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ENDOPLASMIC RETICULUM STRESS AS A PRO-FIBROTIC STIUMULUS

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Abstract

Current evidence suggests a prominent role for endoplasmic reticulum (ER) stress and activation of the unfolded protein response (UPR) in fibrotic conditions affecting a number of internal organs, including the lungs, liver, GI tract, kidney, and heart. ER stress enhances the susceptibility of structural cells, in most cases the epithelium, to pro-fibrotic stimuli. Studies suggest that ER stress facilitates fibrotic remodeling through activation of pro-apoptotic pathways, induction of epithelial-mesenchymal transition, and promotion of inflammatory responses. While genetic mutations that lead to ER stress underlie some cases of fibrosis, including lung fibrosis secondary to mutations in surfactant protein C (*SFTPC*), a variety of other factors can cause ER stress. These ER stress inducing factors include metabolic abnormalities, oxidative stress, viruses, and environmental exposures. Interestingly, the ability of the ER to maintain homeostasis under stress diminishes with age, potentially contributing to the fact that fibrotic disorders increase in incidence with aging. Taken together, underlying ER stress and UPR pathways are emerging as important determinants of fibrotic remodeling in different forms of tissue fibrosis. Further work is needed to better define the mechanisms by which ER stress facilitates progressive tissue fibrosis. In addition, it remains to be seen whether targeting ER stress and the UPR could have therapeutic benefit.

Keywords

unfolded protein response; fibrosis; interstitial lung disease; apoptosis; epithelial-mesenchymal transition

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Introduction

In addition to its role in neurodegenerative diseases and diabetes, endoplasmic reticulum (ER) stress is emerging as a factor in a variety of diseases that result in fibrotic remodeling of internal organs, including liver, gastrointestinal (GI) tract, kidneys, heart, and lungs [1–7]. The ER is an important intracellular organelle whose tasks include facilitating the conversion of nascent proteins to functional forms. Conditions such as calcium depletion, glucose or nutrient deprivation, viral infections, environmental exposures, aging, or expression of mutant proteins can alter the functionality of the ER, resulting in ER stress. To maintain homeostasis, cells rely on protective mechanisms to help them cope with ER stress, pathways referred to collectively as the unfolded protein response (UPR). The UPR encompasses three transmembrane proteins that act as sensors of ER stress with activation of downstream pathways orchestrating a cascade of events that have evolved to protect the cell [1]. Cells with high metabolic activity or with high secretory function rely on the UPR pathways to maintain homeostasis in the setting of ER stress. Perhaps this is best illustrated in the function of plasma cells, which rely on a highly functioning UPR to maintain homeostasis in the setting of immunoglobulin production. However, when ER stress is severe or prolonged, cellular dysfunction can ensue, resulting in injury or death, inflammatory signaling, and/or phenotype transition. This review focuses on structural cells, primarily epithelial cells, where evidence indicates that ER stress can enhance vulnerability to injury and facilitate fibrotic remodeling in a variety of tissues. Although the mechanisms linking ER stress and fibrosis are incompletely understood, we will discuss the current state of knowledge regarding the contribution of ER stress and the UPR to cellular dysfunction and fibrosis.

Endoplasmic Reticulum Stress and the Unfolded Protein Response

The endoplasmic reticulum (ER) is an organelle found in all eukaryotic cells. It is crucially involved in protein folding, lipid synthesis, glycogen production and storage, and calcium metabolism [1]. Under normal physiological conditions, chaperone proteins assist in folding of nascent proteins, thereby preventing aggregation of proteins in the ER. Immunoglobulin heavy-chain-binding protein (BiP), also referred to as glucose regulated protein 78 (GRP78), is an important chaperone that is typically increased when ER stress is encountered. In fact, upregulation of BiP can serve as an indicator of ER stress [8–10]. BiP binds to transmembrane sensor proteins PKR-like endoplasmic reticulum Kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring enzyme 1 (IRE-1), maintaining each in its inactive state [11]. With protein accumulation in the ER, BiP interacts with these nascent proteins and is released from these transmembrane sensors. Once unbound from BiP, these three transmembrane proteins can then assume their activated state, leading to the cascade of events known as the UPR (Figure 1). PERK and IRE-1 are activated by phosphorylation while ATF6 is activated by proteases. These UPR proteins then act to maintain homeostasis and normal functioning of the ER and the cell through attenuating protein translation, increasing cytoprotective factors, enhancing production of folding chaperone proteins, and up-regulating expression of pro-degradation factors (Figure 1) [12,13]. However, when these UPR mechanisms fail or when the UPR is hyperactivated, apoptotic cell death can occur [14].

Activation of PERK occurs by trans auto-phosphorylation and dimerization. Activated PERK phosphorylates the α-subunit of eukaryotic translational initiation factor 2 (peIF2α), which hinders global protein synthesis [15]. The importance of PERK in cell survival in the setting of ER stress was established by studies in which cells with mutant PERK were shown to be unable to phosphorylate eIF2α leading to increased sensitivity to induction of ER stress and greater cell death [16]. In addition to inhibition of protein translation, peIF2α

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induces the expression of ATF4, which has been shown to increase expression of protective redox proteins as well as the pro-apoptotic protein CCAAT/enhancer binding protein (EBP) homologous protein (CHOP) [17]. As a result, a delicate balance appears to dictate whether phosphorylated eIF2α dependent mechanisms are pro- or anti- apoptotic [15–17]. Along these lines, cells from ATF4 knockout mice are more sensitive to oxidative damage [16,18].

ATF6 is an important component of the UPR pathways and has been implicated in several diseases. When activated by ER stress, ATF6 translocates to the golgi, where it is cleaved by site1 and site2 proteases into an NH2 terminal domain and a cytosolic domain. The cleaved cytosolic domain is then transported into the nucleus where it activates the transcription of several ER proteins such as BiP, X-box binding protein 1 (XBP1), GRP94, calreticulin, calnexin, protein disulfide isomerase (PDI), and CHOP[10,19–21]. ATF6 has two isoforms - ATF6α and ATF6β. Of the two isoforms, ATF6α has been shown to be most important for cell survival under ER stress. Double knockouts of ATF6α and ATF6β results in embryonic lethality while single gene deletion of ATF6α or ATF6β does not result in an obviously abnormal phenotype. However, when ATF6α knockout mice are challenged with intraperitoneal injection of the ER stress inducing agent tunicamycin, survival of mice is reduced compared to wild type controls [22].

IRE-1 is a transcription factor in the UPR pathway and its importance in the UPR is well established in studies from mammalian cells. It exist in two isoforms - IRE-1α and IRE-1β When activated, IRE-1 undergoes dimerization, with the RNase domain of IRE-1 cleaving XBP1 into its spliced (and active) form. Spliced XBP1 acts as a transcription factor and promotes the transcription of ER associated degradation (ERAD) target genes such as ER degradation enhancing α-mannosidase-like protein (EDEM) [23,24]. Knockouts of IRE-1 and XBP1 are embryonic lethal with defects in liver development. Intestinal epithelial specific deletion of XBP1 results in loss of paneth cells by apoptosis [25,26]. Together, these studies suggest a protective effect of XBP1 in liver and intestine. Recent studies also indicate a prominent role for IRE-1 in cell death associated with chronic ER stress. Prolonged exposure of both primary mouse β-cells and rat insulinoma cell line (INS-1) to glucose results in increased expression of IRE-1α leading to insulin mRNA degradation and apoptosis [27]. IRE-1α degrades mRNAs encoding membrane bound and secretory proteins through a mechanism called regulated IRE-1- dependent decay (RIDD) [28,29]. In studies by Han et al. in INS-1 cells [30], the RNase domain of IRE-1 was shown to function not only by generating the protective spliced form of XBP1, but also by mRNA degradation and effects on cell survival independent of XBP1 splicing. Thus, IRE-1 pathway activation may improve cell survival, but can also contribute to cell death in situations of chronic ER stress.

An important mechanism by which accumulated unfolded proteins in the ER are eliminated is through the ERAD pathway. Both ATF6 and IRE-1 pathways play a role in the ERAD pathway as ATF6 and IRE-1 knockout mice both show defects in the ability to activate ERAD components [19,31–33]. In addition to ERAD, cells can also eliminate proteins that accumulate in the ER through activation of autophagy, which is a catabolic process mediated through the lysosomal pathway [34,35]. However, it is not clear whether ER stress mediated autophagy protects cells from undergoing apoptosis, as some data suggest that it also induces cell death [36–38].

When a cell encounters prolonged ER stress or is overloaded with accumulation of proteins in the ER, cell death pathways such as caspase-4 (and its murine homolog caspase-12), CHOP, and c-Jun NH2-terminal kinase (JNK) are activated [1,39–41]. Caspase-4 is found in the ER membrane, providing a direct link between ER stress and activation of the caspase pathway [39]. CHOP is also a major regulator of cell death under ER stress. *In vitro* overexpression of CHOP results in apoptosis, while deficiency of CHOP protects cells from

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apoptosis [42,43]. However, apoptosis can be induced in CHOP knockout mice after exposure to ER stress inducing agents, indicating that CHOP independent pathways are also important in ER stress associated cell death.

Pulmonary Fibrosis

Idiopathic pulmonary fibrosis (IPF) is the most common and severe of the idiopathic interstitial pneumonias with a mortality rate greater than 50% within 3 years of diagnosis [44]. Over the past decade, multiple lines of evidence have indicated a role for ER stress in the pathogenesis of IPF and its familial form, known as familial interstitial pneumonia (FIP). Initial insights into the role of ER stress in IPF came from the observation that mutations in the gene encoding surfactant protein C (*SFTPC*) were associated with FIP in both children and adults [45,46]. Type II alveolar epithelial cells (AECs) secrete surfactant protein C (SP-C), a highly hydrophobic protein, into the alveolar space [45,46]. Because of its marked hydrophobicity, SP-C must be processed through the ER as a pro-protein, with subsequent cleavage steps in the secretory pathway yielding the mature protein that is secreted into the alveolar space [47,48]. Folding of the carboxy-terminal region of the pro-protein occurs in the ER, and mutations in this region of the gene result in a protein product that is misfolded, leading to protein accumulation and ER stress [6,49]. In 2008, we evaluated human lung tissues samples from FIP patients with a mutation in *SFTPC* (L188Q), FIP patients without *SFTPC* mutations, and sporadic IPF patients for expression of BiP, EDEM and XBP1[6]. These ER stress markers were prominently expressed in AECs lining areas of fibrosis in patients with *SFTPC* mutations as well as patients with lung fibrosis in the absence of *SFTPC* mutations. Subsequently, Korfei et al reported that AECs lining areas of fibrosis in IPF lung tissue sections not only had expression of ER stress markers, but these same cells also had activation of pro-apoptotic pathways [7].

In vitro studies in which carboxy-terminal *SFTPC* mutations are expressed in lung epithelial cell lines revealed mutant protein accumulation in the ER, leading to ER stress and UPR pathway activation [6,49–51]. Similar to *SFTPC*, mutations in the gene encoding surfactant protein A2 (*SFTPA2*) have been linked to cases of FIP [52]. Modeling of these *SFTPA2* mutations *in vitro* suggests that the resultant mutant proteins cause ER stress and UPR pathway activation [52,53].

Recently, we developed a mouse model in which the L188Q *SFTPC* mutation is inducibly expressed in type II AECs [51]. Such expression leads to ER stress in the type II AEC population, but these cells are able to maintain homeostasis in the setting of ER stress without the development of lung fibrosis. However, following low dose bleomycin, these mice develop excessive lung fibrosis. In a separate model, we induced ER stress in the lungs of wild type mice by intratracheal administration of tunicamycin. As with mutant *SFTPC* expression, ER stress alone did not cause lung fibrosis, but did predispose to greater bleomycin induced fibrosis [54]. Taken together, these studies suggest that ER stress leads to a vulnerable AEC population with predisposition to fibrosis after injury.

Of the potential mechanisms linking ER stress to fibrosis in the lungs, induction of apoptosis is the best characterized. Increased apoptosis is observed in lung epithelial cell lines when mutant forms of *SFTPC* (Δexon4 and L188Q *SFTPC*) are expressed, with studies revealing this process is partially mediated through a caspase-4 (caspase-12) mechanism [6,49,50]. *In vivo*, transgenic mice expressing the Δexon4 *SFTPC* mutation under the human *SFTPC* promoter had abnormal branching morphogenesis in the lung, with evidence supporting a prominent role for protein misfolding and abnormal surfactant protein processing, as well as lung epithelial cell apoptosis[55]. In our model in which we conditionally expressed mutant L188Q *SFTPC* exclusively in type II AECs, these mice did not have increased AEC

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apoptosis with transgene expression alone; however, when challenged with a low dose of bleomycin, L188Q *SFTPC* expressing mice had greater AEC death as detected by TUNEL staining, along with evidence of increased caspase-12 and caspase-3 activation [54].

Another mechanism through which ER stress in epithelial cells may contribute to fibrosis is through shift to a mesenchymal phenotype. Hepatocytes, kidney tubular epithelial cells, and AECs have all been described to undergo epithelial-mesenchymal transition (EMT) under pathological conditions [56–58]. A relationship between ER stress and epithelial cell plasticity was first demonstrated in thyroid epithelial cells where induction of ER stress via tunicamycin administration led to EMT [59]. Consistent with this finding, we and others have shown recently that induction of ER stress in lung epithelial cells, either through expression of mutant *SFTPC* or following exposure to tunicamycin, leads to EMT via Src and Smad signaling [51,60]. Further, we showed that this mechanism is likely mediated primarily through IRE-1α signaling [51]. Recently, a study by Baek et al revealed that UPR activation may also be involved in myofibroblast differentiation of lung fibroblasts [61], extending this potential association between ER stress and cellular plasticity.

Taken together, multiple lines of evidence implicate ER stress in the AEC population as having a prominent role in the pathogenesis of IPF. Interestingly, however, mouse modeling suggests that induction ER stress alone is not sufficient for the development of fibrosis, but when present, ER stress generates an AEC population that is vulnerable to the effects of additional injurious stimuli.

Liver Fibrosis

Liver fibrosis is seen in many types of chronic liver diseases [62]. ER stress pathway components have been observed in several forms of liver diseases [63]. Alpha 1-antitrypsin deficiency is a genetic disorder caused by defective production of α1-antitrypsin (α1AT), with lung manifestations in adults and liver disease in adults and children [64]. Liver disease manifestations result from hepatocyte accumulation of a mutant form of α1AT with resultant ER stress. ER stress pathway components BiP and CHOP have been noted to be markedly increased in experimentally induced liver fibrosis in transgenic mice expressing the α1AT Z mutant protein [65]. In this model, α1AT Z mutant protein accumulates in the ER of hepatocytes, suggesting that ER stress sensitizes hepatocytes to injury and fibrosis. Furthermore, studies in liver cells demonstrate both proteosome and non-proteosomal pathways are activated to degrade the α1AT accumulated in the ER of liver cells [12].

In another model, liver fibrosis and acute liver injury were greatly attenuated in CHOP deficient mice following bile duct ligation [2,65–67]. Transforming growth factor β1 (TGFβ1) is a potent profibrotic cytokine secreted by many different cells [68,69], and its expression was completely abolished in CHOP deficient mice [2,64]. Similarly, αSMA expression, a marker of myofibroblasts, was markedly decreased in CHOP deficient mice [2,67]. More recently, Mu et. al.[3], using a rat model of fibrosis induced by methionine–choline-deficient diet (MCDD), demonstrated that ER stress markers, such as BiP and PDI, were markedly enhanced in rats fed with MCDD compared to those fed a control diet [3]. In addition, αSMA, TGFβ1, and collagen were increased in MCDD fed rats. Both ER stress and fibrosis were reversed when MCDD fed rats were given a normal diet.

When ER stress was induced in primary hepatocytes by exposure to fatty acids, CHOP expression was found to be up-regulated [67]. SiRNA knock down of CHOP in a hepatocyte cell line protected these cells from apoptosis when challenged with the ER stress inducing agent thapsigargin and this protective effect was mediated via inhibition of the JNK pathway [67]. Similarly, hepatocytes from CHOP null mice had reduced apoptosis when exposed to toxic stimuli such as glycochenodeoxycholic acid or intragastric ethanol feeling [2,66,67].

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Taken together, these studies suggest that the ER stress pathway contributes to genetic and injury-induced models of hepatic fibrosis and that CHOP may be an important player in liver fibrosis through effects on hepatocyte survival.

Inflammatory Bowel Disease

Intestinal fibrosis results from chronic inflammation in the pathogenesis of Crohn's disease (CD) and ulcerative colitis (UC), the two prominent forms of inflammatory bowel disease (IBD) [70]. Several studies strongly correlate ER stress with inflammation in IBD. Analysis from human biopsy samples with CD and UC demonstrated that BiP mRNA expression and XBP1 splicing are markedly increased not only in inflamed mucosa but also in areas of mucosa without inflammation, suggesting that ER stress might be a common underlying abnormality in IBD [25]. In the dextran sodium sulphate (DSS) colitis model, IRE-1β knockout mice developed more severe colitis compared to wild type controls as measured by infiltration of mononuclear leukocytes on tissue sections and prominent immunostaining for Intercellular Adhesion Molecule 1 (ICAM-1) in the mucosa of the distal colon. Consistent with these findings, it was recently demonstrated that intestinal epithelial cell specific deletion of XBP1 resulted in spontaneous inflammation with increased levels of tumor necrosis factor α (TNFα) as well as polymorphonuclear cell infiltrates in the small intestine (but interestingly not in the colon), which were accompanied by paneth cell apoptosis mediated via the JNK pathway. Interestingly, ER stress markers BiP and CHOP were also markedly increased in these mice [25,26]. The role of ER stress in intestinal inflammation is well documented in mice with mutations in the *Muc2* gene. Muc2 is a component of mucin secreted by goblet cells in the small and large intestine. Heazelwood et al. generated two strains of mice with a missense mutation in *Muc2* using chemical mutagenesis [71]. In both mice strains, Muc2 protein accumulated in the ER with concomitant increased BiP expression and increased XBP1 splicing in the intestinal epithelium. These mice developed spontaneous inflammation in the large intestine and supernatants from explant cultures of distal colon from these mice had increased inflammatory cytokines such as interleukin-1β (IL-1β), TNF-α, and interferon-γ (IFN-γ)compared to wild type controls Thus, multiple avenues of investigation implicate ER stress as having a key role in the pathogenesis of IBD, likely through effects on inflammation and apoptosis of epithelial cells.

Renal Fibrosis

Tubular interstitial injury is a major cause of chronic kidney diseases [72], and the role of ER stress in kidney fibrosis has been explored recently. Kimura et al. [73] developed mutant mice in which BiP does not have the carboxyl terminal Lys-Asp-Glu-Leu (KDEL) ER retention sequence that functions to retain BiP in the ER lumen. Interestingly, homozygous mice died a few hours after they were born with defects in surfactant protein C synthesis and respiratory failure [74,75]. Heterozygous mice, however, developed renal fibrosis and tubular atrophy with age [73].

In the kidney, apoptosis, and EMT have been associated with ER stress. Apoptosis has been noted in tubular epithelial cells in animal models of albumin proteinuria, puromycin aminonucleoside nephropathy, and cyclosporine induced nephropathy, with *in vitro* models suggesting this effect is mediated via CHOP [76,77]. Furthermore, using the unilateral ureteral obstruction rat model, Chiang et al. showed that tubular epithelial cells have evidence of ER stress with subsequent apoptosis through activation of the JNK pathway, culminating in fibrosis [4]. Proximal renal tubular epithelial cells have also been noted to differentiate into a mesenchymal phenotype under ER stress induced by cyclosporine, with both TGFβ dependent [78] and TGFβ independent signaling [79], potentially contributing to fibrosis. Thus, based on experimental evidence to date, the impact of ER stress on kidney

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fibrosis appears to be prominently mediated through regulation of cell survival and plasticity, findings which are similar to those observed in pulmonary fibrosis.

Cardiac Fibrosis

In the heart, ER stress and the UPR pathway are activated in several disease models resulting in cardiac remodeling and fibrosis. ER stress markers BiP, PDI and CHOP were found to be up-regulated in a rat model of cardiac fibrosis induced by subcutaneous injection of isoproterenol. Treatment with the chemical chaperone 4-phenylbutyric acid (4-PBA) not only prevented expression of BiP, PDI and CHOP, but also attenuated cardiac fibrosis in this model [5,80]. In another model of cardiac fibrosis induced by angiotensin II, Kassan et al. showed that ATF4, peIF2α, and CHOP were up-regulated, along with increased levels of TGFβ1, Smad signaling, and collagen I expression. Interestingly the chemical chaperone 4-PBA attenuated cardiac fibrosis, as well as TGβ1 expression and Smad signaling [81]. These findings show that ER stress in cardiac myocytes affects fibrotic remodeling, but further work is needed to identify the mechanisms involved. Unlike other organs where ER stress in the epithelium appears important in fibrotic diseases, these studies highlight the potential importance of ER stress in other cell types with high metabolic activity and suggest that ER stress in these cells could also render them vulnerable to fibrotic stimuli.

Potential Causes of ER stress in Fibrosis

While genetic causes of ER stress have provided important insights into the connection between ER stress and fibrosis, genetically determined expression of misfolded proteins appears to be a rare cause of organ fibrosis. For example, while ER stress pathways are commonly activated in IPF, only a very small proportion of these cases are related to misfolded proteins resulting from *SFTPC* mutations. Likewise, misfolded α1AT underlies only a small proportion of liver fibrosis. However, several other factors that induce ER stress have been identified that could be relevant to fibrosis [8,82–84].

Viral infections can result in ER stress and UPR pathway activation related to viral protein synthesis with subsequent processing through the ER. In hepatocytes, hepatitis C virus and hepatitis B virus infections result in activation of the UPR pathway [85,86]. In the lung, herpesviruses, most notably Epstein Barr Virus (EBV) and cytomegalovirus (CMV), can be found in epithelial cells lining the areas of fibrosis, a pattern which is not observed in normal lung tissue controls [6,7]. Interestingly, herpesviruses are known to activate UPR pathways [8], and we have shown that herpesvirus antigens colocalize with ER stress markers in the hyperplastic epithelium in areas of lung fibrosis [6]. In addition, a recent study showed that old mice infected with murine γ-herpesvirus 68 develop lung fibrosis, possibly through an ER stress related mechanism [87]. Taken together, these available data suggest that viral infections may be involved in the pathogenesis of fibrosis through ER stress induction and activation of the UPR.

Oxidative stress is another stimulus that can enhance expression of UPR pathway components [88] and appears to have a role in fibrosis of different organs [89–91]. In addition to the usual effects of ER stress, induction of the UPR by reactive oxygen species can result in activation of Nrf2, which is an important transcription factor involved in induction of Phase 2 enzymes that detoxify carcinogens and oxidants. Activated PERK phosphorylates Nrf2, which is an alternative means to activate this transcription factor [92]. In Nrf2 deficient mice, cyclosporine A treatment induces greater ER stress and renal fibrosis compared to wild type mice with increased expression of αSMA and TGFβ [93].

Environmental exposure to airborne particulates and cigarette smoke has been shown to cause ER stress. Airborne particulates, which have been linked to some forms of pulmonary

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fibrosis [94], activate several pro-fibrotic intracellular signaling pathways [95]. In a recent study using both *in vitro* cell culture and an *in vivo* mouse model, Laing et al. demonstrated that airborne particulate matter up-regulates PERK and IRE1α pathways with resultant CHOP mediated apoptosis in both lung and liver cells. Similarly, particulate matter was also shown to induce expression of ATF4 in bronchial epithelial cells [83,96]. Cigarette smoke exposure up-regulates UPR related genes in both normal and malignant lung epithelial cells [82]. Furthermore, UPR pathways are activated in 3T3 fibroblasts following exposure to aqueous extract of cigarette smoke [97,98]. Currently, the role and importance of ER stress in mediating the long-term effects of airborne particulates and cigarette smoke in humans are unknown.

In addition to environmental stimuli, aging may affect ER function. Most chronic diseases with tissue fibrosis increase in incidence with advanced age. Furthermore, there is evidence that the ER components involved in protein folding are significantly down-regulated with age [99,100]. For example, in the kidney, BiP expression is reduced in aged mice compared to young mice. With age, mice with heterozygous deletion of BiP develop severe interstitial kidney injury with fibrotic lesions and apoptosis of renal tubular cells mediated through caspase -12 activation [73]. Taken together, it appears likely that multiple metabolic and environmental factors have the ability to induce ER stress and activate UPR pathways. As individuals age, the cumulative nature of these repeated insults may compound the effects of ER stress. Furthermore, age related decrements in UPR responses may impact the ability of cells to maintain homeostasis in the setting of ER stress. In concert, these factors may, at least in part, explain why fibrotic diseases increase in incidence with aging.

Conclusions and Future Directions

In this review, we have provided evidence from recent studies of lung, liver, GI tract, kidney, and heart that ER stress may play an important mechanistic component in the development and/or progression of tissue fibrosis. We hypothesize that ER stress predisposes to a vulnerable cell population, typically the epithelium, in many forms of fibrosis. In this setting, additional or repetitive exposure to injurious agents likely ignites a series of events that lead to progressive fibrosis (Figure 2). Many questions, however, remain unanswered related to the mechanisms of ER stress induction and the specific means by which the UPR pathway contributes to development of fibrotic diseases. An improved understanding of these mechanisms could lead to new targeted therapies designed to alleviate the impact of ER stress on critical cell populations and thus attenuate fibrosis. However, as we move forward, we must remember that the ER stress response evolved to protect the cell and that attenuating UPR pathways could have deleterious effects. As a result, future approaches designed to improve protein processing or ameliorate the down-stream effects of ER stress (like increased apoptosis) may actually be the best avenues to pursue in targeting ER stress induced fibrosis. Despite the current gaps in knowledge, we remain hopeful that an improved understanding of the functions of ER stress and UPR pathway activation in fibrotic disorders will ultimately lead to new therapies for these devastating diseases.

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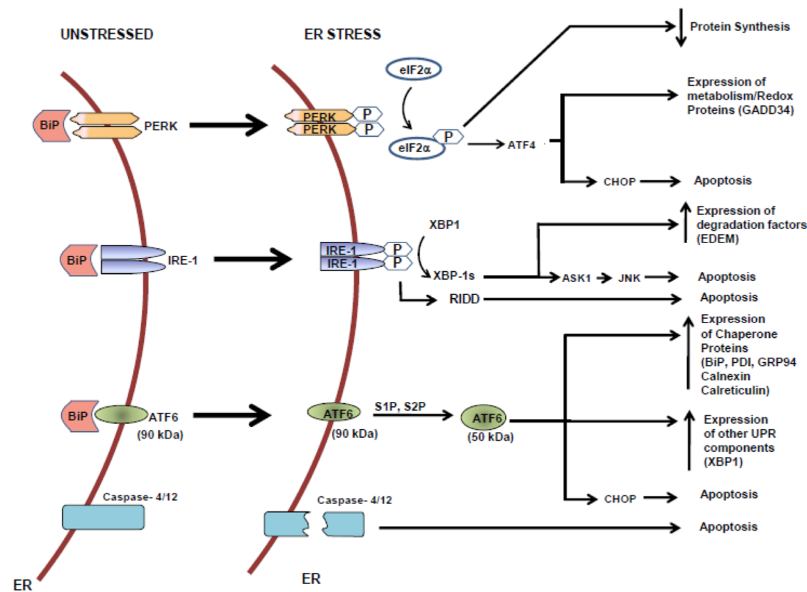

Figure 1.

Schematic illustration of ER stress and the UPR. ATF = activating transcription factor; BiP = immunoglobulin heavy-chain-binding protein; EDEM = ER degradation enhancing α-mannosidase-like protein; eIF2α = eukaryotic initiation factor 2α; ER = endoplasmic reticulum; GADD34 = growth arrest and DNA damage protein 34; IRE = inositol-requiring enzyme 1 (IRE-1); PERK = PKR-like ER kinase; XBP1 = X-box binding protein 1; GADD34 = Growth arrest and DNA damage-inducible protein; PDI = Protein disulphide isomerase; GRP94 = Glucose-Regulated Protein 98; CHOP = C/enhancer binding protein (EBP) homologous protein; ASK1 = Apoptosis signal-regulating kinase 1; JNK = c-Jun N-terminal kinases; S1P = site-1 protease; S2P = site-2 protease; RIDD= regulated IRE1-dependent decay.

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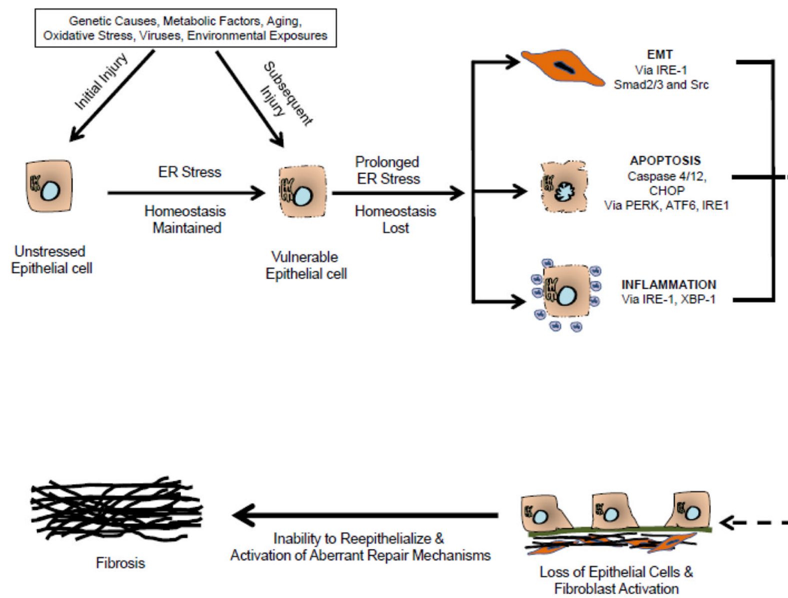

Figure 2.

Schematic of proposed mechanisms by which ER stress may contribute to the development of pulmonary fibrosis.