Endoplasmic reticulum stress in the regulation of liver diseases: Involvement of Regulated IRE1α and β-dependent decay and miRNA

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Abstract
Compromised protein folding capacity in the endoplasmic reticulum (ER) leads to a protein traffic jam that produces a toxic environment called ER stress. However, the ER smartly handles such a critical situation by activating a cascade of proteins responsible for sensing and responding to the noxious stimuli of accumulated proteins. The ER protein load is higher in secretory cells, such as liver hepatocytes, which are thus prone to stress-mediated toxicity and various diseases, including alcohol-induced liver injury, fatty liver disease, and viral hepatitis. Therefore, we discuss the molecular cues that connect ER stress to hepatic diseases. Moreover, we review the literature on ER stress-regulated miRNA in the pathogenesis of liver diseases to give a comprehensive overview of mechanistic insights connecting ER stress and miRNA in the context of liver diseases. We also discuss currently discovered regulated IRE1 dependent decay in regulation of hepatic diseases.

Introduction
The liver is the main organ for lipid metabolism and maintains whole-body lipid homeostasis. Furthermore, it is the hub for fatty acid and lipoprotein synthesis. Fatty acids mainly originate from de novo lipogenesis, triglycerides or lipoprotein remnants, adipose tissue, and cytoplasmic-triglycerol stores. Several transcription factors, including peroxisome proliferator-activated receptors (PPARs), sterol-regulatory-element-binding proteins (SREBPs), stearoyl CoA desaturase-1, and carbohydrate-responsive-element-binding protein, enhance the expression of genes involved in hepatic lipogenesis and fatty acid synthesis and oxidation. It is well known that endoplasmic reticulum (ER) stress can alter hepatic lipid metabolism and the expression of SREBP-1c and PPARα.

The ER is the entry point for the secretory pathway and a distinct metabolic compartment. Among its various physiological functions, protein synthesis and folding are the most important; its highly sophisticated quality control system allows only correctly folded proteins to leave the ER. Its Ca2+ storage ability and the oxidative environment of the ER lumen make the ER a perfect protein folding milieu and regulate various intracellular signals. The ER chaperone proteins, calreticulin, GRP78, and GRP94, stabilize protein folding intermediates in a Ca2+-dependent manner. In addition to the ER’s protein folding activity, the ER membrane works in the biosynthesis of lipids and sterols.

Proteins enter the ER in an unfolded form and get folded by burying their hydrophobic part through interactions with various molecular chaperones. However, this process can be interrupted by various extracellular and intracellular challenges, and that leads to the inflation of unprocessed protein in the ER. The accumulated proteins disrupt the interaction of chaperon protein GRP78 with the three ER transmembrane proteins, inositol requiring enzyme
l (IRE1), protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6). These proteins are called ER stress sensors, and their activity is minimized by GRP78 interaction in an unstressed ER. In the presence of an overwhelming number of unfolded proteins in the ER, GRP78 dissociation and the subsequent activation of ER stress sensors has been cumulatively called the unfolded protein response (UPR). The main aim of the UPR is to reduce stress by enhancing the protein folding rate, degrading misfolded proteins through a process called ER-associated degradation, and reducing new protein synthesis, thereby minimizing the overall stress-induced toxicity. However, persistent ER stress-mediated UPR modulates various inflammatory genes at the transcriptional level and connects ER stress with the pathogenesis of metabolic and inflammatory disorders.

Among the three UPR branches, IRE1α, a type I transmembrane protein, is evolutionarily conserved and available in both yeast and mammals. It has two mammalian homologs: one is the ubiquitin-protein, is evolutionarily conserved and available in both yeast and mammals, and the other is the intestinal-epithelium-specific IRE1β. Upon ER stress, IRE1α harbors both kinase and endoribonuclease activity, whereas role of IRE1β has not yet been clarified. Unfolded proteins in the ER impairs GRP78 and IRE1 interaction which in turn dimerizes and autophosphorylates IRE1α, followed by activation of both kinase and endoribonuclease activity of IRE1α. The cytosolic kinase domain of IRE1α recruits TRAF2 and ASK1, followed by phosphorylation of c-Jun N-terminal kinases (JNK) and subsequent activation of downstream regulators. The endoribonuclease activity of IRE1α regulates splicing of the X-box binding protein 1 (Xbp1) by deleting a 26 base-pair intron and producing a potent transcription factor, s-XBP1, followed by transcriptional activation of several genes involved in protein folding and ER-associated degradation. Furthermore, activated IRE1α reduces the ER protein load by degrading ER-associated mRNAs that encode membrane and secreted proteins (which present an extra challenge to the ER folding machinery) through a process called regulated IRE1-dependent decay (RIDD). Selective degradation of mRNA reduces new protein flow to the crowded ER and thereby minimizes the imbalance between the ER folding capacity and the burden of incoming proteins, leaving time for the ER to deal with the accumulated proteins. Interestingly, the number of RIDD target proteins is higher in Drosophila than in mammalian cells. RIDD suppresses various RNAs by 5–10 fold in Drosophila S2 cells, whereas in mammalian expression, the target genes were suppressed by twofold or less. Notably, IRE1α cleaves mRNAs encoding both secretory and ER resident proteins in mammalian cells, whereas in insect cells, only secretory proteins are targeted by RIDD. Interestingly, the kinase and nuclease properties of IRE1α are dispensable for RIDD. Furthermore, RIDD can be activated in the presence or absence of ER stress. RIDD has constitutive activity at the basal level that is augmented proportionally with the intensity and duration of ER stress. Persistent ER stress decreases the splicing of XBP1, whereas RIDD processes remain active and eventually induce apoptosis by decaying pre-microRNAs and pro-survival protein-encoding mRNAs. In mammalian cells, RIDD-targeted mRNAs have some specific criteria, such as a cleavage site with a consensus sequence (CUGCAAG) and a stem-loop structure similar to that of the IRE1-targeted CD59 and Xbp1 mRNA. RIDD activity is compromised when the stem-loop is mutated or deleted. Fifty-three percent of validated RIDD substrates are cell-death specific, whereas 21% either protect cells in the basal RIDD condition or are linked with pro-death RIDD when ER stress is persistent. Various current studies have demonstrated the physiological relevance of RIDD in several different biological models. Notably, RIDD controls the expression of lipogenic enzymes and the serum lipid level. Furthermore, RIDD protects the liver from drug-induced toxicity by degrading the mRNAs that encode enzymes involved in the metabolism of parent drugs into toxic metabolites.

Similar to IRE1, PERK is a type I ER transmembrane protein that gets phosphorylated under ER stress and then attenuates the translation of many mRNA transcripts through phosphorylation of the universal translation regulator eukaryotic initiation factor 2α (eIF2α). Phosphorylated eIF2α preferentially enhances the translation activity of select transcripts, such as ATF4. ATF4 escapes from eIF2α-mediated translation blockage by overlapping short open reading frames in the untranslated region (UTR) of ATF4 5′UTR. The transcriptional activity of ATF4 merges, paradoxically, both pro-survival and pro-death cellular programs. The pro-survival effect is mediated at least in part by stimulating the expression of genes involved in redox control and amino acid transport. Conversely, the apoptotic stimuli largely depend on the ATF4-dependent expression of CCAAT-enhancer-binding protein homologous protein (CHOP), a key pro-apoptotic transcript that in turn activates GADD34 and forms a complex with the catalytic subunit of protein phosphatase 1, which specifically dephosphorylates eIF2α, enabling the recovery of protein translation. Therefore, the expression of CHOP appears to be the key factor in the cellular commitment to apoptosis. Interestingly, it has also been suggested that eIF2α can regulate mRNA translation in a RIDD-dependent manner. However, that exact mechanism remains to be discovered.

ATF6, a type II transmembrane transcription factor, travels to the Golgi apparatus in a stress-dependent manner: the N terminal portion of ATF6 is liberated by two Golgi resident enzymes S1P and S2P, and thereby nuclear accumulation of the N-terminal transcriptional regulatory domain of ATF6 (Fig. 1). ATF6 transcriptionally upregulates various genes that encode proteins involved in ER chaperone activity, reducing reactive oxygen species and amino acid metabolism. Furthermore, the expression of XBP1 is also enhanced by the transcriptional activity of ATF6 and is followed by splicing via the nuclelease activity of IRE1α. Interestingly, XBP1 and ATF6 are both necessary for hepatocyte-specific apolipoprotein B secretion and the subsequent activation of the fatty acid oxidation pathways (PPARα and PGC1α) under ER-stress conditions. In this way, XBP1 and ATF6 participate in the reduction of severe ER-stress-associated lipogenesis and fatty liver disease. In contrast, ATF6 interacts with SREBP2, which recruits HDAC1 to the complex, which in turn negatively regulates the lipogenic function of SREBP2.

Depletion of IRE1/XBP1 liver phenotype

The genetic ablation of IRE1α or XBP1 is embryonic lethal, mainly because of the severe anemia associated with depleted hematopoiesis and hypoplastic fetal livers. XBP1 deficiency leads to reduced hepatocyte development and enhanced apoptosis. XBP1, a liver-specific XBP-1 target gene, was identified as a regulator of
develops a hypo-lipogenic phenotype; IRE1α, XBP1 in hepatocytes. Furthermore, liver-specific Xbp1 deletion can rescue the embryonic lethality associated with expression of a transgene encoding spliced XBP1 can rescue the embryonic lethality is caused by a requirement for spliced α expression is compromised. In addition, hepatocyte-specific expression of a transgene encoding spliced XBP1 can rescue the embryonic lethality associated with Xbp1 deletion, suggesting that the embryonic lethality is caused by a requirement for spliced XBP1 in hepatocytes. Furthermore, liver-specific Xbp1 deletion develops a hypo-lipogenic phenotype; IRE1α knockdown can rescue that phenotype. Interestingly, liver-specific knockdown of Ire1α (Ire1α<sup>Hepfe<sup>-/-</sup></sup>) is non-lethal and causes no gross morphological or developmental defects. However, rough ER content is less in Ire1α<sup>Hepfe<sup>-/-</sup></sup> livers than in littermate control (Ire1α<sup>Hepfe<sup>+/+</sup></sup>) livers. More specifically, hepatocyte-specific deletion of Ire1α causes compromised ER-to-Golgi protein transport via the repression of genes encoding the functions in this transport system.

Ire1α-null mice in stress free condition experience modest hepatosteatosis, and the condition is exacerbated following ER stress. This could be caused by the decreased expression of metabolic transcriptional regulators such as C/EBPβ, C/EBPδ, PPARγ, and triglyceride-biosynthesis-associated enzymes. In other words, those proteins could potentially be regulated by Ire1α and its downstream regulators, thereby maintaining ER homeostasis following ER stress. Strikingly, Ire1α plays a crucial role in liver regeneration following damage caused by various toxic agents. Mice with CCl4-induced liver injury or partial hepatectomy show exacerbated liver injury condition and impaired proliferation of hepatocytes when Ire1α is knocked out. Conversely, the regenerative proliferation of hepatocytes is higher in wild type mice. However, no one has yet identified how Ire1α regulates liver regeneration.

Depletion of PERK/eIF2α/ATF4 and liver phenotypes

Liver-specific knockdown of PERK deregulates both the transcriptional and translational phases of the UPR, which in turn reduces the expression of chaperone proteins, disrupts lipid metabolism, and increases apoptosis. Furthermore, PERK knockdown impedes the perpetuated expression of the lipogenic enzyme fatty acid synthase, hepatic stearyl-CoA desaturase-1 (SCD1), and adenosine triphosphate (ATP)-citrate lyase. In vivo, eIF2α phosphorylation seems to be the key regulator for proper functioning of the liver to maintain glucose homeostasis. eIF2α-Null mice undergo postnatal death within 18 hr of birth, probably because of the severe hypoglycemia associated with defective gluconeogenesis. Impaired gluconeogenesis could be the consequence of repressed expression of several liver-specific rate-limiting enzymes, including phosphoenolpyruvate carboxykinase, which...
is essential for gluconeogenesis in the liver. At the end stage of gestation, enhanced glycogen synthase expression in the liver is essential for the storage of maternal glucose as glycogen for survival of neonates just after birth, when they have no other source of energy. However, mutated eIf2α Ser51A, unable to get phosphorylated in the liver, cannot synthesize glycogen or use high glycogen. Reduced eIf2α phosphorylation causes decreased expression of the transcription factor CCAAT/enhancer-binding proteins α and β (C/EBPα, C/EBPβ) and its downstream regulator, the adipogenic nuclear receptor PPARγ2 (Fig. 1). In addition, severe osteopenia and spinal curvature, skeletal dysplasia, and compromised locomotor activity are also consequences of in vivo eif2α deletion. Furthermore, eif2α knockdown results in aberrant ER stress.29

Atf4 knockout mice are neither embryonic lethal nor vulnerable to postnatal death.18 In fact, ATF4 deficiency in mice protects against fructose-induced hypertriglyceridemia by attenuating lipogenesis in the liver.28 Thus, ATF4 could be a potential regulator of hepatic lipid metabolism. Similarly, ATF4 knockout heightens high-carbohydrate diet (HCD)-induced hepatic steatosis condition through inhibition of triacylglycerol accumulation in livers. HCD-induced SCD1 expression is downregulated in ATF4-deficient mice, thereby reducing carbohydrate-induced adiposity and hepatic steatosis.29 Taken together, these studies suggest that ATF4 could be the main culprit in HCD-induced hepatic steatosis, caused at least in part by suppression of SCD1 expression. Moreover, liver tissues from Atf4-null mice have reduced amino acid levels, suggesting that amino acid metabolism is altered in the Atf4 knockout condition. The activity of the amino acid sensing protein mTOR and its downstream regulator S6 k are also repressed in the livers of Atf4-null mice.30

**Regulated IRE1α and β-dependent decay in liver disease**

Several current studies indicate that RIDD plays significant physiological roles in various biological models of specific cellular and developmental conditions.9 RIDD targets various liver-specific mRNAs and protects hepatocytes from metabolic and drug-induced toxicity. So, JS and colleagues demonstrated that XBP1 knockdown provokes feedback stimulation of its upstream regulator, IRE1α, thereby enhancing the degradation (through RIDD activity) of various cytosolic mRNAs responsible for lipoprotein metabolism and lipogenesis.22 In that process, IRE1 protects against liver damage and ameliorates hepatosteatosis and hypercholesterolemia in an XBP1 knockout dyslipidemic animal model. Thus, the splicing of XBP1, which functions as a prolipogenic transcription factor, has a profound effect on lipid metabolism.22 Furthermore, RNA interference–mediated repression of RIDD or siRNA-mediated knockdown of IRE1α provokes hyperlipidemia in XBP1-deficient mice.22 A comprehensive microarray analysis revealed that a group of genes responsible for lipogenesis are regulated by RIDD, including Acacb, Angptl3, Ces1, Dagat2, and Pcsk9 (Fig. 2). Among those lipogenic enzymes, Dagat2 and Acacb, Pcsk9 are involved in low density lipoprotein clearance, Angptl3 suppresses lipoprotein lipase-mediated triglyceride clearance, and Ces1 possesses triglyceride hydrolyzing activity, and the Ces1 is implicated in fatty acid mobilization from lipid droplets to nascent very low density lipoprotein.

Hence, suppression of those genes by IRE1α is likely to contribute to the striking hyperlipidemic phenotype mediated by liver-specific loss of XBP1. IRE1α deletion in Xbp-1 deficient mice causes upregulation of those genes, which are substrates of RIDD.

Hepatic lipid metabolism, in the absence of Xbp-1, seems to be regulated by two distinct mechanisms of IRE1α. Primarily, IRE1α enhances mRNA decay of proteins essential for lipid metabolism, such as Acacb, Angptl3, Ces1, Dagat2, and Pcsk9, through RIDD activity. Additionally, IRE1α-dependent splicing of XBP1 triggers upregulation of the genes for certain lipogenic enzymes. Thus, reduced plasma lipid accumulation in XBP1-null mice is the integrated effect of XBP1 deficiency and IRE1α hyperactivation. Moreover, XBP1-mediated expression of lipogenic genes and IRE1α-mediated mRNA degradation are impaired in IRE1α-null mice.22 Interestingly, IRE1β represses protein translation by cleaving 28S ribosomal RNA under ER stress condition.31 IRE1β has an indirect effect on hepatic cholesterol. IRE1β-knockout mice showed an approximately 30% elevation in hepatic cholesterol, along with a 50% increase in plasma. This might be due to the enhanced lipid absorption from intestine and the delivery of absorbed dietary cholesterol and fatty acids primarily to the liver followed by other peripheral tissues. High-fat or high-cholesterol-induced hyperlipidemia is more pronounced in Ire1β−/− mice.37 Furthermore, IRE1β mRNA level is decreased following high-cholesterol and high-fat diets fed wild type mice. Mechanistically, IRE1β reduces intestinal lipid absorption in high fat or high cholesterol diet mice.32 Furthermore, the ER chaperone microsomal triglyceride transfer protein, which is essential for the assembly of apolipoprotein B (Apo B) and chylomicrons biosynthesis, is mainly post-transcriptionally degraded by Ire1β.32 Hence, the term “regulated IRE1β-dependent decay (RIDD for mRNA)” has also been suggested by current studies. It is notable that Xbp-1 knockdown leads to hypolipidemia, whereas Ire1β knockout results in hyperlipidemia. Thus, the absence of Ire1β could have a negative role in Ire1α-dependent mRNA decay, or Ire1α and β could work together in mRNA degradation. This notion is further supported by Y. Imagawa, et al., who showed that Ire1β is 10 times more enzymatically active than IRE1α in RIDD reactions. However, IRE1β is not ubiquitously expressed, and its action is organ specific. Thus it can be assumed that IRE1β functions in association with IRE1α in the intestine and regulates mRNA decay there and that it could have a secondary role in hepatic lipid metabolism. IRE1β-dependent RIDD activity plays a crucial role in maintaining the normal function of intestinal goblet cells by negatively regulating ER stress.33 Contrarily, IRE1β−/− null mice showed elevated Xbp-1 splicing in mucin-secreting goblet cells, accompanied by ER extension and increased ER stress.33 Moreover, mucin secretion in the lungs is Ire1α dependent, and that UPR pathway can be activated in that specific cell type following Ire1β knockdown.

Current evidence suggests that RIDD is associated with inflammatory responses through activation of retinoic acid-inducible gene 1 (RIG-I).34 RIG-I is a cytosolic pathogen recognition receptor that initiates immune responses against microbe-associated stress. It is one of the major pathogen recognition receptors within hepatocytes. Upon viral RNA recognition, RIG-I is redistributed from the cytosol to membranes where it binds the mitochondrial antiviral-signaling protein adaptor protein.35 RIG-1,
in association with IRF-3 and NF-κB, initiates a host defense mechanism that limits hepatitis C virus (HCV) RNA replication. Mechanistically, RIG-1 facilitates the phosphorylation of IRF-3 and subsequent activation of NF-κB by degradation of IkB. Notably, inflammation is one of the main causes of liver disease. HCV, master culprit for liver fibrosis, encodes the NS3/4A protease, which directs lysosomal degradation of RIG-1 and thereby evades RIG-1-mediated signaling. HCV infection triggers ER stress, but interestingly does not induce UPR-responsive genes. However, the genes involved in liver proliferation, inflammation, and apoptosis are drastically induced. Furthermore, certain non-structural proteins of HCV stimulate IRE1 and ATF6 to help the HCV sub-replicon and HCV viral replication. In other words, HCV infection selectively induces the cell death program by bypassing UPR-mediated liver homeostasis, and HCV manipulates the UPR to benefit its own replication and survival. Following ER stress, mRNAs are cleaved into single-strand fragments through RIDD activity. Single-strand mRNAs lack a self-recognition motif, and that can activate RIG-I (Fig. 2). However, RIDD activity has not been studied in HCV-infected livers. It can be assumed that forced activation of RIDD in HCV-infected livers could stimulate a RIG-I-mediated inflammatory response, disrupting HCV’s innate defenses and heightens the disease condition.

Regulated IRE1-dependent decay seems to play crucial role in liver protection from drug-induced toxicity. The liver is the main route of metabolism for most drugs because it contains almost all types of drug metabolizing enzymes. However, persistent exposure or overdose can cause accumulation of toxic metabolites and thereby trigger drug-induced liver toxicity. For instance, an
overdose of N-acetyl-p-aminophenol (APAP) causes acute liver failure by the production of hepatotoxic metabolites. Several drug-metabolizing enzymes in the liver, including CYP1A2, CYP2E1, and CYP3A4, participate in APAP-induced liver toxicity by converting the APAP into toxic metabolites. Interestingly, genetic ablation of XBP1 protects hepatocytes from APAP-induced toxicity. Mechanistically, XBP1 deletion enhances IRE1 activity through a negative feedback mechanism that in turn degrades (through RIDD) the mRNAs encoding the Cyp1a2 and Cyp2e1 genes and reduces JNK phosphorylation. Furthermore, pharmacological ER stress inducers activate IRE1α and suppress the expression of Cyp1a2 and Cyp2e1 in WT but not in IRE1α-deficient mouse livers, indicating the essential role of IRE1α in the downregulation of these mRNAs upon ER stress. The kinase activity of IRE1 facilitates JNK phosphorylation by recruiting TRAF2 and ASK1. On the other hand, JNK activity is compromised when Cyp1a2 and Cyp2e1 enzymes are degraded through RIDD in the liver. However, it remains to be determined how Cyp1a2 and Cyp2e1 reduce JNK activation. IRE1 itself is its own substrate, and there is no other substrate. Hence, the possibility that RIDD interferes with the kinase activity of IRE1 is dispensable; RIDD inhibits JNK phosphorylation without affecting the kinase activity of IRE1. Thus, different activating stimuli can trigger different IRE1 outputs and customize IRE1α’s biological functions.

ER-stress-regulated miRNA in the pathogenesis of liver diseases

MicroRNAs (miRNAs), non-protein coding genes of about 22 nucleotides in duplex RNAs, are generated from intronic or intergenic transcripts and further processed by the RNase III enzyme Drosha in the nucleus to form a precursor microRNA (pre-microRNA). Subsequent transport of pre-microRNA to the cytoplasm is mediated by exportin-5, which allows it to mature through further processing by a multi-protein complex composed of the RNase III enzyme Dicer, the Argonaute protein family (such as Ago1–4 or Ago2), and trans-activation-responsive RNA-binding protein. Single-stranded mature miRNAs are then integrated into the RNA-induced silencing complex, which leads to gene silencing or mRNA transcript degradation by binding to the 3’UTR (and in some cases, the coding sequence) of the targeted mRNA molecule. MiRNAs regulate various developmental and physiological processes by controlling the levels of specific mRNAs. Therefore, tight regulation of the expression and processing of miRNAs is essential for normal cell function. The significance of miRNAs in liver development has not been well studied; nevertheless, the existing evidence suggests that cell lineages and differentiation are likely regulated by these small molecules. Mice with a lack of functional Dicer1 in hepatocytes show normal liver physiology and hepatic function. However, chronic miRNA deficiency causes portal inflammation, hepatocyte regeneration, and hepatocyte apoptosis, processes associated with chronic liver disease. Still, evidence for the involvement of miRNA in ER stress–associated liver disease is limited.

Among the huge number of miRNAs, miR-122, a negative regulator of the UPR pathway, is most abundant in the liver, where it regulates enzymes involved in cholesterol and lipid-metabolism and plays a substantial role in liver homeostasis. Through functional interaction with the HCV genome, miR-122 regulates HCV translation and subsequent stabilization. Therefore, miR-122 facilitates disease progression. Conversely, miR-122 suppresses hepatocellular carcinoma (HCC) by repressing UPR pathway activation (Table 1). UPR activation is protective for HCC cells under chemotherapy-mediated stress.

Table 1  List of miRNAs involved in ER stress-mediated liver disease

| Name   | Function and mechanism                                                                 | ER stress regulation                        |
|--------|---------------------------------------------------------------------------------------|---------------------------------------------|
| miR-33 | - Chronic inhibition of miR-33 leads to the development of hepatic steatosis and hypertriglyceridemia | miR-33 expression remains unchanged following thapsigargin treatment |
| miR-34a| - Involved in NAFLD and ALD                                                            | Degraded under ER stress by RIDD activity of IRE1 |
| miR-122| - Regulates cholesterol and lipid-metabolizing enzymes                                 | A negative regulator of the UPR pathway      |
|        | - Regulates HCV replication                                                             |                                             |
|        | - Involved in NAFLD                                                                    |                                             |
| miR-181| - Involved in the regulation of liver organogenesis                                    | Negatively regulates ER chaperone protein GRP78 |
|        | by degrading GATA6, a transcription factor essential for liver organogenesis–encoding mRNA |                                             |
| miR-199a-5p| - miR-199a-5p protects hepatocytes from bile acid–triggered prolonged ER stress     | Reduces ER stress by directly repressing the 3’UTRs of mRNAs for IRE1, GRP78, and ATF6 |
| miR-150| - Prevents hepatic fibrosis by reducing cMyb and αSMA levels                           | IRE1 cleaves miR-150                        |
| miR-214| - Downregulates HCC                                                                    | Represses pro-survival factor sXBP-1 and inhibits tumor formation |
| miR-221/222| - Upregulated in HCC                                                                  | Undermines ER stress and thereby reduces ER stress–associated apoptosis in HCC model |
| miR-1291| - Enhances accumulation of GPC3 in Huh7 cells                                         | Directly binds with 5’UTR of IRE1 and silences its activation, thereby accumulating GPC3 in HCC |

αSMA, alpha-smooth muscle actin; ATF6, activating transcription factor 6; ER, endoplasmic reticulum; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; NAFLD, Nonalcoholic Fatty liver disease; RIDD, regulated IRE1 dependent decay; UPR, unfolded protein response.
and cholesterol metabolism. Similarly, tunicamycin-induced 7alpha-hydroxylase by posttranscriptional degradation in human with miR-422 inhibits the bile-acid synthesis regulator cholesterol

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thapsigargin or bile acid treatment. Furthermore, bile-duct ligation in an animal model causes an increased level of miR-199a-5p, which in turn reduces ER stress by directly repressing the unfolded protein response in hepatocellular carcinoma (HCC), thereby enhancing cancer progression. Similarly, miR-199a-5p is most abundant in hepatocytes. During bile duct ligation or pharmacologically induced ER stress, the expression of miR-199a-5 is enhanced, and those miRNAs bind with the 3′UTRs of the mRNAs for IRE1, GRP78, and ATF6, degrading them. miR-1291 directly silences IRE1 expression through 5′UTR binding in HCC. Pro-oncogenic protein glypican-3 (GPC3) is degraded by IRE1α and accumulates in HuH7 cells because of the miR-1291-mediated degradation of IRE1α. Thapsigargin treatment of HCC reduces miR-221/222 expression. CHOP downregulation can prevent the attenuation of miR-221/222 following ER stress. The miR199a/miR214 cluster is implicated in XBP1 downregulation in HCC. IRE1α is involved in degrading miR-34 and miR-150 through regulated IRE1-dependent decay (RIDD) activity. RIDD-dependent degradation of miR-150 enhances the expression of cMyb, which in turn inhibits the activation of myofibroblasts. Myofibroblasts are notorious in the progression of liver fibrosis. PERK, PKR-like endoplasmic reticulum kinase. [Color figure can be viewed at wileyonlinelibrary.com]

miR-122 and the UPR markers GRP78, XBP1, and CHOP are upregulated in many HCC tissues compared with normal liver tissues, suggesting that miR-221/222 is associated with the UPR in HCC patients. Similarly, downregulation of miR-221/222 is protective against ER stress-induced apoptosis in HCC through the accumulation of p27kip, which promotes G1 arrest.63 In clinical HCC samples, miR-221 mimics accelerated cell growth, and miR-221 over-expression is related to the status of tumor capsular infiltration.64 Therefore, inhibition of miR-221 could be a potential therapeutic strategy to suppress proliferation and induce apoptosis in HCC cells. Furthermore, CHOP suppression can sustain the miR-221/222 level in HCC cells following thapsigargin treatment, suggesting a role for this UPR transcription factor.

Hepatocyte nuclear factor (HNF) 4α, a liver-specific transcription factor essential for liver development, is negatively regulated by miR-24 and miR-34. Similarly, XBP-1 regulates hepatocyte growth by upregulating αFP. Furthermore, IRE1 promotes liver regeneration by sustaining phosphorylated STAT3. Thus, it is obvious that XBP-1 and HNF4α have interrelated functions. Supporting this notion, Benjamin D. Moore and colleagues demonstrated that XBP-1 is directly upregulated by HNF4α in pancreatic β-cells. HNF4α depletion is associated with reduced glucose stimulated insulin secretion.65 XBP-1 overexpression can rescue glucose stimulated insulin secretion in HNF4α-depleted β-cells.66 Similarly, tunicamycin-induced ER stress elevates expression of miR-34 in chondrocytes, which in turn leads to apoptosis.

Figure 3 Speculated diagram depicting the involvement of miRNA in the regulation of endoplasmic reticulum (ER) stress–mediated liver diseases. Many miRNAs are involved in liver disease and ER stress. However, only a few of them have so far been found to be directly involved in ER stress–associated liver disease. miR-122 is highly expressed in the liver and represses the unfolded protein response in hepatocellular carcinoma (HCC), thereby enhancing cancer progression. Similarly, miR-199a-5p is most abundant in hepatocytes. During bile duct ligation or pharmacologically induced ER stress, the expression of miR-199a-5 is enhanced, and those miRNAs bind with the 3′UTRs of the mRNAs for IRE1, GRP78, and ATF6, degrading them. miR-1291 directly silences IRE1 expression through 5′UTR binding in HCC. Pro-oncogenic protein glypican-3 (GPC3) is degraded by IRE1α and accumulates in HuH7 cells because of the miR-1291-mediated degradation of IRE1α. Thapsigargin treatment of HCC reduces miR-221/222 expression. CHOP downregulation can prevent the attenuation of miR-221/222 following ER stress. The miR199a/miR214 cluster is implicated in XBP1 downregulation in HCC. IRE1α is involved in degrading miR-34 and miR-150 through regulated IRE1-dependent decay (RIDD) activity. RIDD-dependent degradation of miR-150 enhances the expression of cMyb, which in turn inhibits the activation of myofibroblasts. Myofibroblasts are notorious in the progression of liver fibrosis. PERK, PKR-like endoplasmic reticulum kinase. [Color figure can be viewed at wileyonlinelibrary.com]
Role of endoplasmic reticulum stress signaling in hepatitis B virus-mediated pathogenesis

The hepatitis B virus X (HBx) protein, transcriptional activators of hepatitis B virus (HBV) gene products, and Pre-S mutant large HBV surface antigens induce ER stress. Pre-S mutant proteins accumulate in the ER and HBx reduces cellular ATP level by activating eIF2α/ATF4 arm of UPR and subsequent reduction of cellular ATP level. Moreover, ATF4 directly binds to the cyclooxygenase-2 (COX-2) promoter. ER stress, following the accumulation of Pre-S mutant, leads to the genomic instability through oxidative stress, DNA damage and frequent mutation. In one hand, this retention of mutant Pre-S can cause ER stress mediated hepatocellular apoptosis. On the other hand, COX-2 and cyclin A can be upregulated to induce cell cycle progression and proliferation of hepatocytes. The proliferating cells may progress to tumor formation because of the DNA damage and genomic instability.

Hepatocellular carcinoma can be developed under persistent infection by HBV. miRNAs are implicated in the HBV infection and the development of the HBV-related diseases. HBV can modulate the expression of several cellular miRNAs in order to promote a favorable environment for its replication and survival. HBV gene expression and replication is negatively regulated by miR-122 that directly binds to a highly conserved sequence of HBV. Furthermore, HBx protein is also involved in miR-122 dysregulation. However, the role of miR-122 in HBV replication is enigmatic. It may either promote or inhibit HBV replication. Conversely, the miR-17-92 cluster is associated with liver oncogenesis.

Conclusion and future directions

Endoplasmic reticulum stress is associated with a series of hepatic diseases. Here, we have discussed a few of them, integrating miRNAs and IRE1α-dependent decay into ER stress–associated liver diseases. Liver disease can develop through many molecular pathways. ER stress is a vital one of them. The current trend is to study miRNAs and use them as biomarkers to diagnose diseases and develop therapeutic interventions that use their mimics and antagonists. miRNAs have recently been implicated in liver disease; some of them can protect the liver from damage, and others aggravate disease conditions. Despite comprehensive studies, the exact mechanisms of miRNAs in the regulation of liver disease progression and prevention remain to be determined. Furthermore, no global studies have screened for miRNAs in response to various hepatic diseases. Most studies we have emphasized here are individual ones. Despite their huge scope, studies on how miRNAs are regulated by ER stress in liver diseases are limited. Thus, researchers who focus on ER stress–regulated miRNAs might discover potential therapeutic strategies for treating liver diseases. The duration of stress is the main determinant of cell fate. Therefore, researchers should determine which miRNAs are activated during short-term stress and which are specific to persistent ER stress. Therapeutic strategies based on the duration of stress and the upregulation or downregulation of miRNAs in that particular timeframe could be crucial to protecting the liver from ER stress-mediated toxicity. However, creating such therapies has not been easy, partly because of their cell type and their specific activity and wide range of targets. The efficacy of miRNA-based therapies in the prevention of HCC has been tested in recently using several approaches involving miRNA repression or replacement.

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