BiP/GRP78 disassociation with ER stress sensors, IRE1, PERK, and ATF6. Prolonged or excessive ER stress can increase calcium release from the ER and activate caspase-2/4/12 in triggering ER-mediated apoptosis (16, 43, 48). BiP/GRP78 chaperones unfolded/misfolded proteins and maintains ER homeostasis. Induction of BiP/GRP78 can protect cells from apoptotic cell death, whereas decreased BiP/GRP78 can cause ER dysfunction and result in apoptotic cell death (12, 50). Our study has indicated that knockdown of ERp57 increased BiP/GRP78 induction, whereas overexpression of ERp57 decreased BiP/GRP78 levels, suggesting that ERp57 regulates apoptosis at least in part through BiP/GRP78 protein. A recent study has shown that chronic cigarette smoke can induce ER stress in human lung, and UPR compensatory failure could contribute to the development of chronic obstructive pulmonary disease (18). Additionally, ER stress occurs in alveolar epithelium and leads to UPR activation in lung tissue in patients with idiopathic pulmonary fibrosis (IPF); ER stress-induced apoptosis may also play an important role in IPF (21, 22). ER stress mediators in UPR, such as ATF6, ATF4, and spliced X-box binding protein-1 (XBP-1), are significantly increased. The proapoptotic markers such as Bax and C/EBP-homologous protein (CHOP) in ER stress-mediated apoptosis.

Fig. 8. Overexpression of ERp57 decreases tunicamycin-induced BiP/GRP78 levels and augments tunicamycin-induced caspase-3 activation in human endothelial cells. pQCPXIP-EA.hy926 and ERp57-EA.hy926 cells were cultured and treated with tunicamycin (5 μg/ml) for 0, 6, 16, and 24 h. A: BiP/GRP78 expression was determined by Western blotting analysis with a BiP/GRP78 antibody. α-Tubulin was used as a loading control. The fold changes are denoted based on the ratios of BiP/GRP78 to α-tubulin. B: caspase-3 activation was determined by Western blotting analysis with a specific antibody against cleaved caspase-3. β-Actin was used as a loading control. The fold changes are denoted based on the ratios of cleaved caspase-3 to β-actin.

It has been reported that ERp57 is a major target for oxidative stress induced by hydrogen peroxide. It seems that hydrogen peroxide can decrease ERp57 level and modify ERp57 protein (13, 49). We also observed that ERp57 protein shifted to a lower pI after hyperoxic exposure in two-dimensional gel electrophoresis; this alteration could be due to protein phosphorylation of ERp57 since it has been shown that phosphorylation of ERp57 at amino acid serine 150 shifts ERp57 to a lower pI and exhibits a similar pattern we observed in our two-dimensional gel electrophoresis (19, 46). The exact physiological role of phosphorylated ERp57 is unknown, although it has been postulated that phosphorylated ERp57 might interact with STAT3 to modulated intracellular signal transduction (10).

ERp57 is a member of the family of PDI and specifically binds to calnexin and calreticulin, which are involved in protein folding (26, 27). PDI activity of ERp57 is to catalyze disulfide bond formation of interchain and intrachain of polypeptides (2, 11). To our surprise, when we knocked down ERp57 by siRNA, we found that cells with decreased level of ERp57 were more resistant to hyperoxia or tunicamycin-induced apoptosis. Tunicamycin is an inhibitor of N-glycosylation in glycoprotein synthesis and is an ER stress inducer. The inhibition of glycoprotein synthesis causes ER stress, activates UPR, and results in apoptosis. Overexpression of ERp57 exacerbated hyperoxia- and tunicamycin-induced apoptosis. ERp57 is not only present in the ER, but also in the cytoplasm, mitochondria, and nucleus (4, 47). ERp57 forms complexes with mitochondrial α-calpain in the mitochondria, which will cleave apoptosis-inducing factor (AIF), release AIF from the mitochondrial inner membrane, and result in apoptotic cell death. Inhibition of ERp57 activity with PDI inhibitors eliminates AIF release and apoptosis (28). Our observation showed that overexpression of ERp57 increased tunicamycin-induced caspase-3 activation and apoptosis in human endothelial cells. Moreover, knockdown of ERp57 reduced hyperoxia or tunicamycin-induced caspase-3 activation and decreased apoptosis, which strongly suggests that ERp57 plays an important role in regulation of apoptosis. Recent reports have also indicated that ERp57 is implicated in immunogenic apoptotic cell death in cancer therapy because the immunogenicity of apoptotic cell death depends on quantity of calreticulin on cell membrane surface (25, 29). Calreticulin has been known to interact with ERp57 in the ER, and the interaction of calreticulin and ERp57 is required for their cotranslocation to cell membrane. Knockdown and knockout of ERp57 can decrease translocation of calreticulin to the cell membrane and reduce immunogenicity-induced apoptotic cell death (25, 29).
can be detected in IPF lungs. We have also found that hyperoxia can elevate BiP/GRP78 protein levels in bronchial and alveolar epithelia in neonatal rat lung (unpublished data). We speculate that hyperoxia initially activates ER stress and increases BiP/GRP78 induction for cell protection, and prolonged exposure will result in persistent UPR activation and increase ER stress-induced apoptosis. The modulation of ER stress will assist in treatment development for hyperoxia-induced lung injury and BPD.

In summary, our study demonstrates that ERp57 can regulate apoptosis in human endothelial cells. It appears that knockdown of ERp57 confers cellular protection against hyperoxia- or tunicamycin-induced apoptosis by inhibition of caspase-3 activation and induction of BiP/GRP78.

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