The Role of Endoplasmic Reticulum Stress Response in Liver Regeneration

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Semin Liver Dis

Graphical Abstract

Partial hepatectomy and toxicant exposure can activate endoplasmic reticulum stress which affect liver regeneration.

A. Inhibiting ER stress response after partial hepatectomy-induced liver damage promotes liver regeneration.

B. Inhibiting ER stress response after chemical induced liver damage impairs liver regeneration.
Abstract
Exposure to hepatotoxic chemicals is involved in liver disease–related morbidity and mortality worldwide. The liver responds to damage by triggering compensatory hepatic regeneration. Physical agent or chemical-induced liver damage disrupts hepatocyte proteostasis, including endoplasmic reticulum (ER) homeostasis. Post–liver injury ER experiences a homeostatic imbalance, followed by active ER stress response signaling. Activated ER stress response causes selective upregulation of stress response genes and downregulation of many hepatocyte genes. Acetaminophen overdose, carbon tetrachloride, acute and chronic alcohol exposure, and physical injury activate the ER stress response, but details about the cellular consequences of the ER stress response on liver regeneration remain unclear. The current data indicate that inhibiting the ER stress response after partial hepatectomy–induced liver damage promotes liver regeneration, whereas inhibiting the ER stress response after chemical-induced hepatotoxicity impairs liver regeneration. This review summarizes key findings and emphasizes the knowledge gaps in the role of ER stress in injury and regeneration.

Keywords
- liver regeneration
- ER stress response
- unfolded protein response
- liver injury
- chemical-induced liver injury
- partial hepatectomy

Lay Summary
Liver injury induced by chemicals is a global and common problem. The liver is vulnerable to damage because of its central role in chemical detoxification. Following injury, the liver tries to repair itself by regenerating the damaged portion. The endoplasmic reticulum (ER) is part of cells that is necessary for normal functioning if cells including those in the liver. During liver injury, ER activates a stress response that affects the expression of genes, affecting how well the liver can regenerate. Various drugs like acetaminophen, alcohol, and even physical injuries can activate ER stress. A limitation in our understanding is how ER stress plays a role during liver regeneration. Studies suggest blocking the ER stress response can help the liver heal after a physical injury. In the case of chemical injuries, blocking the stress response can worsen the outcome. This review highlights the role of ER stress response in liver regeneration and highlights potential lines of future investigation.

The liver is one of the largest organs in human body and performs several important functions including macronutrient metabolism, detoxification of xenobiotics, bile acid synthesis, and lipid and cholesterol metabolism. The liver is anatomically divided into four lobes and functionally into eight by the portal vein and hepatic artery. It is made up of different cell types including approximately 60% hepatocytes and approximately 40% nonparenchymal cells, which include cholangiocytes, hepatic stellate cells (HSCs), Kupffer cells, and liver sinusoidal endothelial cells. Hepatocytes are the workhorse of the liver and perform all the major liver functions described earlier. The anatomical and physiological arrangement of the liver exposes it to significant concentrations of toxicants and infectious agents which can result in hepatocyte damage and liver injury. However, the liver has a remarkable capacity to undergo regeneration to refurbish the damaged liver and regain normal function.

Maintenance of cellular protein homeostasis (proteostasis) is a key to cellular health. To maintain cellular proteostasis, cells employ various mechanisms to control protein synthesis, folding, intracellular trafficking, and compartmentalization along with regulated protein degradation. The endoplasmic reticulum (ER) plays a critical role in regulating proteostasis, protein synthesis, and protein trafficking. An imbalance between newly synthesized polypeptide chains entering the ER and folded proteins exiting the ER can result in the accumulation of unfolded or misfolded proteins in the ER. This accumulation of unfolded proteins can trigger an ER stress response—unfolded protein response (UPR). The severity and duration of stress experienced by the cells guide whether the cells will adapt to the stress or undergo cell death.

Liver injury caused by hepatotoxicant exposure or by physical damage as in partial hepatectomy (PH) is associated with increased histological damage and necrosis. This damage activates proliferation in the remnant–uninjured hepatocytes to compensate for and restore the lost liver tissue. Hepatocytes are under an active state of proliferation during liver regeneration which is associated with an increase in cellular protein synthesis. It is likely that the rise in protein synthesis during liver regeneration can result in the accumulation of unfolded proteins in the ER lumen which can activate hepatocyte UPR. In hepatocytes, many genes are repressed due to activated ER stress response signaling, whereas expression of stress response genes, which support cell survival during stress conditions are selectively upregulated. The cellular and hepatological effects of ER stress on liver regeneration is an underexplored area of research. In this review, we attempt to summarize the key findings and highlight the knowledge gaps in our understanding of the role ER stress plays in liver regeneration. Although a large part of our discussion...
focuses on the role of ER stress response in liver parenchymal cells, readers are encouraged to visit Maiers and Malhi\textsuperscript{15} for a thorough discussion on the role of ER stress in HSCs and Zhou et al\textsuperscript{16} for a discussion on the role of ER stress response in Kupffer cells. Finally, since chronic liver injury and constitutively active ER stress is associated with cellular apoptosis, readers are directed to visit the recent review by Zhang et al\textsuperscript{17} on ER stress-mediated cell death in conditions of liver injury.

**Mechanistic Insights into ER Stress Response**

The ER provides conditions that support protein folding, which is monitored by the members of ER protein folding machinery which ensures proteins are properly folded and packaged in ER exit vesicles. When unfolded proteins accumulate in the ER lumen, the ER protein folding machinery activates the UPR. The active UPR also augments the targeted degradation of unfolded proteins through ER-associated degradation (ERAD).\textsuperscript{18,19} UPR functions by relaying the information of unfolded proteins to the cell by three transmembrane protein sensors: protein kinase R-like endoplasmic reticulum kinase (PERK) encoded by eukaryotic translation initiation factor 2 alpha kinase 3 (EIF2AK3); inositol-requiring enzyme 1 (IRE1\textalpha) encoded by endoplasmic reticulum to nucleus signaling 1 (ERN1), and activating transcription factor 6 (ATF6).\textsuperscript{10} Each of these protein sensors has a unique mechanism of propagating the ER stress response (\textcircled{1}).

Under normal physiological conditions, these UPR sensors are inactive and bound to binding immunoglobulin protein (BiP), a member of the heat shock protein family, on their ER luminal domains. Accumulation of unfolded proteins in the ER lumen beyond a threshold dissociates BiP from the luminal side of the ER stress sensors activating them to trigger ER stress response.\textsuperscript{20,21} An alternative mechanism proposed for BiP dissociation involves activation of UPR sensors by direct binding of unfolded proteins to their luminal domains and dissociating BiP.\textsuperscript{22,23} UPR activation has significant cellular consequences including transcriptional reprogramming, translation inhibition, selective translation of stress response genes, and depending upon the severity and duration of stress, cell death.\textsuperscript{10,11,24}

**ATF6**

ATF6 is an ER membrane-localized transcription factor bound to BiP under inactive conditions.\textsuperscript{10} ATF6\textalpha functions as a transcriptional activator, while ATF6\textbeta has not been shown to have an effect on the ATF6\textalpha-mediated gene expression.\textsuperscript{25} Accumulation of unfolded proteins activates ATF6 by dissociation of BiP from its ER luminal domain translocating ATF6 from ER membrane to Golgi.\textsuperscript{26} Two Golgi proteases S1P and S2P (site-1 and site-2 proteases) cleave the translocated ATF6 at two sites liberating its N-terminal domain to migrate into the nucleus and activating UPR response genes (\textcircled{1A}).\textsuperscript{27,28} Golgi proteases used in the processing of ATF6 are also used by the liver in lipid metabolism and processing of sterol response element binding proteins (SREBPs).\textsuperscript{28–30} Although important in proteotoxic stress response, ATF6 is also activated by sphingolipids which trigger ER lipid biosynthetic genes through mechanisms distinct from the proteotoxic stress response.\textsuperscript{33} In the liver, ATF6 activation has been seen to play a role in hepatocarcinogenesis and liver regeneration.\textsuperscript{32,33}

**IRE1**

IRE1 is a bifunctional transmembrane protein with kinase and endoribonuclease activities.\textsuperscript{34,35} Under inactive conditions, its monomeric form is bound to BiP on its ER luminal domain.\textsuperscript{21} Unfolded protein accumulation in the ER lumen dissociates BiP from IRE1’s ER luminal domain allowing the oligomerization of IRE1 followed by trans-autophosphorylation which activates the protein.\textsuperscript{36} The now active IRE1 acts as an endoribonuclease, splicing the UPR-specific transcription factor X-box binding protein 1 (XBP1) mRNA, which is followed by ligation of 5’ and 3’ ends of spliced mRNA (\textcircled{1B}).\textsuperscript{37,38} In metazoans, both precursor and spliced XBP1 mRNAs upon translation have different functional properties. Precursor mRNAs encode a protein that represses the expression of UPR target genes, whereas the protein produced from spliced mRNA acts as a potent activator of UPR target genes.\textsuperscript{39} This newly spliced XBP1 mRNA on translation acts as a transcription activator for UPR response genes associated with ER chaperone and ER secretory genes along with genes for ERAD. In addition to activating UPR target genes, IRE1 plays an important role in mediating the death of ER-stressed cells by recruiting tumor necrosis factor receptor (TRAF) and activating Jun N-terminal kinase (JNK) while also interacting with other components of cell death machinery like caspase-12.\textsuperscript{40} It has been seen that activated IRE1 can degrade nonessential mRNAs in a process called regulated IRE1\textalpha dependent decay (RIDD).\textsuperscript{41–43} RIDD has been noted to be associated with cell survival and cell death in nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH).\textsuperscript{44} Transport and Golgi organization (TANGO1) is an ER membrane exit site resident protein required for the secretion of collagen and expansion of transport vesicles to accommodate large cargo from the ER.\textsuperscript{45} TANGO1 is one of the downstream targets of the spliced XBP1. Under ER stress conditions, spliced XBP1 upregulates TANGO1 expression in the liver resulting in liver fibrosis. Experiments with the abrogation of TANGO1 or chronic unresolved ER stress have resulted in apoptosis.\textsuperscript{46} These observations suggest that ER stress plays an active role in liver fibrosis.

**PERK**

PERK is a transmembrane protein kinase which inhibits protein synthesis under stress conditions. It is an ER resident transmembrane protein kinase with ER luminal domain similar to IRE1 and a cytoplasmic kinase domain.\textsuperscript{47} Under normal physiological conditions the ER luminal domain of PERK is bound to BiP. With the accumulation of unfolded proteins in the ER lumen, PERK is activated by oligomerization and trans-autophosphorylation and acts as a kinase phosphorylating Ser51 residue on the alpha subunit of eukaryotic translation initiation factor 2 (eIF2) (\textcircled{1C}). eIF2 is an
Fig. 1 Summary of three branches of ER stress response signaling pathways. On accumulation of unfolded proteins in the ER lumen, BiP dissociates from the ER luminal domain of three ER membrane-localized sensors: ATF6, IRE1, and PERK resulting in their activation. (A) BiP-dissociated ATF6 translocates to the Golgi where it is activated by proteolytic cleavage and the activated ATF6 travels to the nucleus and induces expression of downstream ER stress response genes. (B) PERK is activated by oligomerization and trans-autophosphorylation upon BiP dissociation. Active PERK phosphorylates eIF2α resulting in inhibition of protein synthesis and selective translation of ATF4 which induces ER stress response genes. Phosphorylated eIF2α is dephosphorylated by CREP and GADD34 phosphatases. (C) IRE1 is activated by oligomerization and trans-autophosphorylation which selectively affect mRNA splicing. IRE1-mediated splicing activates XBP1 which induces the expression of ER stress response genes. Both spliced and precursor mRNAs are expressed in cells; spliced XBP1 acts as an activator of UPR target genes while its unspliced precursor represses UPR gene expression.
important translation initiation factor required for the delivery of initiating tRNA (Met-tRNAi) to the translation initiation complex. Eukaryotic translation initiation factor 2B (eIF2B) is a guanine exchange factor for eIF2. Phosphorylated eIF2\(\alpha\) acts as an inhibitor by binding to eIF2B irreversibly and not undergoing guanine exchange causing a drop in the available active pools of eIF2 ultimately resulting in inhibition of translation.\(^{48}\) Such conditions of translation inhibition due to phosphorylated eIF2\(\alpha\) selectively favor the translation of stress response genes like activating transcription factor 4 (ATF4; \(\rightarrow\) Fig. 1C) which activates yet another set of stress response genes including inducible eIF2\(\alpha\) phosphatase, growth arrest, and DNA-damage-inducible protein-34 (GADD34) and a transcription factor C/EBP homologous protein (CHOP).\(^{48}\) Experiments with overexpression of CHOP have been shown to arrest the cell cycle and increase cellular apoptosis, while CHOP deletion has been shown to reduce apoptosis under conditions of ER stress. Furthermore, an inverse apoptotic relationship is observed between CHOP and antiapoptotic factor Bcl-2 expression. Taken together, these and many other observations highlight the prominent role of CHOP in apoptosis.\(^{50}\) To cope with the translation inhibition conditions, a constitutively expressed eIF2\(\alpha\) phosphatase, a constitutive repressor of eIF2\(\alpha\) phosphorylation (CReP), continues to dephosphorylate eIF2\(\alpha\) to restore protein synthesis independent of stress.\(^{50}\) –\(^{53}\) The retention of phosphorylated eIF2\(\alpha\) and inhibition of translation protect the cell by reducing the damaging effects of ER stress. Pharmacological modulators such as GSK2606414 and GSK2656157 inhibit PERK, eIF2B inhibitor ISRIB, and eIF2\(\alpha\) phosphatase inhibitors: Salubrinal, Guanabenz, and Sephin1 protect cells from the adverse effects of ER stress.\(^{54}\) Mouse models of hepatic steatosis– and obesity-induced NAFLD have shown that consumption of a high-fat diet can trigger ER stress response through PERK-mediated phosphorylation of eIF2\(\alpha\).\(^{55}\) Administration of Salubrinal showed attenuation of obesity and hepatic steatosis by reducing the severity of ER stress through increased levels of phosphorylated eIF2\(\alpha\). Rise in ATF4 levels due to phosphorylation of eIF2\(\alpha\) promoted autophagy.\(^{55}\) On similar lines, the PERK-eIF2\(\alpha\)-ATF4 branch of ER stress response has also been shown to play a hepatoprotective role in alcohol-induced liver damage.\(^{56}\) This suggests that the PERK-eIF2\(\alpha\)-ATF4 branch of ER stress response is important in protecting the liver from damage caused by ER stress.

Overall, in a cell under ER stress, the three branches of UPR (ATF6, IRE1, and PERK) work in a complex interconnected manner and together contribute to cell survival against ER stress.\(^{57}\) –\(^{60}\) More research is needed to elucidate the complex interrelationship between the three UPR branches and their role in cell survival under stress conditions.

### The Role of ER Stress during Liver Pathogenesis and Regeneration after Chemical-Induced Liver Injury

Toxicant-mediated tissue injury and tissue’s response to the inflicted injury are key aspects in determining the progression or regression of the toxicant-induced liver damage. The physiological effect of pharmacological agents and toxicants is determined mainly by their dose. Once toxicant exposure occurs, the toxic effects depend on the absorption, distribution, metabolism, and excretion of the chemical. It is probable that the majority of toxins at some level can disrupt the cellular ER homeostasis and activate UPR. More investigation into the involvement of the ER stress response due to chemical exposure is therefore necessary. Since reviewing the involvement of ER stress in liver injury induced by every chemical is beyond the scope of this review, we have chosen to review ER stress modulation by chemicals that have either clinical (acetaminophen \([\text{APAP}]\) and alcohol) or experimental (thapsigargin, tunicamycin, and carbon tetrachloride \([\text{CCl}_4]\)) significance (\(\rightarrow\) Fig. 2).

#### Thapsigargin and Tunicamycin-Induced ER Stress

A product of the Mediterranean plant \(\text{Thapsia garganica}\), thapsigargin is a potent inducer of ER stress. Thapsigargin induces ER stress by inhibiting sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) which causes depletion in ER calcium pools affecting ER homeostasis and triggering ER stress response while also initiating downstream ER-mediated apoptotic signaling (\(\rightarrow\) Fig. 2A). Inhibition of SERCA increases the cytosolic calcium deposition, which in turn triggers apoptotic signaling.\(^{61}\) Detailed insights into the role of thapsigargin in inducing ER stress in liver hepatocytes are limited and will require further investigation. A product of \(\text{Streptomyces lysosuperficus}\), tunicamycin is a potent antibiotic against many gram-positive bacteria, fungi, yeast, and viruses. Tunicamycin interferes with the first ER step in the synthesis of N-glycoproteins causing impaired protein glycosylation in the ER and resulting in misfolded proteins, triggering UPR in the ER (\(\rightarrow\) Fig. 2B).

Due to their ability to induce ER stress, thapsigargin and tunicamycin have been explored as potential candidates against cancer.\(^{67}\) –\(^{69}\) However, given their physiological toxicity, thapsigargin and tunicamycin are not of pathophysiological relevance; however, they have been widely used experimentally as a model compounds to induce ER stress in liver and in cultured hepatocytes to study the role of ER stress response signaling.\(^{65,66}\)

Due to their ability to induce ER stress, thapsigargin and tunicamycin have been explored as potential candidates against cancer.\(^{67}\) –\(^{69}\) However, given their physiological toxicity, there still needs to be a deeper understanding of their effects on human physiology and their concentration-dependent effects on the biological system. For example, it has been seen that subtoxic concentrations of thapsigargin have a cytoprotective role against the influenza virus.\(^{70}\) It is therefore necessary to develop temporal dose–response studies to address irreversible slow changes in cellular functioning in response to low-concentration doses of thapsigargin and tunicamycin. Such studies also need to be developed for other toxins discussed later in this section.

#### Acetaminophen-Induced ER Stress

APAP is the most widely used analgesic and antipyretic agent in the world. Overdose of APAP is the most common cause of acute liver failure in the Western world leading to thousands of hospitalizations and hundreds of deaths.\(^{71}\) APAP is metabolized by the drug-metabolizing enzyme cytochrome
P450 2E1 (CYP2E1) into a reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI; Fig. 2C) which under normal conditions is detoxified by cellular processes through conjugation with glutathione (GSH). Under overdose conditions, NAPQI accumulates at toxic levels due to rapid GSH depletion and alters cellular redox balance. The distorted redox balance results in imbalanced mitochondrial membrane potential, increasing reactive oxygen species (ROS) and peroxynitrite species concentration while disrupting Ca$^{2+}$ homeostasis, and cellular proteostasis, causing DNA damage, impairing mitochondrial function, and finally culminating into hepatocyte necrosis. Studies have indicated that ER stress response signaling is active in APAP-induced hepatotoxicity. Spliced XBP1 activates the expression of CYP1A2 and CYP2E1 which convert APAP to its reactive intermediate NAPQI (Fig. 2C). XBP1-deficient mouse models have shown constitutively active IRE1α signaling which had hepatoprotective effects by suppressing the expression of CYP1A2 and CYP2E1 and cleavage of existing CYP1A2 and CYP2E1 mRNA through RIDD. APAP overdose resulted in increased expression of CHOP. Whole-body CHOP knockout mice showed decreased APAP toxicity and had a better regenerative response.

A common observation in these studies is the latent activation of ER stress response against APAP toxicity. Multiple factors can underlie this latent activation. One possible explanation can be that the ER stress response is a result of the cellular damage happening immediately after APAP exposure. Another aspect of latent ER stress activation can be the unspecific binding of accumulated NAPQI to ER proteins interfering with ER homeostasis. Mechanistic insights into ER stress response activation and downstream...
consequences of ER stress signaling on hepatocyte recovery or death or recovery from APAP and other toxin exposure will be an important and interesting area for further investigation.

**Carbon Tetrachloride-Induced ER Stress**

CCl₄ is an organic solvent heavily used in industry for degreasing and is a well-studied hepatotoxicant. Treatment of CCl₄ is also used as an experimental model to study acute and chronic liver injury. CCl₄ toxicity is associated with centrilobular hepatocyte necrosis, which results in subsequent dose-dependent induction of liver regeneration. However, repeat dosing of CCl₄ can induce liver fibrosis and scar formation which can develop into cirrhosis. The progression of scar tissue results in cessation of liver regeneration, whereas cessation of CCl₄ administration is associated with reactivation of liver parenchyma regeneration. CCl₄ exposure leads to activation of ER stress signaling, but mechanisms are not completely known (→ Fig. 2D).

Following CCl₄ exposure, cytoplasmic interferon regulatory factor 3 (IRF3) and BAX (a proapoptotic factor) complex formation is detected which is seen to migrate to the mitochondria and activate caspase-mediated hepatocyte apoptosis and subsequent liver fibrosis. [82]

**Alcohol-Induced ER Stress**

**Metabolism of Alcohol in the Liver**

Uncontrolled consumption of alcohol is recognized as a global issue of public health. Hepatocyte resident alcohol dehydrogenase (ADH) and CYP2E1 are the main enzymes involved in metabolism of alcohol. ADH catalyzes alcohol oxidation through NAD⁺ reduction converting alcohol to acetaldehyde. This reaction results in the production of acetaldehyde and NADH which are heavily reactive and toxic (→ Fig. 2E). Acetaldehyde is further oxidized to relatively safer acetate by mitochondria resident aldehyde dehydrogenase 2 (ALDH2) through the reduction of NAD⁺. Excess consumption of alcohol can result in increased activity of ADH and ALDH2 which is associated with increased concentration of NADH resulting in imbalanced cellular redox potential (NAD⁺:NADH ratio). This is followed by a metabolic shift from oxidative to reductive and increased fatty acid synthesis which contributes to fatty liver disease. CYP2E1 follows a similar oxidative reaction converting alcohol to acetaldehyde. Besides ADH and CYP2E1, cellular catalase has also been noted to oxidize alcohol to acetaldehyde.

**Acute Alcohol Consumption and Its Effect on the Liver**

Acute alcohol consumption is a major cause of alcoholic liver damage; however, the damage is reversible. Acute alcohol consumption can impact liver function by overwhelming liver's capacity to process alcohol efficiently. This can result in oxidative stress, inflammation, and damage to liver cells. Acute alcohol consumption can disrupt the balance of lipid metabolism in the liver, leading to the accumulation of fat droplets and the development of alcoholic fatty liver disease (AFLD). Moreover, the breakdown of alcohol by the liver enzyme ADH generates toxic byproducts, such as acetaldehyde, which can cause DNA and protein damage and impair liver function. Another consequence of acute alcohol consumption is the activation of immune cells in the liver, triggering an inflammatory response. This chronic inflammation can lead to the development of alcoholic hepatitis, characterized by liver cell injury and inflammation. If left untreated, alcoholic hepatitis can progress to more severe conditions such as liver fibrosis and cirrhosis. Interestingly, variations in genes involved in alcohol metabolism and antioxidant defense systems can influence an individual's risk of developing liver diseases associated with alcohol consumption. Understanding these genetic and other host-associated factors can help identify individuals who may be more vulnerable to the harmful effects of acute alcohol consumption. An interesting gut–liver axis is seen to function in alcohol-induced liver damage where consumed alcohol coupled with lipopolysaccharide (LPS) from the gut microbiome translocates to the liver and activates Kupffer cells inducing liver inflammation. Furthermore, the LPS–alcohol combination can activate ROS formation which results in the worsening of hepatocellular damage.

**Chronic Alcohol Consumption and Its Effect on the Liver**

Chronic alcohol consumption has profound and detrimental effects on the liver and has been associated with the development of alcoholic liver disease (ALD). ALD encompasses a spectrum of conditions, ranging from alcoholic fatty liver to alcoholic hepatitis and, ultimately, liver cirrhosis. Chronic alcohol consumption disrupts lipid metabolism, resulting in the accumulation of fat within liver cells, a characteristic feature of AFLD. Oxidative stress induction has been seen as one of the consequences of chronic alcohol consumption, leading to the production of ROS and lipid peroxidation, contributing to liver cell injury and inflammation. Additionally, chronic alcohol exposure activates HSCs, promoting collagen deposition, and fibrosis, which are key mechanisms underlying the development of liver cirrhosis. Furthermore, chronic alcohol consumption and subsequent ALD are also associated with impaired liver regenerative potential through disruption of the balance between liver cell proliferation and cell death. This impaired regenerative process contributes to the development of liver fibrosis, cirrhosis, and, ultimately, liver failure. Excessive alcohol consumption is initially followed by liver steatosis which can progress to steatohepatitis. Continued consumption of alcohol can lead to liver fibrosis which can progress to liver cirrhosis and hepatocellular carcinoma (HCC).

**Role of the ER Stress Response**

Acute and chronic alcohol consumption triggers ER stress response in the liver and can contribute to ALD. Alcohol disrupts ER homeostasis, leading to the accumulation of unfolded or misfolded proteins within the ER lumen, activating ER stress. It has been suggested that ER stress can contribute to the development of liver steatosis or fatty liver disease, an early manifestation of ALD. ER stress-induced activation of the transcription factor SREBP-1c promotes
lipid synthesis and accumulation, contributing to the development of hepatic steatosis. Additionally, ER stress-mediated dysregulation of lipid metabolism pathways, such as lipolysis and fatty acid oxidation, further exacerbates liver lipid accumulation. Prolonged and excessive ER stress can overwhelm the UPR, resulting in sustained activation of inflammatory pathways and cell death. It has been seen that alcohol-induced oxidative stress and lipid accumulation can trigger ER stress by disrupting calcium homeostasis and impairing protein folding machinery resulting in the development of hepatic steatosis and ALD.

Multiple ER stress response genes have been identified to be upregulated during alcohol-induced liver toxicity indicating ER stress response signaling as an active contributor to alcohol-mediated liver injury. Alcohol toxicity causes hepatocyte apoptosis which is driven by the PERK-eIF2-ATF4 branch of ER stress signaling leading to CHOP activation followed by hepatocyte apoptosis. Furthermore, studies in primary cultured human hepatocytes exposed to alcohol showed activation of the integrated stress response (ISR). ISR is a cellular stress response system in which various extracellular and intracellular stresses are identified by four kinases: PERK, PKR, HRI, and GCN2, which give a common output of eIF2 phosphorylation and inhibition of cellular protein synthesis. It will be interesting to see how active ISR can have detrimental effects on liver regeneration.

Role of ER Stress in Liver Regeneration after Chemical-Induced Liver Injury
The liver responds to chemical-induced injury by compensatory proliferative activation of hepatocytes and nonparenchymal cells through complex signaling networks. These newly formed cells make up for the lost liver tissue and restore normal liver function. Multiple studies discussed in this section highlight the role of ER stress response signaling in liver regeneration after chemical-induced liver injury.

Studies conducted using chemical-induced hepatotoxicity models suggest that abrogating ER stress response results in impaired liver regeneration. CCL4-induced liver toxicity models on the background of IRE1α deletion showed poor regenerative response and diminished STAT3 phosphorylation. However, when IRE1α expression was restored, sustained STAT3 phosphorylation levels were detected. STAT3 is an important signal transducer in hepatocyte proliferative signaling and, therefore, these results suggest that ER stress signals interact with other cellular signaling pathways and play an important role in activating cell proliferation following chemical-induced liver injury. Mechanistic insights into why IRE1α signaling is important come from studies on downstream promotion of genes due to IRE1α spliced XBP1 signaling. Interleukin-24 (IL-24) is a known-negative regulator of cell proliferation and has a hepatoprotective effect. Spliced XBP1 is seen to promote the expression of IL-24 which accumulates on the ER membrane and inhibits PERK-eIF2-ATF4 branch of ER stress. This PERK-eIF2-ATF4 inhibition has two consequences; first, it allows for continued protein synthesis necessary for hepatocyte adaptation to the incurred chemical injury and liver regeneration; second, it downregulates the CHOP expression and subsequent apoptosis-protecting remnant hepatocytes and allowing liver regeneration.

The Role of ER Stress in Regeneration after Partial Hepatectomy
Liver Injury and Regeneration Dynamics after Partial Hepatectomy
Due to the lobular structure of the liver, surgical resection of one of its lobes provides a clean model to study liver regeneration. Seminal experiments demonstrated the potential of the liver to regenerate to normal physiological size after two-thirds PH making it the most widely used model to study liver regeneration. Although two-thirds PH is widely used, studies exceeding this limit have shown to have lethality due to inefficiency and lack of functionality of the remnant liver to sustain normal physiological functioning and regenerative potential. PH is followed by recalibration of hepatic blood flow with the blood now circulating through the remnant liver. This can increase the blood pressure in the remnant liver causing portal hyperperfusion and focal hemorrhage which can progress in the adjacent parenchyma. Progression of poor hepatic circulation and associated damage can cause functional dearterialization, overall, drastically damaging the liver, and impairing liver regeneration.

The liver undertakes multiple regenerative processes to modulate its size corresponding to the host’s physiological conditions. For example, liver size increases during pregnancy but decreases in cases of cachexia. Furthermore, liver size is seen to be modulated by the host physiology through yet unexplored factors. This phenomenon of regulated liver size during regeneration in correspondence to the host physiology suggests a “hepatostat”-like regulatory function. The restored liver after PH does not involve the regrowth of its lost liver lobe, but instead is associated with the expansion of existing liver lobes to the critical mass before heptectomy. Multiple signaling pathways have been identified initiating and regulating liver regeneration after PH.

In a healthy liver, majority of cells are in a quiescent state with a minimal population in an active state of proliferation, dividing with long intervals. However, upon injury compensatory regeneration is stimulated and a significant number of cells, both hepatocytes and nonparenchymal cells, enter the cell cycle and undergo cell proliferation to replace the dead cells. Liver regeneration is a complex process relying on internal and external signals that control the nature and extent of regeneration. The purpose of liver regeneration is to retain the lost stability of liver functioning which is critical for maintaining physiological homeostasis. Interestingly, under conditions of excessive liver parenchymal cell damage, nonparenchymal cells are seen to undergo active regeneration and transdifferentiate into hepatocytes through genome-wide alterations (epigenetic and transcriptomic) and signaling pathway rewiring.
Role of ER Stress in Liver Regeneration after PH

PH is associated with an increased risk of hepatic steatosis and inflammatory liver failure due to the generation of ROS, and excessive apoptosis and disturbed hepatic circulation. Chemicals that modulate ER stress such as 4-phenyl butyric acid (PBA) and tauroursodeoxycholic acid (TUDCA) has noted hepatoprotective effects against PH and ischemia–reperfusion injury.130–132 Experiments investigating the roles of PBA and TUDCA on ER stress response and liver regeneration post-PH suggested that all three branches of ER stress response and their associated downstream signaling are activated in livers post-PH and ischemia–reperfusion.133,134 Steatotic livers exhibit a reduced response to ER stress signaling than non-steatotic livers. PBA and TUDCA resulted in suppression of IRE1 and PERK branches of ER stress response subsequently inhibiting apoptosis and inflammation and improving liver regeneration.135 Although these experiments highlight a correlation between ER stress inhibition and improved liver regeneration, it is important to note that the mechanism of action of PBA and TUDCA is not completely elucidated and a deeper investigation into their mechanism will help in uncovering how they modulate ER stress in hepatocytes and affect the liver regeneration post-PH. PBA has emerged as a promising therapeutic agent with diverse applications in neurodegenerative diseases, cancer, and metabolic disorders. As a chemical chaperone and modulator of cellular stress response pathways, PBA exhibits multiple beneficial effects. It has the ability to reduce protein aggregation in neurological conditions, alleviate ER stress, induce cellular differentiation and apoptosis, and enhance metabolic function. These findings highlight the potential of PBA as a targeted intervention for improved patient outcomes.135 TUDCA has been extensively studied for its hepatoprotective effects in liver diseases such as nonalcoholic fatty liver disease (NAFLD), cholestasis, and liver fibrosis. Moreover, TUDCA has demonstrated promising neuroprotective properties, making it a potential therapeutic intervention for neurodegenerative disorders like Parkinson and Alzheimer disease. Additionally, TUDCA’s anti-inflammatory and antiapoptotic characteristics offer potential therapeutic avenues for various inflammatory and apoptotic-related conditions. The diverse therapeutic applications of TUDCA underscore its significance as a candidate for further research and clinical exploration in different disease contexts.136

Mouse models with 90% hepatectomy followed by administration of prostaglandin and somatostatin combination showed their combinatorial hepatoprotective effect to be more pronounced than their individual administration. This protective effect was seen to be through inhibition of ER stress response which inhibited cellular apoptosis and promoted liver regeneration.137 It is important to note that prostaglandins and somatostatins carry out a vast spectrum of functions in the body and their hepatoprotective effects seen in these experiments through suppression of ER stress response can be a part of a multifaceted cell-wide action, the effect of which is reduced hepatic damage after PH. We lack detailed mechanistic insights into the action of prostaglandins and somatostatins on the hepatocyte ER and liver and more investigations are warranted.

UFMylation, Cell Death, and Regeneration

A recently identified cyclin-dependent kinase 5 activator, CDK5RAP3, is especially expressed in the liver along with other organs of the body, contributes to a multitude of cellular processes, and interestingly also is a component of the UFMylation system. UFMylation, also known as ubiquitin-fold modiﬁer 1 (UFM1) conjugation, is a posttranslational modification pathway involving the attachment of UFM1 protein to the target proteins.138 UFMylation has been shown to play a role in regulating ER homeostasis and the cellular response to ER stress. Studies have demonstrated that UFM1 and its conjugating enzymes are involved in the maintenance of ER protein folding capacity and the UPR, a cellular mechanism activated during ER stress.138,139 Since UFMylation is a part of posttranslational modification in the ER, CDK5RAP3 is seen to play an important role in ER homeostasis.140 CDK5RAP3 deletion has been shown to disrupt ER homeostasis through an impaired posttranslational modification, altogether contributing to the activation of UPR.140,141 However, it must be noted that the complete understanding of the cell-wide functions of CDK5RAP3 and the ramifications of its deletion in relation to ER stress induction and liver regeneration need to be further investigated.

Overall, such experiments suggest that hepatocytes have upstream regulatory factors, the loss of which triggers ER stress-mediated hepatocyte apoptosis to perhaps avoid errors that might result in unregulated hepatocyte proliferation and HCC.

Actively proliferating hepatocytes in response to PH-induced liver damage require continued synthesis of proliferation-dependent proteins. Studies in fibroblast cell lines showed that activation of the PERK-elf2-ATF4 branch of ER stress response results in a drop in cellular protein synthesis and cause cell cycle arrest at the G2/M phase.142,143 ER stress response is also associated with proliferation-promoting and inhibitory signaling. Liver regeneration is associated with active cell proliferation through oncogenic Ras signaling. It has been seen that IRE1α-mediated ER stress response signaling is activated by Ras-mediated proliferative signaling. This observation comes from experiments that showed that abrogating ER stress through the reduction of IRE1α-mediated Xbp1 splicing resulted in growth arrest and premature senescence.144 Corresponding to this, it has been seen that ER stress is associated with ubiquitin
proteasomal degradation of p53 and further supporting cell proliferation.\textsuperscript{145}

Canopy homolog 2 (CNPY2) is a recently identified mediator of PERK signaling. Under stress conditions, CNPY2 binds to the ER luminal domain of PERK by dissociating BiP and activates the PERK-eIF2-ATF4 branch of ER stress response. CNPY2 expression is activated by CHOP which is activated by ATF4 signaling. The expressed CNPY2 then gets localized in the ER lumen.\textsuperscript{146} Recently, mechanistic insights into CNPY2-mediated cell cycle enhancement were revealed which suggest the activation of PERK by CNPY2, activates multiple signaling pathways that inhibit p53, alleviating its proliferation inhibitory effect and promoting rapid cell proliferation in the liver.\textsuperscript{147}

It is important to note that impaired liver regeneration in response to toxicant-induced injury or PH or error-prone cell proliferation is associated with the development of HCC. These studies highlight the importance of maintaining a fine balance of proliferative and proliferation inhibitory signaling in liver regeneration and the important role played by ER stress response signaling in this process.

**Future Lines of Investigation on the Role of ER Stress in Liver Regeneration**

ER plays a central role in cellular homeostasis and different environmental conditions like toxicant exposure and physical injury have been associated with distorting cellular homeostasis. Recent studies have begun to elucidate the role of ER stress response in modulating cellular processes in response to stress conditions. Many observations in the past decade have suggested that ER plays an important role in regulating liver regeneration. Based on the limited literature on the topic, we highlight the following as important areas of investigation.

1. Previously discussed studies have largely applied single dose or repeat dosing to induce liver injury following which the process of liver regeneration is monitored. Future studies need to focus on dose-dependent ER stress response prior to associated damage. Furthermore, continued monitoring of ER stress response through liver regeneration can help elucidate the liver regeneration regulatory role of ER stress response.

2. As previously discussed, a complete understanding of how different toxins affect hepatocytes and directly or indirectly result in activating ER stress response followed by its implications on liver regeneration is necessary. Furthermore, how toxicants targeting cellular locations other than ER can trigger a cascade of response upstream of ER eventually activating ER stress needs to be further resolved.

3. Multiple studies have highlighted context-dependent PERK-eIF2-ATF4 signaling-associated cellular changes in response to oncogene and tumor suppressor gene signaling.\textsuperscript{144,145,148,149} Since oncogene and tumor suppressor gene signaling is active during liver regeneration and considering their links with the PERK-eIF2-ATF4 branch of ER stress response, this signaling crosstalk needs to be further explored.

4. Our discussion highlights that inhibition of ER stress response in PH supports liver regeneration, while inhibition in chemical-induced liver injury results in impaired regeneration. The three arms of UPR act in a sequential fashion with PERK-eIF2-ATF4 acting last.\textsuperscript{158,157,156} It would be interesting to explore how sequential activation of the three branches of ER stress response and associated downstream effects in PH and chemical-induced liver injury contribute to the contextual nature of ER stress in liver regeneration. This apparently contextual nature demands further elucidation of differential mechanisms involved in PH and toxicant damage hepatocyte response. Furthermore, we need to understand how the remnant injured or uninjured cells perceive the damage and in turn modulate their ER stress response and initiate liver regeneration.

5. Mitochondria-mediated metabolism and energy production is important in regulating liver regeneration. Important links in ER-mitochondria crosstalk have been observed at different levels from physical contact, mitochondria-associated membranes (MAMs) between the two organelles to signaling crosstalk.\textsuperscript{150–152} ER-mitochondria calcium crosstalk is important in maintaining cellular calcium homeostasis. ER release of calcium signals to mitochondria is crucial to regulate mitochondrial functions like metabolism, energy production, and apoptosis.\textsuperscript{153} ER stress response signaling proteins like PERK are seen to interact with mitochondria under stress. This is seen to regulate mitochondrial protein homeostasis, ER mitochondria calcium signaling, and apoptosis.\textsuperscript{10,154–156} Recently, it has been seen that mitochondria to ER crosstalk can be mediated by NADPH production and redox regulation of GSH. Mechanistic insights suggest active redox cycling of GSH is associated with the inhibition of ER stress.\textsuperscript{157} This highlights the potential importance of investigating the processes involved in ER-mitochondria crosstalk and their broader implications on regulating liver regeneration.

6. Finally, we believe that it is important to elucidate the mechanistic role of ER stress response in liver regeneration under different liver insults.

**Conclusion**

Over time, we have gained good insights into the role of ER stress response in PH and drug-induced liver toxicity.\textsuperscript{109} We believe that in the future, an even clearer picture of ER stress in various other liver diseases will emerge.\textsuperscript{158} ER plays a central role in cellular homeostasis and different environmental conditions like toxicant exposure and physical injury have been associated with distorting cellular homeostasis. Recent studies have begun to elucidate the role of ER stress response in modulating cellular processes in response to stress conditions. Multiple observations in the past decade have suggested that ER plays an important role in regulating liver regeneration. Based on the limited literature on this topic, multiple studies suggest that inhibiting the ER stress response after PH-induced liver damage promotes liver regeneration, whereas chemical-induced hepatotoxicity demonstrated that inhibiting the ER stress response impairs liver regeneration. Therefore, we see
an apparent contextual nature to the role of ER stress response signaling in liver regeneration. Finally, we would like to highlight that while extensive mechanistic data are available in rodent models, more research on the role of UPR is needed for human liver diseases.

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Conflict of Interest
None declared.

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