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Endoplasmic reticulum stress in the pathogenesis of fibrotic disease

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Eukaryotic cells contain an elegant protein quality control system that is crucial in maintaining cellular homeostasis; however, dysfunction of this system results in endoplasmic reticulum (ER) stress and activation of the unfolded protein response (UPR). Severe or prolonged ER stress is associated with the development of degenerative and fibrotic disorders in multiple organs, as evidenced by the identification of disease-causing mutations in epithelial-restricted genes that lead to protein misfolding or mistrafficking in familial fibrotic diseases. Emerging evidence implicates ER stress and UPR signaling in a variety of profibrotic mechanisms in individual cell types. In epithelial cells, ER stress can induce apoptosis, inflammatory signaling, and epithelial-mesenchymal transition. In other cell types, ER stress is linked to myofibroblast activation, macrophage polarization, and T cell differentiation. ER stress–targeted therapies have begun to emerge using approaches that range from global enhancement of chaperone function to selective targeting of activated ER stress sensors and other downstream mediators. As the complex regulatory mechanisms of this system are further clarified, there are opportunities to develop new disease-modifying therapeutic strategies in a wide range of chronic fibrotic diseases.

Introduction

Tissue fibrosis is a pathologic hallmark of many chronic diseases (1). Progressive architectural remodeling characterized by extensive production of collagen and extracellular matrix commonly accompanies organ dysfunction and failure. Acute or insidious injury almost invariably precedes the development of tissue fibrosis (2). Diverse antecedent events, including tissue ischemia, infection/inflammation, and toxic exposures, can lead to fibrotic remodeling, suggesting that convergent molecular mechanisms culminate in the pathology of tissue fibrosis (1). There is great interest in defining these molecular mechanisms and identifying novel therapeutic targets for a wide range of chronic fibrotic diseases (3).

Abnormalities in protein folding and quality control are cardinal features of aging (4) and have been detected in many chronic degenerative and fibrotic disorders. Initial indications that the protein quality control system may play a direct role in tissue fibrosis emerged from studies of Mendelian forms of fibrotic disease, including familial interstitial pneumonia (the familial form of idiopathic pulmonary fibrosis [IPF]) (5), familial forms of chronic kidney disease (6), and α_1-antitrypsin–related (α1AT-related) cirrhosis (7). In each case, germline mutations were identified that result in defects in folding and/or processing of a nascent peptide, leading to induction of endoplasmic reticulum (ER) stress and activation of a signaling network known as the unfolded protein response (UPR). As described in more detail below, subsequent work has demonstrated UPR activation in progressive fibrotic diseases involving different organs.

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The ER quality control system

The ER contains an elegant system designed to facilitate the proper folding and trafficking of proteins, particularly those destined for secretory pathways. Within the ER, a variety of chaperone proteins are involved in protein folding and trafficking. In physiologic states of rapid cellular proliferation (for example, malignancy) or in highly secretory cells (plasma cells, pancreatic β cells, and alveolar type II epithelial cells), the activation of a well-coordinated series of transcriptional and translational changes promotes homeostasis. UPR signaling is mediated through three effector pathways that involve activation of PKR-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring enzyme 1α (IRE1α). Activation of UPR signaling can modulate new protein synthesis, increase production of ER chaperones to improve protein folding, and induce components of the ER-associated degradation (ERAD) system.

The immunoglobulin heavy chain chaperone protein Bip (also known as glucose-related peptide 78, GRP78) is a heat-shock protein family member that is central to UPR regulation. Under normal conditions in the ER, Bip is constitutively bound to the three ER sensors (PERK, ATF6, and IRE1α) and suppresses their signaling (2). Bip also binds to misfolded proteins in the ER, and thus, as misfolded proteins accumulate, Bip binding to the three UPR sensors is reduced (3, 4).

PERK undergoes dimerization and autophosphorylation upon dissociation from Bip, which in turn leads to phosphorylation of eukaryotic translation initiation factor 2α (EIF2α) at Ser51, resulting in a global reduction in mRNA translation (8). However, eIF2α phosphorylation can also increase translation of selected mRNAs, including activating transcription factor 4 (ATF4) (9). ATF4 then acts to increase expression of ATF3, which in turn promotes expression of genes related to antioxidant responses, amino acid synthesis, and autophagy (13).

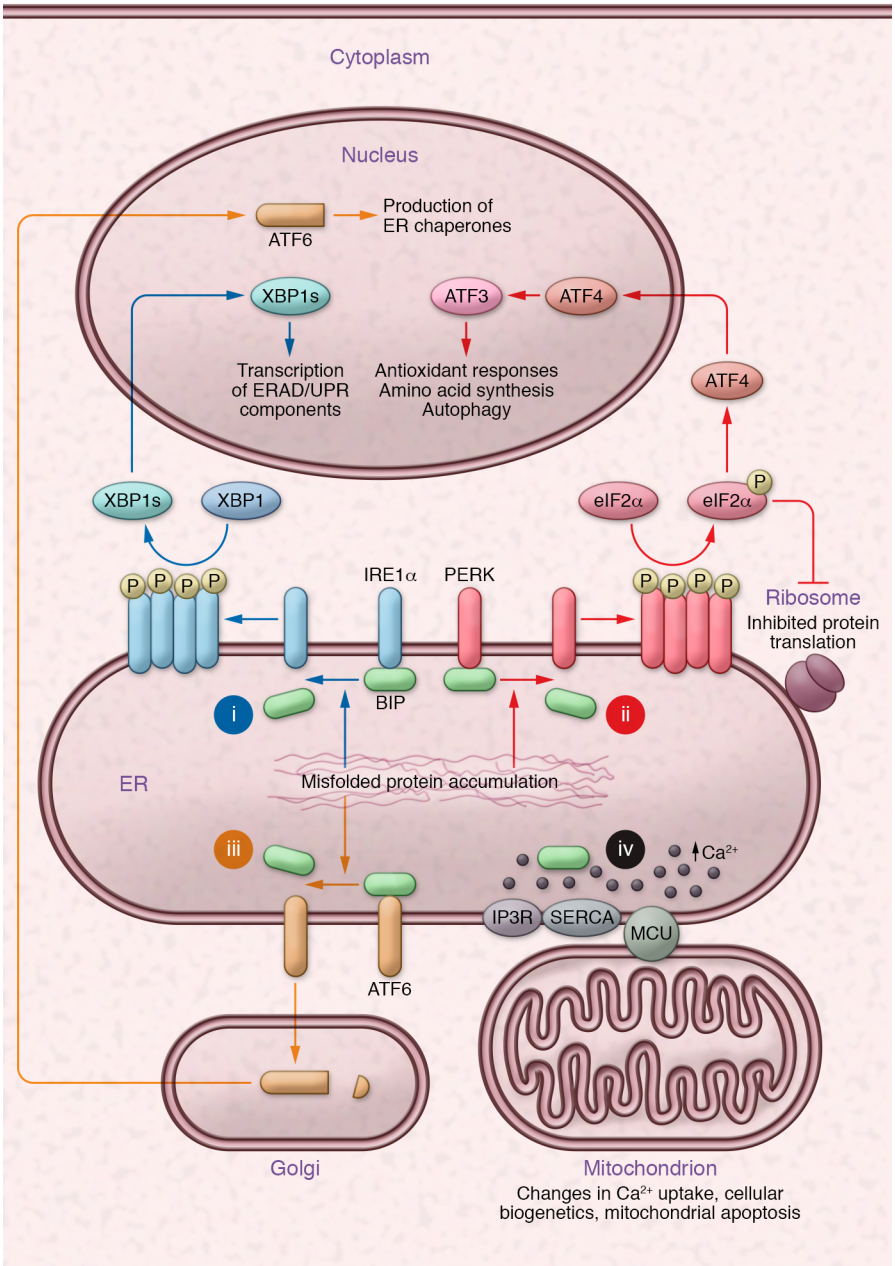

Figure 1. Overview of ER stress–related signaling. Bip binds to accumulating misfolded proteins in the ER, leading to its dissociation from the three ER stress sensors, IRE1α, PERK, and ATF6. (i) Dissociation from Bip allows IRE1α to multimerize and autophosphorylate, activating endoribonuclease activity that leads to alternative splicing of the transcription factor XBP1. Spliced XBP1 (XBP1s) then translocates to the nucleus and promotes transcription of components of the ERAD system. Oligomerized IRE1α loses stringency of endoribonuclease activity and activates regulated IRE1-dependent decay (RIDD), thereby degrading mRNA and miRNAs. (ii) Bip dissociation leads to dimerization and autophosphorylation of PERK, which phosphorylates eIF2α to inhibit protein translation and signals for ATF4 nuclear translocation. Once in the nucleus, ATF4 activates ATF3, which induces adaptive antioxidant responses, promotes amino acid synthesis, and promotes autophagy. (iii) Bip dissociation from ATF6 permits its transit from the ER to the Golgi, where further processing allows trafficking to the nucleus and subsequent increases in production of ER chaperones. (iv) Bip and other ER chaperones serve as calcium-binding proteins. The ER tightly controls the cytosolic calcium pool available for mitochondrial uptake through the mitochondrial calcium uniporter (MCU) via sarcoendoplasmic reticulum Ca^{2+} ATPase (SERCA) and the inositol triphosphate receptor (IP3R). Through its regulation of calcium flux, the ER plays a central role in the regulation of cellular bioenergetics and mitochondrial mechanisms of apoptosis.

Following its release from Bip, ATF6 is trafficked to the Golgi apparatus, where it undergoes a cleavage event that releases the cytosolic domain. This activated form of ATF6 enters the nucleus and acts to enhance transcription of target genes, including the ER chaperones Bip, GRP94, calreticulin, and components of the ERAD system (14–16).

Activated IRE1α possesses endoribonuclease activity, which leads to selective removal of 26 base pairs from the mRNA encoding transcription factor X-box protein-1 (XBP1), producing a transcriptionally active form (XBP1s). XBP1s then migrates to the nucleus, where it promotes transcription of components of the ERAD system, including ER degradation–enhancing α-mannosidase–like protein (EDEM). Activated IRE1α can either homodimerize or oligomerize and autophosphorylate. Oligomerized polyphosphorylated IRE1α appears to have less stringent mRNA endonuclease activity and acts to promote generalized degradation of mRNAs, as well as microRNAs (miRNAs), through a process known as IRE1-dependent decay (RIDD) (17).

These three arms of UPR signaling work in concert to maintain cellular homeostasis in the setting of ER stress (Figure 1). However, under severe or prolonged ER stress, this process may become maladaptive and promote cellular dysfunction and death.

Profibrotic cellular phenotypes associated with ER stress

Although the mechanisms connecting ER stress with fibrosis have been challenging to unravel, most studies to date have focused on proapoptotic (18) or proinflammatory effects of ER stress. In addition, direct profibrotic effects related to mediator production and acquisition of mesenchymal characteristics (epithelial-mesenchymal

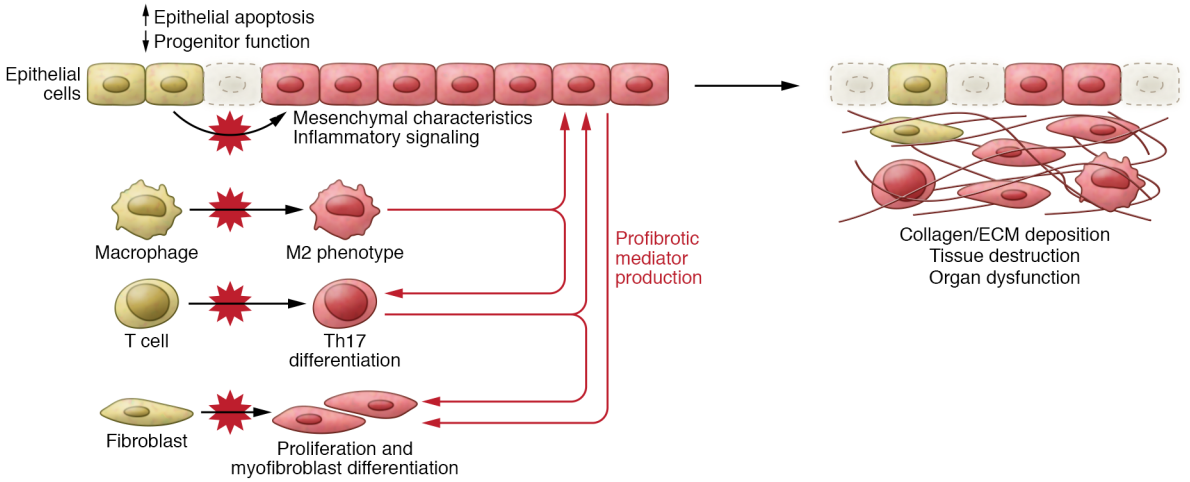

Figure 2. Mechanisms of fibrosis related to ER stress. In epithelial cells, ER stress induces a profibrotic microenvironment by promoting apoptosis, suppressing progenitor cell function, activating inflammatory signaling pathways, and inducing production of profibrotic mediators that promote fibroblast proliferation and myofibroblast differentiation. ER stress signaling in T lymphocytes suppresses Th1 and Th2 polarization and drives Th17 polarization, which can promote fibrosis through interactions with epithelium and fibroblasts. In macrophages, ER stress facilitates acquisition of the M2 phenotype, which is accompanied by enhanced production of profibrotic mediators.

transition) (19, 20) may also contribute to fibrotic remodeling in a context-specific manner.

Apoptosis. Apoptosis of epithelial cells in the lungs and other organs is linked to fibrotic remodeling, presumably by inhibiting re-epithelialization after injury or by impairing barrier functions of the epithelium. The ER is intricately associated with mitochondria through a large number of mitochondria-associated membranes (reviewed in ref. 21) and plays a critical role in regulating cellular bioenergetics and cell-death signaling through its sequestration of intracellular calcium. Uptake of calcium by the mitochondria through the low-affinity mitochondrial calcium uniporter complex (MCU) is crucial for maintaining the mitochondrial membrane potential (22, 23); the availability of cytosolic calcium for uptake through the MCU is highly regulated by the ER membrane–resident sarco/endoplasmic reticulum Ca^{2+}/ATPase (SERCA) pump, ryanodine receptors, and inositol 1,4,5-triphosphate receptors (IP3Rs) (21). Numerous ER-resident chaperones, including Bip and calreticulin, function as calcium-binding proteins (21), and impairment of SERCA function increases calcium leakage, promotes ER stress, and enhances apoptotic susceptibility (24, 25). Impairment of IP3R-dependent calcium transfer also leads to reduced mitochondrial ATP production and promotes autophagy (26).

In addition to direct ER-mitochondrial interactions, ER stress can initiate proapoptotic signaling through each of the three arms of the UPR cascade. ATF4 induction downstream of PERK can activate C/EBP homologous protein (CHOP), a well-studied inducer of apoptosis (reviewed in ref. 27). IRE1α and ATF6 pathways can also contribute to CHOP induction in some settings (28). CHOP is a bifunctional transcription factor that can promote cell death by influencing expression of pro- and antiapoptotic factors, including BCL-2 and BH3-only family members (29). IRE1α phosphorylation also leads to activation of c-Jun NH_2-terminal kinase (JNK), which can promote apoptosis as well as increase RIDD, which can have proapoptotic effects. Also, caspase-4 (and its murine homolog, caspase-12) is an ER membrane–resident caspase that becomes activated in the setting of ER stress (30) and induces apoptosis through activation of caspase-9 (14). The precise mechanism of ER stress–induced caspase-4 activation remains uncertain, but it may be mediated through calcium signaling (32).

Inflammatory signaling. Persistent inflammation can result in tissue injury and aberrant repair that facilitates fibrotic remodeling of affected tissues. The UPR has been shown to activate proinflammatory transcription factors such as NF-κB and AP-1 (33) through IRE1α-mediated induction of TRAF2 signaling (17). TRAF2 can activate NF-κB in a NOD1/2- and RIP1-dependent cascade (18) and can activate AP-1 through JNK phosphorylation (17). PERK activation can also result in increased NF-κB activity through translational suppression of IκBα (36). However, activation of inflammatory signaling appears to be context and cell type dependent, as NF-κB activation is not observed in all models of ER stress (20, 21). Suppression of TNF-α and LPS-induced NF-κB activation (22), as well as inhibition of NF-κB–driven cytokine production, has even been reported in some systems (23).

In addition to transcriptional effects on inflammatory pathways, ER stress can alter the phenotype of immune/inflammatory cells, particularly macrophages. NF-κB activation supports production of a variety of mediators associated with classically activated (M1) macrophages (41). In addition, ER stress has also been linked to activation of NLRP3 inflammasomes (42), which process the M1-associated cytokines IL-1β and IL-18 for secretion. A recent study showed that genetic deletion of IRE1α in macrophages limited M1 polarization and increased the alternatively activated (M2) macrophage phenotype (43). Similarly, CHOP deficiency was shown to prevent M1 macrophage polarization in a model of high-fat diet–induced obesity (44). In contrast, however, genetic deletion of CHOP reportedly decreased M2 macrophage polarization in a model of pulmonary fibrosis (45), and ex vivo M2 polarization of mouse peritoneal macrophages has been shown to depend on ER stress–induced JNK activation (46).

In addition to innate immunity, UPR signaling plays a role in the adaptive immune system. Plasma cell and dendritic cell maturation and differentiation requires ER stress–induced activation of IRE1α (47, 48). ER stress may also play a role in regulating T cell responses, in particular, polarization of Th17 cells (49), which have been implicated in lung and skin fibrosis (50, 51). Induction of ER stress by hypoxia or nutrient deprivation promotes Th17 cell differentiation independent of TGF-β, which is mediated at least in part through XBP1 (49).

Thus, available evidence indicates that ER stress modulates inflammatory signaling and immune/inflammatory cell phenotypes in complex ways that depend on the cell type and stimulus.

Epithelial-mesenchymal signaling and differentiation. In epithelial cells, induction of ER stress has been shown to cause a phenotypic shift characterized by adoption of mesenchymal cell–like morphology and reduced expression of epithelial markers. This phenotype shift includes induction of vimentin, N-cadherin, and α-smooth muscle actin (αSMA), markers typically associated with mesenchymal cells (33, 34). Acquisition of mesenchymal characteristics may allow these cells to more directly contribute to wound repair and tissue remodeling. In addition, recent work indicates that ER stress can suppress Wnt-driven epithelial stem/progenitor cell function downstream of β-catenin nuclear localization (52), suggesting that ER stress may inhibit the self-renewal capacity of local progenitor cell niches.

UPR activation has been shown to alter the function and activation of fibroblasts (53), the cell type primarily responsible for collagen and matrix deposition. UPR activation promotes TGF-β–mediated myofibroblast differentiation (54, 55) at least in part through IRE1α-mediated regulation of miRNAs (56). For example, activated IRE1α can degrade miR-150, which negatively regulates αSMA expression (57).

Together, available data indicate that ER stress promotes fibrosis by enhancing apoptosis of epithelial cells, promoting profibrotic cytokine production from epithelial and immune cells, and enhancing activation of myofibroblasts (Figure 2).

Mechanisms of ER stress induction in fibrotic diseases

The case for a mechanistic relationship between ER stress and fibrosis is bolstered by the identification of rare disease-causing mutations that result in expression of misfolded proteins. In the lung, mutations in the genes encoding surfactant protein C (*SFTPC*) (58–60) and surfactant protein A2 (*SFTPA2*) (61), which result in ER stress in type II alveolar epithelial cells, have been identified in families with IPF. In the kidney, nephrin (62), laminin β2 (63), and podocin (64) mutations that result in ER stress in podocytes have been identified in families with chronic kidney disease. In the liver, Z-allele α1AT produces a misfolded protein product in hepatocytes that accumulates in the ER and is associated with development of cirrhosis in some individuals homozygous for the mutant allele (65). Each of these disease-causing genetic variants shares the common feature of producing highly expressed protein products that are misfolded and mistrafficked, resulting in ER stress in epithelial cells of the target organ.

In addition to expression of defective proteins, expression of exogenous viral proteins can cause ER stress and fibrotic remodeling in target organs. Hepatitis C virus (HCV) infection is associated with ER stress in hepatocytes (66, 67). Before the development of effective antiviral therapies, HCV infection frequently progressed to cirrhosis and end-stage liver disease. In the lung, chronic infection of alveolar epithelial cells with human herpesviruses has been associated with ER stress and development of IPF (60).

Despite the examples given above, the proximate cause of ER stress in fibrotic diseases is often not apparent; however, numerous metabolic and bioenergetic stressors are known to cause ER stress and activate the UPR in vitro. Cellular aging has also been associated with reduced ER capacity and increased susceptibility to ER stress, likely mediated at least in part through mitochondrial dysfunction (68, 69). In addition, oxidative stress (70), altered calcium homeostasis (71), and cellular hypoxia (72–75) can impair protein folding and processing, culminating in ER stress.

ER stress in chronic fibrotic disorders

Pulmonary fibrosis. IPF is a progressive form of tissue fibrosis that typically leads to respiratory failure within 3–5 years of diagnosis (76). Familial clustering of IPF, known as familial interstitial pneumonia (FIP), has been observed since the 1950s (77). In 2002, the first genetic cause of FIP was identified in a large family that included IPF patients and individuals with childhood onset of interstitial lung disease (78). Affected individuals shared a rare missense variant (L188Q) in the gene encoding surfactant protein C (SP-C), which is expressed exclusively in type II alveolar epithelial cells. This variant is adjacent to a cysteine residue in pro–SP-C that is required for proper folding (79). After studies showed that misfolded pro–SP-C aggregates in the ER and activates the UPR (60, 80), additional studies from several groups demonstrated that other disease-associated mutations in the C-terminal portion (or BRICHOS domain) of pro–SP-C result in expression of a misfolded protein (20, 38, 58, 59, 80). In addition to heterozygous *SFTPC* mutations, heterozygous mutations in another surfactant protein gene, *SFTPA2*, have been linked to aberrant protein processing, ER stress, and pulmonary fibrosis in families (61).

The identification of ER stress in alveolar epithelial cells expressing mutant surfactant proteins prompted studies in sporadic IPF patients, which identified ER stress as a common feature in IPF lungs (60). Increased expression of a number of ER stress markers, including Bip, XBP1, ATF4, ATF6, and CHOP, has been reported in IPF patient samples, primarily localized to hyperplastic type II alveolar epithelial cells in areas of fibrotic remodeling (60, 68, 81, 82). In addition, some asymptomatic first-degree relatives of FIP patients have evidence of ER stress in alveolar epithelial cells (83), suggesting that ER stress could be an early driver of disease pathogenesis. While these studies provide compelling evidence of ER stress in the IPF lung, the etiology of ER stress is uncertain. In vitro and preclinical animal models indicate that cigarette smoke (84–86), asbestos (87), and environmental particulates (88) can induce ER stress in alveolar epithelial cells. In addition, the colocalization of ER stress markers with herpesvirus antigens in the alveolar epithelium of IPF patients (60) suggests that viral protein expression could be a factor.

Animal models have helped to clarify a role for ER stress in development of pulmonary fibrosis. Constitutive expression of

a mutant form of *SFTPC* or overexpression of the mature SP-C peptide disrupts lung development and results in perinatal mortality with disrupted lung development, accumulation of protein in the ER, and evidence of ER stress (58, 89). We used an inducible transgenic model to overexpress the L188Q mutant form of human *SFTPC* in alveolar epithelial cells using the murine SP-C promoter (20). Following doxycycline treatment, the transgene localized to the ER and activated the UPR, but no evidence of lung fibrosis was found even with long-term transgene expression. However, following treatment with low-dose bleomycin, lung fibrosis and alveolar epithelial cell apoptosis were markedly exacerbated in animals expressing mutant *SFTPC*. Similarly, intratracheal treatment with the ER stress–inducing agent tunicamycin failed to spontaneously cause fibrosis, but worsened bleomycin-induced lung fibrosis. Together, these studies indicate that induction of ER stress in lung is not sufficient to cause lung fibrosis; rather, ER stress exacerbates the response to fibrogenic stimuli.

Although epithelial cell apoptosis is implicated as an important factor in determining the development and progression of lung fibrosis, the relevant UPR pathway(s) and effector molecules that determine epithelial cell death and survival in the presence of fibrotic stimuli remain uncertain. For example, the role of CHOP has been investigated with conflicting results. In two reports, CHOP-deficient mice exhibited comparative reductions in hydroxyproline content and histologic fibrosis after bleomycin treatment (45, 90). In contrast, another group reported markedly worse survival and increased fibrosis in CHOP-deficient mice following bleomycin treatment (91). The latter study also showed that mice with heterozygous loss of Bip (which would be expected to exacerbate ER stress) were protected from lung fibrosis via increased CHOP-dependent macrophage apoptosis.

IPF is a disease that occurs with aging (median onset at age 65), and altered proteostasis is a hallmark of aging. One study using murine herpesvirus 68 infection found that ER stress in the lung was substantially greater in old mice after infection, and this correlated with development of lung fibrosis (69). While the reason for increased susceptibility to ER stress in aging is not well understood, an important clue may be the finding that expression of the mitochondrial protective factor PINK1 is reduced in aging and IPF lungs (68). ER stress was shown to downregulate PINK1 in mitochondria, thus altering bioenergetics in affected epithelium and promoting apoptosis. In turn, loss of PINK1 can induce ER stress (92), potentially leading to a feedback loop with persistent ER stress that facilitates fibrotic remodeling.

Available data indicate that ER stress contributes to a vulnerable epithelial state in IPF, resulting in increased susceptibility to apoptotic stimuli and impaired epithelial regeneration following injury. However, the impact of ER stress on disease pathogenesis remains incompletely understood, and more information is needed regarding the causes and consequences of ER stress in IPF.

Chronic kidney disease. ER stress related to both genetic and environmental factors has been identified in chronic kidney disease (CKD) (6, 93–98). Aberrant gene products associated with mutations in several genes expressed by podocytes, including collagen IV (97), α-actinin-4 (99), laminin (63), nephrin (62), and podocin (64), can induce ER stress and UPR activation, which have been shown to contribute to podocyte injury/apoptosis, proteinuria, and CKD. In addition, albumin has been shown to induce ER stress in tubular epithelial cells by altering intracellular calcium levels, resulting in apoptosis via UPR-dependent upregulation of lipocalin 2 (100). Together, these studies implicate ER stress as a factor in both induction of proteinuria and mediation of its toxic effects. In another form of familial CKD, autosomal dominant tubulointerstitial kidney disease, genetic mutations in uromodulin (98, 101, 102) have been reported to induce ER stress in epithelial cells in the thick ascending limb of the loop of Henle and cause progressive tubulointerstitial fibrosis. A recently published transgenic mouse model with knock-in of a human missense uromodulin mutation showed that ER stress–regulated factors, including tribbles-3, can determine pathology by sensitizing cells to TNF-α– and TRAIL-induced apoptosis (103).

In addition to genetic causes of ER stress, environmental insults such as hypoxia, increased glucose, and drugs (e.g., cyclosporine) can induce ER stress in the kidney (6, 96). ER stress is well documented in diabetic kidneys (104–106); however, it is not entirely clear whether it is protective or pathogenic. While diabetic CHOP-deficient mice develop less proteinuria compared with controls (107), podocyte-specific XBP1 deficiency or overexpression of ATF6 worsens diabetic nephropathy (108).

Recent studies have identified a new mechanistic connection between ER stress and kidney disease through the ER-associated protein reticulon 1 (RTN1) (104, 109–112), which is associated with acute kidney injury and progression to CKD in animal models and in humans. Increased expression of RTN1 (particularly the RTN1A isoform) induces apoptosis of renal epithelial cells through ER stress–induced activation of PERK and downstream induction of CHOP (104). Knockdown or inhibition of RTN1A expression attenuated ER stress, apoptosis, and renal injury fibrosis in models of unilateral ureteral obstruction (104), diabetic nephropathy (111), and albumin-overload kidney disease (112). It remains to be seen whether this mechanism of ER stress–dependent injury and remodeling is relevant in other organs.

Another mechanism by which ER stress signaling affects diabetic nephropathy is regulation of long noncoding RNA (lncRNA) (105). In a murine model, a cluster of several dozen miRNAs encoded by a host lncRNA was found to be upregulated in the setting of ER stress, both in glomeruli of diabetic mice and in cultured mesangial cells (105). This miRNA cluster is predicted to regulate a variety of signaling processes relevant to fibrosis, including the TGF-β pathway (105). Although more work is needed, it is likely that future studies will identify a larger role for lncRNAs in regulating ER stress–related signaling.

Hepatic fibrosis. ER stress has been observed in several forms of chronic liver disease, including cirrhosis associated with HCV infection (113) or mutant forms of α1AT (65), nonalcoholic steatohepatitis (NASH) (114), and primary biliary cirrhosis (115). Toxic aggregation of misfolded Z-allele α1AT protein is believed to drive α1AT-related liver disease through pathways involving ER stress, autophagy, and other cellular quality control systems (116–118). In NASH, lipids accumulate in hepatocytes when the influx of fatty acids exceeds the clearance capacity (114). Subsequently, lipid overload results in chronic ER stress that, in turn, increases lipogenesis, drives inflammation, and causes hepatocyte apoptosis (114, 119).

In addition to hepatocytes, hepatic stellate cells (HSCs) are also susceptible to ER stress and may be important for hepatic remodeling. It was recently reported that UPR signaling mediates HSC collagen I secretion through XBP1-dependent induction of transport and Golgi organization 1 (TANGO1), thus stimulating liver fibrosis (120). In addition, ER stress was shown to enhance TGF-β signaling as a result of decreasing levels of miR-18a in HSCs (121). Further, several recent reports have indicated that upregulation of the growth factor FGF21 may hold promise in preventing ER stress–mediated steatosis (122–125). Despite evidence that ER stress in HSCs contributes to collagen deposition, studies using chemical chaperones 4-phenylbutyric acid or tauroursodeoxycholic acid to reduce ER stress in mice with methionine- and choline-deficient diet–induced hepatic steatosis have reported mixed results regarding disease progression (126, 127). Underscoring the complexity of this phenotype, it has also been suggested that ER stress–dependent apoptosis of HSCs could be beneficial in limiting fibrotic remodeling in the liver (119).

A number of studies have suggested that CHOP is an important effector molecule through which ER stress impacts liver fibrosis. Following bile duct ligation, liver fibrosis and acute liver injury are greatly attenuated in CHOP-deficient mice (128). Knockdown of CHOP also protects primary hepatocytes from apoptosis following fatty acid–induced ER stress (129, 130). Similarly, hepatocytes from CHOP-deficient mice display reduced apoptosis when exposed to toxic stimuli such as glycochenodeoxycholic acid (128) or intragastric ethanol feeding (131).

ER stress in other chronic fibrotic disorders. Available evidence links ER stress to fibrotic conditions in a variety or organs. For example, ER stress–regulated chronic inflammation drives fibrotic remodeling in inflammatory bowel disease (IBD). In biopsies from IBD patients, increased levels of Bip and spliced XBP1 have been reported, including in segments of the mucosa relatively devoid of inflammation (132). Mice deficient in IRE1β or XBP1 in the intestinal epithelium develop spontaneous gut inflammation and display enhanced proapoptotic signaling in Paneth cells (132). In contrast, however, inducing ER stress by expression of a mutant mucin 2 (*Muc2*) was shown to cause spontaneous colonic inflammation and an increase in Th1 cytokines (133).

In heart failure, several experimental models have shown induction of Bip, CHOP, and other ER stress markers in cardiomyocytes, along with attenuated fibrosis after treatment with a pharmacologic ER chaperone (134, 135). In the skin, interrupting IRE1α signaling can prevent or reverse myofibroblast activation in cells from patients with systemic sclerosis (57).

ER stress as a therapeutic target in fibrosis

There is emerging interest in components of the ER stress response as therapeutic targets in the fields of cancer biology, neurodegenerative diseases, and fibrosis. Targeting these pathways presents unique challenges, as current evidence suggests that conventional approaches attempting to block or broadly inhibit signaling through one or more arms of the ER stress pathway may have significant toxicities due to the role of the UPR in cellular homeostasis (33). For example, deletion of XBP1 in lymphoid cells leads to failure of plasma cell differentiation and profound suppression of antibody production (47), which could predispose to infectious complications.

As an alternative, there has long been interest in enhancing protein chaperone function in the ER. In support of this approach, treatment with pharmacologic chaperones such as sodium phenylbutyrate has been shown to reduce ER stress and reduce or prevent disease in a variety of preclinical disease models (136–140). Other creative strategies to "fine-tune" ER stress signaling may also hold promise. In one intriguing report, a small-molecule allosteric modulator was shown to selectively inhibit oligomerized IRE1α but permit signaling through dimerized complexes (141), suggesting that it may be possible to specifically target pathologic signaling through this molecule. Leveraging alternative cellular quality control mechanisms such as autophagy may also alleviate pathologic ER stress in certain circumstances (116).

The future: ER stress and the microenvironment

Studies to date have provided remarkable insights into the molecular events that mediate ER stress within a cell; however, the effects of pathologic ER stress in a given microenvironment remain incompletely understood. Given the dueling homeostatic and pathologic functions of the ER stress machinery, targeting the downstream consequences of ER stress may be the most practical and promising therapeutic approach. However, emerging clues suggesting non–cell-autonomous effects of ER stress suggest that a broader context may be required for considering ER stress–targeted therapies (142). For example, transfer of supernatant from mucopurulent material from cystic fibrosis lungs can induce ER stress in normal human bronchial epithelial cells (143). Similarly, conditioned medium from prostate cancer cells was shown to induce ER stress in cultured cells (144). The mediators of this effect have not yet been fully elucidated, but may involve TLR4 signaling (144). In *Caenorhabditis elegans*, neuron-specific expression of spliced XBP1 induced ER stress in adjacent non-neuronal tissue, suggesting that paracrine communications may play a role in ER stress induction (145). Further work is needed to clarify the mechanisms of this "transmissible ER stress." Intriguingly, a recent report indicates that multivesicular body formation and exosome release are increased in the setting of ER stress (146), suggesting that the cargo of ER stress–derived exosomes could contribute to cell-cell interactions in fibrotic tissue. Technological advances in single-cell analytics should allow rapid growth in our understanding of the role of intracellular communications in disease pathology and are likely to uncover downstream mediators that are promising therapeutic targets.

Conclusion

It has been more than two decades since the earliest descriptions of the cellular consequences of ER stress, and there have been significant advances in understanding homeostatic and pathologic signaling through this pathway. The best-studied disease mechanism (ER stress–induced apoptosis) may have high relevance to chronic neurodegenerative diseases and cancer therapy (28), but its contribution to chronic fibrotic disorders is less certain. It appears unlikely that enhanced cell death and/or turnover is sufficient to explain the striking pathologic changes observed in lung,

kidney, liver, and other forms of tissue fibrosis related to ER stress. The challenge for the coming years is to better elucidate how the cellular phenotype of ER stress culminates in chronic fibrotic diseases, and how to leverage these complex signaling mechanisms to promote adaptive tissue repair and homeostasis.

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