Chronic liver disease (CLD) affects 1.5 billion individuals worldwide and can lead to complications such as cirrhosis and liver cancers. Endoplasmic reticulum (ER) stress is a major contributing factor to the pathogenesis of many CLDs, including hepatic biliary diseases, alcoholic liver disease (ALD), nonalcoholic fatty liver disease (NAFLD), and hepatocellular carcinoma (HCC). The ER is responsible for several vital intracellular functions, including calcium homeostasis and steroid hormone and lipid biosynthesis. Importantly, the ER is also a key player in the synthesis, folding, trafficking, and degradation of at least one-third of all eukaryotic proteins. Disruption of ER homeostasis creates a unique cellular state termed ER stress. Several physiological or pathological stimuli are identified as ER stress inducers, including ER accumulation of unfolded or misfolded proteins and free cholesterol, depletion of ER calcium stores, oxidative stress, and nutrient deprivation. ER stress is sensed by the unfolded protein response (UPR), a set of intrinsic signal transduction pathways evolved in mammalian cells. The adaptive UPR reduces unfolded protein load in the ER to maintain cell viability and function. However, extended UPR activation triggers cell death. Recent advances in the UPR field indicate that ER stress–mediated activation of UPR signaling pathways plays a key role in regulating protein folding, lipid biosynthesis, and cell death.

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K.G.J. and G.W.W. contributed equally to this work.

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This article is part of a review series focused on the role of cellular stress in driving molecular crosstalk between hepatic cells that may contribute to the development, progression, or pathogenesis of liver diseases.
metabolism, and the immune response. This review addresses the current understanding of ER stress and the UPR signaling cascades and its crucial role in combating CLD.

The ER

ER Structure and Composition

The ER has a unique intracellular membrane structure, is one of the largest organelles in eukaryotic cells, and is integral for proper cell function. It has a large dynamic structure with a continuous lumen and membrane. The ER membrane consists of the nuclear envelope and the peripheral ER, defined by its flat sheets and branched tubules. These rough sheets exist in a stacked conformation with luminal thicknesses of 50 nm in mammalian cells. The rough ER (RER) has flat sheets studded with ribosomes on the cytosolic surface and is the main location of ER protein synthesis and folding. Conversely, smooth ER (SER) tubules have far fewer membrane-bound ribosomes and appear smooth and highly curved. Although the phospholipid composition of the RER and SER is very similar, the SER has twice as much cholesterol as the RER. The SER smooth tubules are formed by a special class of curvature-stabilizing proteins, reticulon, and transcription factor DP1. Not only are the structural differences between sheets and tubules important in ER function, but also the ratio between the two confers differences in discrete cellular functions. For example, specialized cells that synthesize large amounts of secreted proteins have an ER structure composed primarily of RER sheets. On the other hand, cells involved in lipid synthesis and calcium signaling, and which form ER contact sites with other organelles, have a greater proportion of SER tubules. Thus, these ER structural components form distinct domains that facilitate cell type-specific functions.

ER Function

As a distinct membrane-bound organelle, the ER participates in various biological processes, including protein synthesis, folding, modification and transport, lipid metabolism, and calcium homeostasis. Although free ribosomes in the cytosol synthesize most proteins for use within the cell, almost all secreted and membrane-bound organelle proteins are translated on ribosomes in the RER, including proteins bound to the ER or plasma membrane, Golgi apparatus, and lysosome. Polypeptides that bind to the ER membrane possess an N-terminal signal sequence recognized by a signal recognition particle and are inserted into the ER lumen by the ER translocon complex. The ER is also responsible for the secretion of >30% of intracellular proteins. Various signals and stresses are transmitted across the brush border of the ER and other organelles such as mitochondria, lysosomes, the Golgi apparatus, and the nucleus. The synthesized proteins are translocated to the ER-Golgi intermediate compartment, a tightly juxtaposed ER region of the Golgi apparatus that is abundant in tubules and vesicles and facilitates biochemical protein modifications. Finally, the ER is the main intracellular calcium storage site and plays an important role in calcium signaling. Deviation from normal ER function due to various physiological or pathologic stimuli leads to ER stress, which has been linked to the progression of several CLDs. Therefore, understanding ER stress mechanisms is fundamental to effectively treating and reversing these diseases in patients.

ER Stress and the UPR

The UPR is activated as a compensatory response to ER stress and alleviates ER stress by restoring ER homeostasis. During the last two decades, extensive studies have elucidated the three major UPR signaling pathways. Three ER transmembrane proteins have been identified that regulate the three major arms of the UPR: protein kinase R-like endoplasmic reticulum kinase (PERK), inositol requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6) (Figure 1). Under normal physiological conditions, the luminal surface of these transmembrane proteins is bound by binding immunoglobulin protein (BiP), also called heat shock protein family A member 5. BiP is an ER chaperone protein that is regarded as the master UPR regulator for all three UPR pathways. When misfolded and unfolded proteins accumulate in the ER lumen, BiP binds to the hydrophobic regions of these proteins, freeing the luminal surfaces of the UPR sensor proteins PERK, IRE1, and ATF6. Once the luminal surfaces of IRE1 and PERK are exposed, they are activated by dimerization and phosphorylation.

The type 1 transmembrane protein IRE1 contains a serine/threonine kinase and endoribonuclease domains on its cytosolic surface. During ER stress, misfolded proteins bind to the luminal surface of IRE1, causing dimerization, auto-phosphorylation, and domain activation. The activated endoribonuclease of IRE1 then cleaves the unspliced mRNA of X-box binding protein 1 (XBP1), removing a 26-base sequence, leading to a frameshift mutation that generates spliced XBP1, which contains a C-terminal transactivation domain absent in unspliced XBP1. Spliced XBP1 is translocated to the nucleus and contributes to the adaptive UPR by up-regulating genes associated with ER protein entry, protein folding, and ER-associated degradation (ERAD). Thus, the IRE1 endoribonuclease activity predominantly exerts its effects through spliced XBP1. However, IRE1 also negatively regulates gene expression via Ire1-dependent decay of mRNA, which targets the mRNA of IRE1 and several lipid metabolism genes. Despite its role in adaptive UPR, the cytosolic kinase domain contributes to the maladaptive UPR by recruiting tumor necrosis factor receptor-associated factor 2 and mitogen-activated protein kinase 5. Tumor necrosis factor...
receptor−associated factor 2 and mitogen-activated protein kinase 5 phosphorylate and activate both c-Jun N-terminal kinase (JNK) and NF-κB. Sustained JNK activation is well known to cause apoptosis. However, more recent evidence suggests that it may also play a role in necroptosis and autophagy.26,27

PERK is also a type 1 transmembrane protein, similar to IRE1, and a member of the eukaryotic translation initiation factor 2α (eIF2α) kinase family. During ER stress, the luminal domain of PERK binds to misfolded proteins, leading to oligomerization and activation via autophosphorylation.28 Activated PERK phosphorylates multiple substrates, including eIF2α, nuclear factor erythroid 2−related factor 2, forkhead box O, and diacylglycerol.28 Phosphorylation of eIF2α attenuates translation initiation by inhibiting delivery of initiator methionyl-tRNA to the initiation complex but enhances mRNA translation with short upstream reading frames, such as DNA damage-inducible transcript 3 protein (DDIT3) and activating transcription factor 4 (ATF4).29,30 Prolonged DDIT3 activation leads to cell death through mitochondrial-dependent or death receptor pathways.31

ATF6, a type 2 transmembrane protein, is a basic leucine zipper transcription factor. During ER stress, ATF6 dissociates from BiP and translocates from the ER to the Golgi apparatus for activation. A recent study reported that after BiP dissociates from ATF6 during ER stress, an ER-resident oxidoreductase, ERp18, associates with ATF6.32 ERp18 dissociates from BiP and translocates from the ER to the Golgi for activation. A recent study reported that after BiP dissociates from ATF6 during ER stress, an ER-resident oxidoreductase, ERp18, associates with ATF6.32 ERp18

Figure 1 The signaling pathways of endoplasmic reticulum (ER) stress. The unfolded protein response consists of three main arms/branches, including protein kinase R-like endoplasmic reticulum kinase (PERK), inositol requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6). These pathways are activated by unfolded or misfolded protein accumulation-mediated sequestration of binding-immunoglobulin protein (BiP). Activating these three branches leads to an adaptive recovery response to mitigate the unfolded protein levels and maintain ER homeostasis. Activated endoribonuclease activity of IRE1 promotes cleavage of unspliced X-box binding protein 1 (XBP1u) mRNA to produce spliced XBP1 (XBP1s). XBP1s then promotes the transcription of ER chaperones, ER-associated protein degradation (ERAD) components, and lipid metabolism−associated genes and may induce autophagy. PERK activation promotes the phosphorylation of nuclear factor erythroid 2-related factor 2 (Nrf2), forkhead box (FOXO) transcription factors, diacylglycerol (DAG), and the eukaryotic translation initiation factor 2α (eIF2α) phosphate. Eukaryotic translation initiation factor 2α (eIF2α) phosphorylation promotes preferential activating transcription factor 4 (ATF4) translation. ATF4 transcribes genes associated with amino acid biosynthesis, redox, and autophagy. Activated ATF6 then migrates and is subsequently cleaved at the Golgi apparatus by site-1 and site-2 proteases. ATF6 cleavage liberates the basic leucine zipper-class transcription factor (ATF6-N), which then translocates to the nucleus and transcribes XBP1, ER chaperones, lipid synthesis, and ER expansion genes to restore ER homeostasis and reduce cell stress. These compensatory responses are effective for the short-term mediation of misfolded or unfolded protein levels. However, chronic or excessive unfolded protein response signaling initiates a maladaptive response closely associated with increased cAMP response element-binding (C/EBP) homologous protein (CHOP) protein levels that can lead to apoptosis. DDIT3, DNA damage-inducible transcript 3 protein; JNK, c-Jun N-terminal kinase; RIDD, IRE1-dependent decay of mRNA; TRAF2, tumor necrosis factor receptor−associated factor 2.
bound to ATF6 prevents premature ATF6 release from the ER. In ERp18 knockout cells, it has been shown that ATF6 activation is attenuated despite the transport of ATF6 to the Golgi, where proteolytic processing necessary for ATF6 activation traditionally occurs. The authors attribute this seemingly contradictory outcome to abnormal ATF6 splicing in the Golgi, which prevents the release of the soluble transcription factor and halts pathway activation.32 Although further research into the exact mechanisms of the role of ERp18 in ATF6 regulation and ER stress is necessary, this finding elucidates a nuanced role for ERp18 in ATF6 trafficking and processing.

At the Golgi complex, ATF6 is sequentially cleaved by site-1 protease and site-2 protease to release the soluble basic leucine zipper transcription factor ATF6-N.33 ATF6-N then translocates to the nucleus and binds to promoters containing ER stress response elements, leading to the transcription of UPR genes, including XBP1 and genes involved in ERAD. Together, ATF6 and XBP1 form a heterodimer that promotes ERAD.34 ATF6 also promotes lipid synthesis and ER expansion in the absence of XBP1.35 It was initially thought that ATF6 primarily leads to adaptive regulation via transcription of ER stress response elements such as XBP1, ER chaperones, and folding enzymes such as BiP. However, more recently, it is believed that ATF6 may play a role in apoptosis.36

**The Role of ER Stress in Cell Death and Inflammation**

**Apoptosis**

Apoptosis is a highly regulated cell death mechanism that removes dysfunctional cells while preventing inappropriate immune responses.37 Hepatocytes are responsible for the synthesis and secretion of many essential proteins, such as albumin and lipoproteins. In addition, lipotoxicity due to the ectopic fat distribution into liver tissue can also lead to UPR activation and apoptosis. For this reason, hepatocytes are especially vulnerable to ER stress, which often drives liver disease progression.3 The UPR helps to maintain proteostasis by halting protein translation, while ERAD facilitates the clearance of misfolded proteins. However, prolonged UPR activation or the persistence of misfolded proteins promotes a switch from these adaptive, pro-survival responses to apoptosis.38

Apoptosis comprises three phases: initiation, commitment, and execution. Both PERK and IRE1, which control the UPR, also regulate the initiation of ER stress—mediated apoptosis. Each of these UPR-related pathways converges at the activation of DDIT3. Although ATF6 up-regulates DDIT3 transcription, it has not been linked to apoptosis and is only recognized for pro-survival signaling.39 DDIT3 activation marks the beginning of the commitment phase of apoptosis, at which point the cascade is considered irreversible. DDIT3 activation is highly reliant on PERK-dependent activation of ATF4, which encodes the C/EBP transcription factor that can circumvent the eIF2α translational block. Once activated, DDIT3 up-regulates pro-apoptotic death receptor 5 (DR5), Bcl-2-like protein 11 (BCL2L11; alias BIM), and tribbles homolog 3 (TRIB3) and down-regulates antiapoptotic B-cell lymphoma 2 (BCL2) expression.3 The cell then undergoes structural changes: cell rounding and retraction from neighboring cells, nuclear condensation, and fragmentation of cellular organelles. A portion of the cellular contents and plasma membrane then extrudes, furrows, and cleaves away from the cell. This process, referred to as blebbing, packages the cellular contents into apoptotic bodies.37 During this process, the apoptotic cells release signals, including sphingosine-1-phosphate, lysophosphatidylcholine, and fractalkine, that promote homing of phagocytic immune cells and Kupffer cell proliferation that helps to clear the cell debris.40 However, apoptotic bodies phagocytosed by hepatic stellate cells promote activation and fibrosis of these cells.31

Although the IRE1/XBP1 axis is pro-survival in the UPR, IRE1 is also important in pro-apoptotic signaling. In ER stress, mitogen-activated protein kinase 5 can be recruited to the IRE1/tumor necrosis factor receptor—associated factor 2 complex and promotes downstream JNK and p38 phosphorylation (pJNK; pp38). pJNK phosphorylates Bcl-2 family proteins to alleviate Bcl-2—mediated restriction of apoptosis. In addition, pJNK phosphorylates the pro-apoptotic Bcl-2 homology domain 3-only (BH3-only) protein, Bim.3 This cascade permits the translocation of Bak and Bax to the mitochondria, beginning the execution phase of apoptosis.37

Despite the well-defined pathways linking ER stress to apoptosis, other cellular signals, such as miRNAs and enzymes, further regulate these mechanisms. For example, ATF4, downstream of PERK, helps promote apoptosis through miR-483 induction.42 The creatine kinase brain-type (CKB) gene, which miR-483 represses, produces phosphocreatine that donates its phosphate group in converting ADP to ATP. Decreased energy availability is known to lead to apoptosis. Enzymes can similarly regulate apoptosis. The activation of ERAD typically promotes the ubiquitination of the enzyme N6-adenosine-methyltransferase-14 (METTL14), a substrate that binds an E3 ubiquitin ligase, HRD-1.43 However, a recent finding through the Feng laboratory at Northwestern University showed that the accumulation of unfolded proteins could competitively bind HRD-1 and up-regulate METTL14 expression, which, in turn, modifies and suppresses DDIT3 mRNA.44 These opposing mechanisms add to the complexity of pro-survival versus pro-apoptotic signals in the ER stress response.

Apoptosis is an important mechanism to remove dysfunctional cells that pose a risk to the hepatic environment. Reduced apoptosis can allow for hepatic cancer development. However, chronic apoptosis is maladaptive in
the context of liver disease. Excessive apoptosis contributes to the loss of parenchymal tissue and increases the risk of fibrosis, cirrhosis, and HCC; this has been recognized as a driving factor of disease progression in acute and chronic hepatitis, cholestatic liver disease, ALD, and nonalcoholic steatohepatitis (NASH).41

The Inflammasome

Immune responses in CLD include dynamic interactions between the complement system and resident and infiltrating immune cells, as well as inflammasome activation.25

Among these, the inflammasome has been an important topic in many recent hepatologic studies involving ER stress. Inflammasomes are cytoplasmic multimeric protein complexes that contain a sensor protein, an adapter protein, and procaspase-1zymogen. The inflammasome evolved to respond to various pathologic or physiological stimuli, including ER stress. Inflammasomes are a vital part of innate immune function whose activation promotes IL-1β and IL-18 secretion. IL-1β and IL-18 are critical for pathogen or damaged cell clearance via apoptotic or pyroptotic cell death, in the latter case, by facilitating membrane pore formation that allows for cellular content expulsion.46–48

Previous studies show that ER stress–associated nucleotide-binding domain and leucine-rich repeat containing inflammasome activation was mediated through UPR-mediated mitochondrial dysfunction and subsequent caspase-2 cleavage and pro-apoptotic factor release.49

It has been shown recently that ER-mitochondria communication plays a crucial role in the innate immune response. Tunicamycin-induced ER stress leads to nucleotide-binding domain and leucine-rich repeat containing inflammasome activation in THP-1 human monocytes. In this context, nucleotide-binding domain and leucine-rich repeat containing activation was reliant on Ca2+-dependent mechanisms and coincided with ER-mitochondria–associated membrane formation and loss of mitochondrial membrane potential.50 Lysosomal transport of taurine-conjugated bile acids via human equilibrative nucleoside transporter 3 alleviates ER stress in mouse hematopoietic stem and progenitor cells.51 In an acute-on-chronic alcohol model, treatment with 4-phenylbutyrate prevented ER stress–induced DDIT3 up-regulation and inflammasome activation.52 Our understanding of the importance of UPR-mediated pathways in liver disease continues to grow; this review evaluates the current literature that relates ER stress and the UPR in CLD pathogenesis.

ER Stress in Chronic Liver Disease

ER stress has been linked to the progression of various liver diseases, including biliary disease, ALD, NASH, and HCC. CLD can be caused by several factors, from metabolic disturbance and alcohol consumption to viral infections and autoimmune disorders. This association has been shown in several published studies.43,53–55

ER Stress in Biliary Pathogenesis

Cholestasis is a CLD in which the bile flow becomes impeded due to bile duct narrowing or clogging. Patients with hepatobiliary diseases commonly have increased bile acid plasma concentrations.56,57 Bile acids increase hepatic inflammatory conditions in high concentrations and are associated with cirrhosis.58 Accumulation of bile acids causes necrotic cell death, inflammation, neutrophil infiltration, and liver injury. Patients with hyperbilirubinemia exhibit increased expression of key UPR genes, as revealed by RNA-sequencing.59 Interestingly, the XBP1, PERK, and ATF6 pathways were significantly correlated with alanine aminotransferase levels, whereas only the PERK pathway was significantly associated with total bilirubin levels. Bile acids also induce ER stress, leading to apoptosis in HepG2 cells in a hydrophobicity–dependent manner.60

Bile duct ligation is a cholestasis mouse model, and research has shown that bile duct ligation induces ER stress and DDIT3-mediated hepatocyte cell death.61 Bile acid and thapsigargin-induced ER stress and bile duct ligation mice up-regulate miR-199a-5p expression, to protect against ER stress by repressing the 3′untranslated regions of mRNAs for heat shock protein family A member 5, IRE1, and ATF6.62,63 Patients with primary biliary cholangitis exhibit miR-506 overexpression in cholangiocytes, which increases reactive oxygen species levels and up-regulates two key ER stress markers, ERN1 (alias IRE1) and DDIT3, while expression of the genes related to other ER stress pathways (eg, ATF6, PERK, XBP1) are unchanged.64

Several pharmacologic interventions have shown efficacy in reducing ER stress associated with CLDs. Recently, scopolotin and umbelliferone were reported to be protective against palmitate and glycochenodeoxycholic acid–induced hepatocyte cell death by decreasing ER stress, reactive oxygen species generation, and JNK phosphorylation, a cell death signaling intermediate.65 Metformin has been shown to reduce bile acid–induced ER stress, inflammation, chemokine expression, and neutrophil infiltration in bile duct ligation mice.66 In the same study, tauroursodeoxycholic acid also reduced inflammation and cell death in hepatocytes, furthering previous knowledge regarding the efficacy of tauroursodeoxycholic acid in biliary cirrhosis treatment. This knowledge may also be beneficial for ER stress–related diseases. Tauroursodeoxycholic acid has been shown to reduce ER stress–associated protein expression, inhibit dissociation of BiP and PERK, reduce ER stress–mediated cell death in mesenchymal stem cells, and protect mesenchymal stem cells from ER stress via an Akt-dependent cellular prion protein pathway.67
ER Stress in ALD Pathogenesis

ALD is a clinical illness caused by excessive or chronic consumption of alcohol that has a widespread impact from the gastrointestinal tract and liver to neurologic tissues in the case of hepatic or alcoholic/Wernicke’s encephalopathy. ALD can be a life-threatening disease; its incidence continues to rise, contributing to 30% of all HCC cases and HCC-related deaths.68,69 Unlike other CLDs, no potential pharmacotherapeutic drug candidates for ALD have progressed to phase 3 clinical trials in the United States within the last 5 years (ClinicalTrials.gov, https://clinicaltrials.gov/ct2/results?cond = ALD+-+Alcoholic+Liver+Disease&age_v = &gndr = &typ e = &rslt = &phase = 2&Search = apply, last accessed March 7, 2023).

The importance of bile acids in ALD progression has been thoroughly examined.70 ER stress has been linked to ALD pathogenesis in several experiments. In the Lieber-DeCarli and NIAAA mouse models, N-nicotinamide methyltransferase overexpression plays an important role in ALD pathogenesis by activating the PERK-ATF4 pathway.71 In a mouse model of alcoholic hepatitis, alcohol increased IL-17A production, ER stress–mediated pro-apoptotic signaling via Bax and Bim, and caspase-3 and caspase-8 activation, as indicated by DDIT3 up-regulation, which was prevented with 4-phenylbutyrate administration, to inhibit ER stress.52 This study also indicated that an IL-17A–blocking antibody attenuates ER stress–mediated apoptosis in vivo. Crossed high alcohol preferring mice, fed 10% ethanol for 7 months, exhibited decreased thiamine levels and increased oxidative stress, ER stress, and neuronal apoptosis.72 ER stress was determined based on increased ER stress markers ATF-6, GRP-78, DDIT3, and p-eIF2α. Chronic alcohol-induced steatohepatitis in the chronic ethanol feeding model using Long Evans rats was associated with enlarged mitochondria and disrupted ER structure as shown by electron microscopy and ER stress–related gene (eg, DDIT3, ATF4, and BAX) up-regulation.73 A recent study using mice gastric mucosal epithelial cells showed that rebamipide could reverse ethanol-induced (400 mmol/L) ER stress, apoptosis, and downstream NF-κB signaling pathway.74 ER stress plays a crucial role in extracellular vesicle release, mediating intercellular communication and contributing to CLD progression. In 2020, the Gao laboratory at the National Institute on Alcohol Abuse and Alcoholism, Laboratory of Liver Diseases, showed that chronic ethanol feeding promoted mitochondrial DNA–enriched extracellular vesicle release from hepatocytes. Mitochondrial DNA is a typical damage-associated molecular pattern; mitochondrial DNA–enriched extracellular vesicles promote neutrophil chemotaxis and enhanced liver injury. Furthermore, the ASK/p38 signaling axis was found to be an essential regulator for mitochondrial DNA–enriched extracellular vesicle release; this may be a potential therapeutic target for patients with alcohol use disorder.75

ER Stress in NAFLD Pathogenesis

NAFLD is the fastest-rising cause of liver cirrhosis-related hospitalizations worldwide. Currently, NAFLD affects 25% to 30% of the global population, including 83 million people in the United States alone.66,67 Metabolic disorder–related fatty liver disease is closely linked with metabolic syndrome, and its prevalence is expected to rise alongside rates of obesity and type 2 diabetes.77 Another etiology of NAFLD, “lean NAFLD,” is closely connected with mutations in hepatic lipid metabolism genes. ER stress is one of the many factors that promotes NAFLD progression from uncomplicated steatosis (nonalcoholic fatty liver) to NASH, distinguished by the onset of inflammation and varying degrees of fibrosis.75 In NAFLD, high serum lipid levels are resolved through ectopic redistribution into muscle and liver tissues. Circulating lipid is either taken up by hepatocytes from the bloodstream (75%) or synthesized de novo (26%) in hepatocytes.73 Here, we discuss the roles of lipotoxic stress and sphingolipid and bile acid signaling in hepatocyte UPR responses in NAFLD.

UPR activation is typically considered a response to proteotoxic stress, traditionally mediated by luminal ER protein activation. However, in NAFLD, cytotoxic lipids modify ER structure and directly activate the UPR; this is believed to lead to subsequent dysregulation of proteostasis.78 Although hepatocytes are tolerant of triglyceride storage, saturated fatty acids and lysophosphatidylcholines induce lipotoxicity.78,77 Once taken up by hepatocytes, these lipids are stored in cytoplasmic droplets.79 These changes perturb many aspects of hepatocyte function, including glucose uptake, mitochondrial function, and ER homeostasis (Figure 2).38,80 Excess saturated fatty acids trigger hepatocyte ER stress through multiple mechanisms. Incorporation of saturated fatty acids into the ER membrane increases membrane rigidity.79 Changes to ER membrane stiffness are sensed by the amphipathic helices of the transmembrane proteins IRE1 and PERK, leading to UPR activation.78,81 Unlike IRE1 and PERK, ATF6 is sensitive to high concentrations of the sphingolipids dihydrosphingosine and dihydroceramide. Each of these cases enables the release of BiP from the UPR transmembrane proteins IRE1, PERK, and ATF6.81

Diet high in fructose also increase the incidence of NAFLD.82 Furthermore, the deletion of XBP1 promotes compensatory increases in IRE1. Recent work has shown that increased IRE1 via hepatocyte-specific deletion of XBP1 in conjunction with dietary fructose (600 mg/g chow) ad libitum for 4 weeks increased lipogenic and pro-apoptotic gene expression and worsened multiple parameters of liver steatosis and inflammation in vivo. Double knockout of both XBP1 and IRE1 in hepatocytes, however, ameliorated liver injury while measures of steatosis were unaffected. These data suggest that IRE1-mediated signaling is essential to liver injury with loss of XBP1.83
In addition to the direct effects of lipotoxic lipids, gene regulation is critical to NASH progression. Lipotoxic free fatty acid up-regulated cytoplasmic, nuclear factor of activated T cells (NFATC1) expression in a murine NAFLD model. In patient samples, NFATC1 was shown to be increased in patients with varying degrees of NASH compared with patients with early, uncomplicated steatosis.84 In lean NAFLD, patatin-like phospholipase domain containing 3 (PNPLA3) mutations are well recognized in patients. Overexpression of mutant PNPLA3 in the gold standard, DIAMOND model of NASH accelerated overall disease progression and increased the expression of the ER stress—associated genes HSPA5, ATF4, and DDIT3.85

Bile acids and sphingolipids play prominent roles in NAFLD-related ER stress.86 Meta-analysis studies have shown that primary and secondary conjugated bile acids are elevated in patients with NASH and correlated with increased NASH severity.87–89 These conjugated bile acids, especially TCA, have been shown to activate sphingosine-1-phosphate receptor 2 in cholangiocytes and promote cholangiocyte proliferation and pro-inflammatory signal activation.90 In addition, sphingolipids such as sphingosine-1-phosphate and ceramide play a significant role in ER stress induction in NAFLD. The mechanisms by which bile acids and sphingolipids contribute to fatty liver disease have been thoroughly described.86 In rare cases, patients with NAFLD develop HCC, which has also been linked to ER stress in its pathogenesis.

Dietary or caloric restriction, especially diets low in saturated fatty acids, in conjunction with exercise, remains the gold standard in treating diet-induced NAFLD.91 However, there is an imperative need to develop therapeutic agents to treat the later, irreversible stages of NASH. Resmetirom (MGL-3196; Madrigal Pharmaceuticals, West Conshohocken, PA) is a selective thyroid hormone receptor beta-selective agonist that has made a promising entrance to the pool of potential pharmacologic candidates. It will be in phase 3 clinical trials until its expected completion in 2024 (ClinicalTrials.gov, https://clinicaltrials.gov/ct2/show/NCT03900429, last accessed March 9, 2023).
NAFLD pathologic progression is regulated, in part, by cooperative ER and mitochondrial stress responses, which have been thoroughly discussed in other works. Previous studies have shown that thyroid hormone receptor beta agonism modulates mitochondrial energetics and reactive oxygen species production in HepG2 cells. Further study into the direct effects of thyroid hormone receptor beta—selective agonists on ER stress responses is needed, but it would be unsurprising if oxidative stress alleviation via thyroid hormone receptor beta agonism also lessened the cellular burden of unfolded or misfolded protein.

ER Stress in HCC Pathogenesis

HCC is one of the most common malignant solid tumors, encompassing 75% to 80% of all diagnosed liver cancers. The morbidity and mortality associated with HCC are increasing annually, becoming a worldwide public health crisis. ER stress drives HCC occurrence and progression. ER stress and UPR biomarkers have emerged as key hallmarks of hepatocarcinogenesis. Several studies suggest that major players in the UPR pathway are up-regulated in HCC and contribute to tumorigenesis and disease progression. It is well known that chronic UPR activation and ER stress in tumor cells occurs due to increased metabolic rate and mutation-driven demands for protein synthesis. ER stress in stromal cells has also been recognized as a key contributor to hepatocarcinogenesis.

Moreover, viral hepatitis, alcoholic hepatitis, metabolic disorder—related fatty liver disease, and related liver cirrhosis are considered the main factors in HCC pathogenesis and progression. For example, ER stress plays a role in the progression of viral hepatitis, and hepatitis B surface antigen can activate the UPR, which may subsequently promote apoptosis and precancerous phenotypic changes in hepatocytes. Li et al. found that the lissencephaly-1 protein, also known as platelet-activating factor acetylhydrolase IB subunit beta, is down-regulated in HCC, and reducing lissencephaly-1 protein level stimulates hepatocytes to develop from steatosis to HCC by activating ER stress. Conversely, the rapid proliferation of tumor cells is accompanied by a dramatic increase in protein synthesis, inevitably leading to UPR activation, while miRNA imbalance exists in both hepatitis and HCC. Some studies have also shown that IRE1 endoribonuclease activity is not only responsible for cleaving XBP1 but also for cleaving and regulating miRNAs. Thus, ER stress leads to miRNA dysregulation in inflammation and cancer, which in turn promotes tumorigenesis and disease progression.

Many studies thus highlight ER stress as an important factor in the process of HCC. In addition, emerging evidence suggests that chronic ER stress is directly involved in hepatocellular tumorigenesis. However, the role of ER stress in HCC development and the exact contribution of discrete UPR pathways are not fully understood. Furthermore, hepatocarcinogenesis is a complex multistep process involving the activation of multiple distinct signaling cascades; the molecular crosstalk between these factors and ER stress requires further investigation.

Future Perspectives

ER stress and the UPR play a prominent role in the pathogenesis of chronic liver diseases. Continued research focusing on the mechanisms involved in the interplay between ER stress signaling and liver disease progression is vital to developing new pharmacologic treatments. Unfortunately, there are no ongoing clinical trials directly focusing on liver disease and ER stress. A clinical trial (ClinicalTrials.gov, https://clinicaltrials.gov/ct2/show/NCT01807910, last accessed January 13, 2023) was proposed in 2013 investigating ER stress in NALFD but was withdrawn in the same year, stating a lack of funding. Research has shown clear involvement of ER stress and UPR in several chronic liver diseases, but lack of interest or funding has hindered the development of potential therapeutic agents. An increase in funding and research is needed in this field and could lead to the development of potential therapeutic agents that will help combat the progression of several CLDs.

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Author Contributions

K.G.J., M.K.L., and G.W.W. created the figures; and H.Z. oversaw all aspects of manuscript preparation. All authors wrote and edited the manuscript.

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