Endoplasmic Reticulum Stress Signaling in the Regulation of Hepatic Pathological Responses

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Abstract: The endoplasmic reticulum (ER) is a vital cell organelle that is primarily involved in the processes of protein folding, maintenance of intracellular calcium storage and lipid synthesis in order to maintain cellular homeostasis. To achieve this meticulous order, several ER-dependent processes have to be in unison and perfect harmony. However, a persistent supply of newly synthesized proteins strains the ER mainly due to the accumulation of unfolded proteins, thus ultimately leading to an imbalance termed ER stress. Although the accumulation of misfolded proteins is a frequent reason for the initiation of ER stress, it is also induced by the hyper-production of reactive oxygen species, aberrant calcium leakage from the ER and due to the effect of cytokines. ER stress signals are conveyed via three arms of ER, namely PERK, IRE1 and ATF6. Signal transduction from these signaling molecules often converges on the transcriptional upregulation of CHOP and its related signaling mechanisms. If the ER stress is unresolved, then it can lead to cell death through different cell death mechanisms, including apoptosis, proptosis, etc. In the liver, it has been observed that ER stress plays a critical role in hepatic damage under different experimental conditions. This review highlights the role of ER stress in liver pathologies.

Keywords: ER stress; liver disorders; inflammasomes; interleukin-1 cytokines

1. Introduction

The ER is a crucial organelle involved in maintaining cellular homeostasis via the synthesis of proteins and lipids. The regular process of protein folding can put a toll on the ER, especially in cells such as hepatocytes that have a major responsibility in synthesizing and secreting several proteins required to achieve a normal physiological response. In this course, sometimes, there is an accumulation of misfolded proteins that cause stress in the ER. To counter this, an adaptive response known as unfolded protein response (UPR) is initiated. Initially, UPR responses are focused on the mitigation of cellular damage via the induction of several chaperones and other protective molecules. However, failure to resolve these issues results in disruption of cellular homeostasis and leads to several pathologies, including neurodegeneration, atherosclerosis and hepatic disorders [1].

ER is an even more vital organelle for the liver because hepatocytes are primary cells involved in metabolism and drug detoxification. In addition, within hepatocytes detoxification activity occurs in the ER. Biotransformation involves an intricate set of enzymatic processes that result in the production of more water-soluble products than the parent compound, which can be easily excreted from the body. This is achieved mainly by the action of the cytochrome P450 system via an intricate set of enzymatic reactions that involve oxidation, reduction, hydrolysis and conjugation with polar compounds to increase their water solubility, consequently facilitating excretion. Unfortunately, these processes can have an untoward effect, too, especially due to their propensity to produce reactive oxygen species (ROS), which can result in an adverse physiological consequence by an attack on membrane phospholipids. Notably, ROS production is one of the well-studied mechanisms leading to ER stress and is also reported to impart adverse effects to hepatocytes [2,3].
The endoplasmic reticulum is a long organelle located in the cytosol. It is an interconnected network of membranous sacs known as cisternae. Although there is varying information in the literature about the exact date on which the organelle itself was noted, available reports indicate it to have been known anywhere between the late 19th Century to the early 20th Century. However, a more elaborate elucidation of its ultrastructure was performed much later, around 1945, by Porter et al. [4]. Furthermore, our understanding of the cellular and molecular events that underlie ER stress was accelerated by many groundbreaking studies in the 90s and early 2000s. Although ER stress can occur in all eukaryotes, this manuscript mainly highlights the role of ER stress in non-plant and mammalian settings. The role of ER stress in plants can be found in an excellent review article elsewhere [5]. The ER is a major site of protein folding, lipid synthesis and storage of intracellular calcium. A well-regulated oxidative process in association with protein disulfide isomerase (PDI) and endoplasmic reticulum oxidoreductin (ERO1) plays an important role in the process of protein folding via the formation of disulfide bonds in the ER [6]. Meanwhile, it is also necessary to promote the degradation of misfolded proteins, which is facilitated via their reduction [7]. In this way, two fundamental systems are at play in the ER that maintain a delicate balance in the functioning of the ER to sustain an elegant harmony at the cellular level. Furthermore, the prevention of misfolded proteins to achieve harmony in ER is carried out by the diligent action of several ER-resident proteins known as ER chaperones. An accord in the redox environment and the immaculate action of chaperones sustain the process of protein folding under normal conditions. However, an overload of proteins in ER leads to an accumulation of misfolded proteins. To accommodate the overload of incoming proteins, the ER undergoes some readjustment in its structure via expansion and reorganization of its volume so as to adapt to the requirement of increased protein folding. This is also required for the proper segregation of misfolded proteins. This also initiates a cytoprotective process termed UPR that encompasses a set of cellular processes such as general translational shutdown but upregulation of chaperone proteins, including BiP. Such action for a prolonged period of time can amplify stress in the ER, thus ultimately leading to devastating consequences because the balance between recovery and cytoprotection tilts towards the cytotoxic end due to overwhelming load. Initiation of the ER stress response is accompanied by the release of chaperone proteins from the three ER stress sensors, namely PERK, IRE1α and ATF6α, which then culminates in the activation of basic leucine zipper (bZIP) transcription factors and genes transcribed by these bZIP transcription factors. UPR induction is a ligand-independent process and mainly occurs via oligomerization and trans-autophosphorylation for PERK and IRE1α, which are type-I transmembrane domains [8], while ATF6-dependent signaling occurs after the release of chaperone protein that promotes its translocation into Golgi apparatus, where it is converted into cleaved forms by proteases site-1 protease (S1P) and site-2 protease (S2P) [9,10]. Phosphorylation of PERK leads to the phosphorylation of eif2α [11], which causes abrogation in global translation. However, despite a global protein shutdown, some proteins, such as activating transcription factor 4 (ATF4), undergo preferential translation. ATF4 then acts as a transcription factor and is involved in the upregulation of several proteins, namely ER chaperones, C/EBP homologous protein (CHOP) and autophagy-related genes. IRE1α phosphorylation, on the other hand, triggers mRNA splicing of X-box binding protein (XBP-1) to generate a spliced form commonly referred to as sXBP-1 [12], which functions as a transcription factor and is involved in the processes akin to ATF4. Similarly, the cleavage of ATF6 leads to the translocation of the cleaved form into the nucleus, which can also behave as a transcription factor in similar processes described previously. Interestingly, upregulation in mRNA levels of XBP-1 occurs under the action of ATF6 [12,13]. These intricate events suggest a close interrelationship of various branches of UPR to modulate cell fate decisions. Consequently, once UPR is initiated, and the signaling events move into action, the ultimate cell fate lies in the balance between the adaptive and cytotoxic responses [14], both of which can be modulated by ER stress-dependent signaling.
The decision of cell fate would also likely depend on the complementary interaction with other signaling pathways. It is also possible that early and late ER stress responses behave differently in deciding cell fate from the outlook of cytoprotection or cytotoxicity [14,15]. For instance, prolonged activation of c-Jun N-terminal kinase (JNK), which is a downstream effector of ER stress beyond 12 h, has been reported to promote apoptosis, while it is protective in the initial stages under ER stress [16]. In addition, it has been postulated that the differential mRNA stability of chaperones such as BiP and CHOP could also account for the varying effect on adaptation and cytotoxicity [17]. Although ER stress can lead to adverse consequences, it can also provide cellular repair function in some conditions. ER stress in an ATF4-dependent manner can also promote the upregulation of DNA repair genes, including growth arrest and DNA damage-inducible gene (GADD45α) [18]. This gene can provide cytoprotection under certain conditions of stress [19]. The fundamental mechanism by which it can repair DNA damages includes nucleotide excision repair, where bulky DNA lesions are removed. In a methionine and choline-deficient diet (MCD)-induced ER stress and hepatotoxicity model, deletion of GADD45α was found to amplify steatohepatitis, suggesting a protective role of GADD45α induction in liver during ER stress [20]. Despite possessing some cytoprotective ability, ER stress has a major adverse consequence in cells and is implicated in a multitude of hepatic diseases, from simple steatosis to HCC (Figure 1). It has also been observed to play a negative consequential effect on viral hepatitis via an increase in the production of viral load [21,22]. Conversely, inhibition of ER stress conferred significant hepatoprotection during various hepatic pathologies [23–26].

![Figure 1. Multipotent role of ER stress in liver disease. ER stress is known to influence a wide variety of liver diseases that can range from simple steatosis, which arises because of excess fat deposition in hepatocytes, to severe pathologies such as HCC. The major causative role of ER stress in promoting liver pathologies lies in the abnormal activation of immune responses and metabolic dysfunction. This illustration was partially created with BioRender.com.](image)

This review highlights the relationship between the regulation of ER stress and other cellular responses with a focus on liver disease.
2. Branches of UPR and Their Signal Transduction

As briefly mentioned previously, induction of ER stress occurs via three transmembrane receptors, PERK, IRE1α and ATF6α. Depending on the experimental condition, it is possible for only one branch to predominate, or in other cases, cooperation of different branches of UPR can also occur to exert a concerted action [27]. Broadly, there is the existence of two major categories of chaperone systems in the ER—the HSP70 chaperone system and carbohydrate-binding chaperone system. Among these various chaperones, BiP, which falls under the HSP70 system, is the most extensively studied chaperone because of its versatility in binding multiple proteins [28,29]. The protein folding activity of BiP is facilitated by its ATPase activity, which promotes binding to the hydrophobic regions of the misfolded proteins in order to prevent their aggregation [30]. This ATPase activity of BiP is further regulated by nucleotide exchange factors (NEFs) that promote the release of ADP from BiP to initiate another cycle of binding with ATP in order to act in the inhibitory process of misfolded protein-aggregation [31]. When ER is overwhelmed with misfolded proteins, BiP is released from the binding with these transmembrane domains, thereby resulting in signal transduction via the three branches of UPR, which is briefly explained in Figure 2.

![Figure 2. Branches of UPR and their signaling response. UPR transduces signal downstream via three transmembrane domains of the ER. This occurs subsequent to the release of BiP, resulting in the transautophosphorylation of PERK and IRE1α and liberation of ATF6α from ER followed by translocation into Golgi. All of these events generate bZIP transcription factors, which are implicated in the gene expression of various molecules, including ER chaperones and proteins associated with ERAD, autophagy, and apoptosis.](image-url)

2.1. Protein Kinase R-like ER Kinase (PERK)

PERK is a serine/threonine kinase and is an important member of the eif2α kinase family. Under normal conditions PERK is kept at an inactive state by the binding of BiP. However, due to an increase in the misfolded proteins inside the ER, dissociation of BiP occurs, thereby promoting the dimerization of PERK [8]. This causes trans-autophosphorylation of PERK, leading to phosphorylation of eif2α at serine51 residue [11]. The repercussion of eif2α phosphorylation is a halt in global protein synthesis. Although global protein synthesis is abrogated, some proteins, such as ATF4, undergo preferential translation courtesy of its 5’-untranslated region that lies upstream of the open reading frame [32,33]. ATF4
is a transcription factor and can induce the upregulation of various proteins associated with countering ER stress, including genes related to amino acid metabolism, antioxidant genes and autophagy-related genes [34]. However, the most relevant role of ATF4 from the perspective of ER stress would be the upregulation of CHOP-a vital protein that possesses a crucial role in the ultimate response of ER stress because of its involvement in both the cytotoxic and adaptive responses [15,35].

2.2. Inositol-Requiring Enzyme Type 1 (IRE1)

IRE1 is a dual-function protein in the sense that it possesses both endoribonuclease and kinase activity, with phosphorylation mainly occurring on serine residue [36]. Furthermore, IRE1α is a ubiquitous protein, while IRE1β is found only in the gastrointestinal tract [37]. The N-terminal luminal domain (NLD) is the one that is implicated in the process of sensing ER stress responses, while the kinase and endoribonuclease activity is present on the cytosolic end [38]. During normal physiological conditions, the interaction of BiP and NLD prevents aberrant activation of IRE1α, while accumulation of misfolded proteins leads to the dissociation of the chaperone and ultimately promotes the dimerization [8] and activation of IRE1α kinase and endoribonuclease activity, leading to the splicing of XBP-1 [39]. Spliced XBP-1 then translocates to the nucleus and induces transcription of various adaptive genes. In addition to splicing of XBP-1 via endonuclease activity, IRE1α kinase activity can also lead to upregulation in CHOP mRNA through JNK and is implicated in neuronal cell death [40]. Notably, the release of BiP is important but not sufficient for the induction of IRE1α activation [41]. Furthermore, it has also been suggested that from the perspective of ER stress, the endonuclease activity of IRE1α would be more critical than its kinase activity because of the associated response in splicing XBP-1 introns and cleavage of selected mRNAs by a process termed regulated IRE1α-dependent decay (RIDD).

2.3. Activating Transcription Factor 6 (ATF6)

Preceding ER stress, ATF6 is confined in the ER membrane. Following the release of chaperone proteins due to the accumulation of misfolded proteins in ER, it translocates to the Golgi apparatus [9,42]. There, it encounters the proteases- S1P and S2P. These proteases cleave ATF6, thereby leading to the formation of an N-terminal truncated protein of 50 kDa. This truncated protein possesses a bZIP domain, and when directed into the nucleus, it binds to ER stress response elements and is implicated in the upregulation of several UPR genes. It has been reported that S2P plays a more important role in the activation of ATF6 than S1P because of the complete lack of ATF6 processing in S2P null cells than S1P null cells [10]. Nonetheless, both of these proteases are involved albeit at a varying degree. Furthermore, it can also lead to the upregulation in mRNA levels of XBP-1 and CHOP [13], suggesting an important role of ATF6 in modulating other branches of the UPR. While two isoforms of ATF6 exist as ATF6α and ATF6β, ATF6α is reported to be more crucial than ATF6β in the regulation of gene expression related to ER stress. In fact, it has been suggested that ATF6β could even act as a suppressor of ATF6α-induced UPR gene expression [43].

3. All Roads Lead to CHOP

Pioneering works in the late 80s and early 90s led to the discovery of CHOP (GADD153) and its regulation, which seems to be activated by several inducers of cellular stress such as methyl methanesulfonate, hydrogen peroxide and UV radiation [44]. Subsequent studies further clarified its involvement in various physiological processes and led to the discovery of its association with ER stress response [45]. The ultimate consequence of ER stress lies in the signaling of newly synthesized genes by the three different branches of UPR. Interestingly, all three branches of ER stress induce transcriptional upregulation of CHOP, which is also a bZIP transcription factor [46,47]. PERK-mediated CHOP upregulation depends on ATF4; IRE1α-dependent CHOP upregulation is associated with its kinase activity and activation of JNK, while cleaved ATF6 itself can upregulate CHOP. Not only this, it has been
observed that its proapoptotic property mainly confines in the bZIP region [46]. Apart from the transcriptional regulation, CHOP is also regulated by several post-transcriptional and post-translational modifications [48–50]. To this end, it has been reported that modifications such as phosphorylation of CHOP at serine 30 residue by kinases such as AMPKα1 can lead to its degradation via the ubiquitin–proteasome system [51].

CHOP has diverse roles in the modulation of cellular physiology under ER stress. In addition, it has also been observed to be implicated in the suppression of metabolic genes [52], but the consequence of this in cell fate decision has not been well defined. Although debatable, it can be considered the most important protein in the whole machinery of UPR because of its diverse ability to induce both cytotoxic and cytoprotective responses as well as negative self-regulation of the UPR [53]. However, these adaptive responses often are overwhelmed when the initiating signal is too strong or persistent for a long duration. Then, CHOP, by virtue of its proapoptotic property, induces cell death via upregulation of apoptotic genes such as BCL2 associated X, apoptosis regulator (BAX) and BCL2 homologous antagonist/killer (BAK) [54]. Furthermore, it can also enhance a hyperoxidative environment in the ER via the ERO1α-dependent mechanism [55]. This creates a favorable environment for the induction of cell death pathways. In addition to cell death pathways such as apoptosis, cell fate is critically determined by matters pertaining to the regulation of cell cycle progression/arrest. In this context, CHOP has been known to induce a decrease in DNA synthesis and induce cell cycle arrest in the G1/S phase [56]. Therefore, in addition to the direct induction of apoptosis, a halt in cell cycle progression by CHOP could also account for consequential cellular decisions in certain conditions. Interestingly, it has been observed that CHOP upregulates autophagy genes at an early time point and induces apoptotic genes at a later time, which highlights its ambivalent role in both autophagy and apoptosis [15]. This also indicates the possibility that failure to resolve ER stress leads to apoptotic death by CHOP. A large body of evidence suggests that inhibition of CHOP can have a beneficial effect on various physiological abnormalities, including hepatic fibrosis, drug-induced liver injury, cytokine-induced hepatocyte death, non-alcoholic fatty liver disease (NAFLD) and lipotoxicity [57–63]. However, it would still be too early to celebrate the encouraging results observed from CHOP inhibition because of its fundamental role in the repair of DNA damage and also studies where it has been observed to promote cytoprotection. For instance, it has been demonstrated that CHOP KO mice are not protected under experimental hepatic dysfunction induced by carbon tetrachloride (CCL4) [64], although a significant hepatoprotection was observed in the case of acetaminophen (APAP) administration due to abrogation of CHOP signaling. While there are some similarities in the hepatotoxic effects and mechanisms between CCL4 and APAP, a silver lining in this case might be the fact that the APAP model is more relevant in human physiological/pathological conditions because of APAPs wide use as a nonsteroidal anti-inflammatory drug (NSAID).

Despite some setbacks, a predominant array of studies suggest a beneficial role of CHOP inhibition. In addition, the conditions that are more relevant to human physiological/pathological settings reinforce this hypothesis regarding the beneficial effect of CHOP inhibition. Therefore, there is a hope that some therapies targeting CHOP in any way might turn out as a boon for the management of those liver disorders where CHOP played a predominantly adverse role.

4. Self-Regulation of ER Stress

It has been observed that several cellular responses are often accompanied by certain negative feedback regulations, too. ER stress is no exception to that. ER stress responses are regulated at various stages of transcription and translation, including several post-transcriptional as well post-translational events. In addition, several feedforward and negative feedback loops are also at play. The negative autoregulation of the PERK-pathway of ER stress basically occurs via growth arrest and DNA damage-inducible protein (GADD34)-dependent dephosphorylation of eif2α [65]. GADD34 is a gene transcribed by CHOP,
which leads to the dephosphorylation of eif2α via recruitment of protein phosphatase 1 (PP1). In addition, slightly complicated information has been obtained from a study employing GADD34 KO mice. Though GADD34 can mediate dephosphorylation of eif2α and supposedly limit UPR, this GADD34-mediated action does not necessarily lead to cytoprotection. It has been observed that GADD34 KO mice had a lower degree of liver damage than their WT counterparts [66], suggesting enhanced cytoprotection is achieved when ER stress is let to run its course rather than by abrogating it in the middle. This indicates that ER stress modulates cell fate in a complex manner and prematurely blocking any simple step in the process of ER stress/UPR does not necessarily provide a theoretically supposed beneficial effect. It has been suggested that such adverse consequences might be because the enhanced protein supply after dephosphorylation of eif2α in an already stressed ER could lead to amplification of damage due to overload in ER [67].

In addition to GADD34-mediated feedback inhibition, PERK activity and further signaling are also dampened by the binding of the p58 inhibitor of protein kinase (p58IPK) [68]. Additionally, bax inhibitor 1 (BI-1) is a negative regulator of IRE1α, which forms a complex with its cytosolic domain to inhibit ER stress signaling [69]. Also, fortlin is another protein that acts in a similar manner to inhibit IRE1α signaling [70]. Furthermore, the RNase activity of IRE1α can also be dampened by S-nitrosylation, but an activation of the PERK pathway occurred in that condition [71]. Moreover, the activity of caspases also subsidizes IRE1α activation [72]. In addition, IRE1β can also act as a negative regulator of IRE1α-dependent signaling [73]. This interesting insight evokes a thought that these could be the yin and the yang of the IRE1 branch. Although this is interesting to speculate about, in-depth research from this perspective is required to ascertain this fact. Additionally, it should be noted that this response would be limited due to the localized presence of IRE1β in the respiratory and intestinal epithelium [74]. Nonetheless, this hypothesis could be extended to the ATF6 branch, too, where the β-isofrom has been found to inversely regulate α-isofrom-dependent gene expression [43]. Apart from these, post-translational modifications such as the sumoylation of ATF6 have rendered it inactive in the process of ER stress induction [75]. Additionally, Wolfram syndrome 1 (WSP1)-dependent degradation of ATF6 via proteasomal pathway is also reported to counter against potential induction of ER stress [76]. Furthermore, an ER stress-inducible protein known as nucleobindin-1 has been reported to possess ER stress-suppressing effect via inhibition of S1P, ultimately resulting in repression of ATF6 cleavage [77].

In addition, aggregation of uXBP-1 can also provide an inhibitory signal for UPR [78]. In a recent study, it was reported that two ER membrane protein complexes had totally contrasting roles in regard to their property of inhibiting ER stress. The authors reported that the soluble form scEMC10 leads to an enhancement of ER stress via the PERK pathway, while the membrane-bound mEMC10 suppressed ER stress [79]. Moreover, this effect was found to be implicated in the pathogenic process of diet-induced obesity in relation to ER stress. On the cumulative assessment of these studies, it can be inferred that several regulatory checkpoints occur in the events related to ER stress to thwart further damage, and ultimate cell fate is dependent on the balance of various factors Figure 3.
5. ERAD and ER-Phagy Two Dual Dynamos of ER Quality-Control

ERAD and ER-phagy are the two major offshoots of the UPR itself that are implicated in the events associated with the degradation of misfolded and dysfunctional proteins. ERAD-mediated removal of misfolded proteins occurs through proteasome. ERAD encompasses a sub-set of specific proteins such as ERdj, Hrd1, etc. and their functions range from recognition of the misfolded proteins to the ubiquitination and subsequent proteasomal degradation. An unusual structural orientation of the misfolded proteins triggers the activation of recognition proteins such as calnexin or BiP and ultimately leads to their removal by the subsequent steps of ERAD machinery with the help of proteasome [80]. ERAD can provide beneficial effects in several situations, such as ERAD-mediated targeting of CYP2E1 can mitigate potential hepatic injury because CYP2E1 is involved in the amplification of hepatic injury, such as during alcohol ingestion. Additionally, liver-specific deletion of SEL1 can lead to the upregulation in IRE1α expression, elevation of FGF21 and alteration in metabolic profile and growth retardation [81]. Along with the degradation of misfolded proteins, ERAD machinery can also downregulate IRE1α expression via its enhanced degradation [82], and mice deficient in ERAD protein SEL1L are more susceptible to experimental colitis induced by DSS administration in an IRE1α-dependent manner. This underscores the important role of ERAD function in the limitation of ER stress and associated damage.

In recent years the lysosomal degradation of a part of ER after trafficking it into a membranous vesicle in a selective manner has been referred to as ER-phagy. It is also synonymous with autophagy and occurs via a delicate action of associated receptors such as a family with sequence similarity 134 (FAM134), Sec62, cell cycle progression gene 1(CCPG1), reticulon 3 (RTN3L), etc. The specific roles of these proteins have been reviewed extensively in excellent articles elsewhere [83,84]. Basically these receptors play an important role in the incorporation of damaged portions of ER into autophagosomes with an intricate interaction with LC3. This facilitates the selective removal of damage ER and can also help in thwarting further cellular damage. It has been suggested that ER-phagy can subsidize ER stress and maintain homeostasis [85]. In addition, the selective degradation of procollagen by the ER-phagy receptor FAM134B in association with calnexin promoted the degradation of misfolded procollagen [86]. The consequence of accumulation in misfolded procollagen can be ER stress; hence, it can be considered that ER-phagy can limit potential stress in the ER by the degradation of misfolded procollagen. Furthermore,
starvation triggers the truncation of FAM134B, which was upregulated by C/EBPβ, and the ultimate consequence lies in the degradation of apolipoprotein C III (ApoC III). Noteworthy, although secreted by the liver, high levels of ApoC III can have detrimental consequences on the cardiovascular system [87]. In this way, hepatic ER-phagy can provide protection beyond the liver as well.

6. ER Stress Relays Signals to Mitochondria for Inducing Cell Death

Although there are many contact sites between ER and mitochondria, some experiments in cell-free systems led to speculation that mitochondrial involvement may not be required or would be minor for ER stress-induced apoptosis as assessed by effective caspase-3 and 9 processing in the absence of mitochondria by thapsigargin-treated microsome extract. Even so, the requirement of mitochondria was not completely ruled out because mitochondria might still be involved in amplifying apoptotic death, especially in the long run [88]. However, other studies have suggested that ER stress can relay signals to mitochondria to induce apoptosis [89]. ER can also communicate with mitochondria via various signaling events, including the release of Ca²⁺ (Figure 4). Noteworthy, the requirement of Ca²⁺ for the proper folding of proteins has been long documented [90]. Additionally, its release might overburden ER via an increase in misfolded proteins. In addition, the release of Ca²⁺ from the ER can cause an overload in mitochondrial calcium via enhanced uptake, thereby disrupting mitochondrial homeostasis [91,92]. In another case, this can also lead to enhanced release of calcium from the mitochondria in a process termed “calcium-induced calcium release”. These processes can lead to impairment in mitochondrial functions and promote apoptosis via the release of cytochrome c, which can amplify the apoptotic pathway. Furthermore, calcium can also be implicated in cell death via activation of calcium-dependent proteases called calpains, which are involved in cell death machinery either independently or via activation of the classical apoptotic pathway [93–97]. Chen and colleagues administered tunicamycin to C57BL/6 mice and observed that, as expected, tunicamycin administration led to the upregulation of ER stress-marker CHOP. Additionally, they also observed a marked activation of calpain 1 in cytosol and mitochondria, as well as a decrease in complex I activity and dysfunction in mitochondria [98]. This verifies that ER stress can induce activation of calpain 1, and this co-exists with a reduction in mitochondrial function and could possibly have a causative role, too. Another mechanism by which ER stress can induce mitochondrial damage involves the upregulation of mitochondrial reactive oxygen species [99]. These can cause an attack on mitochondrial membranes, thereby leading to the release of various mitochondrial proteins which modulate apoptotic cascade. Oxidative stress and ER stress have a close association, and inhibition of one can affect the other in a similar fashion. In one study, inhibition of oxidative stress resulted in the downregulation of ER stress-markers along with a reduction in calpain-1 expression and apoptotic markers [100]. Thus, on cumulative assessment, it would appear that the involvement of mitochondria may not be an absolute requirement for the induction of ER stress-mediated apoptosis, but in certain situations it can play a decisive role with regard to cell fate in an ER stress-dependent manner.
we consider as an upstream signaling event in a cellular setting where several such events follow continuously? Despite clear evidence, this is a complex scenario that needs further research to elucidate the underlying mechanism. In addition, ER stress is known to induce upregulation of tumor necrosis factor (TNFα) to induce cell death [109], while on the other hand, it is also known that TNFα can also lead to ER stress [108,110]. Furthermore, in conditions where TNFα alone is insufficient to induce hepatocyte death, activation of ER stress
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can promote cell death [111], suggesting a cooperative role in inducing cytotoxicity. TNFα released from macrophages can also lead to non-alcoholic steatohepatitis (NASH) and HCC during conditions of over nutrition [112]. This indicates multiple possibilities, including a collaboration of these signaling mechanisms, such as additive, synergistic or sensitizing effect of ER stress with TNFα in its signaling mechanisms leading to cell death. Another cytokine reported to influence hepatic physiology via ER stress is IFNγ. It has been observed that IFNγ can be involved in hepatocyte death in relation to ER stress via mitochondrial dysfunction characterized by the release of cytochrome c into cytosol [113]. Furthermore, antioxidants prevented such damage, which suggests antioxidants as a possible therapeutic agent in liver disorders that arise due to aberrant ER stress by interferon-gamma (IFNγ). In addition, IRE1α can also accelerate HCC development via IL-6 and TNFα-dependent STAT3 activation [114]. Furthermore, interleukin-8 (IL-8) is also reported to be involved in the regulation of ER stress and lipogenesis. It has been observed that although IL-8 may not be directly involved in direct cell death due to palmitate-induced ER stress, it can promote the migration of macrophages and possibly contribute to enhanced inflammation [63]. A collective assessment of these reports strongly indicates a feedback loop between ER stress and cytokine signaling to modulate cell death under different experimental conditions.

Notably, there is also some evidence suggesting no connection between ER stress and cytokines in modulating hepatic physiological responses, such as suppression of bile acid synthesis [115]. Also, in some cases, TNFα released by hepatic macrophages in an ER stress-dependent manner can direct towards the possible resolution or prevention of hepatic fibrosis by inducing apoptosis of activated hepatic stellate cells [116]. To sum up, an aggregate analysis of these studies indicates close cooperation of ER stress and various cytokines in modulating hepatic physiological responses, with some exceptions.

8. Involvement of ER Stress in Liver Diseases

ER stress has been implicated in a wide range of liver diseases. These include metabolic disorders such as insulin resistance caused by disrupted insulin signaling resulting in AKT inactivation. Inactivation of AKT or its upstream kinase PI3K can have detrimental effects on various cell types, including hepatocytes [117–119]. Additionally, it can accompany hepatic steatosis, as well as conditions stemming from the interplay of inflammation and metabolic disorders such as steatohepatitis, fibrosis and cirrhosis [120,121]. ER stress can lead to NASH through a diverse set of mechanisms, including inflammasome activation [122], calcium overload leading to alteration in mitochondrial dynamics and function [99], oxidative stress and dampening of the antioxidative responses [123,124]. Although there are multiple mechanisms that could lead to NASH by ER stress, the fundamental essence lies in the requirement of lipid accumulation and the presence of inflammation driven by any of the aforementioned processes associated with ER stress. ER stress-dependent lipotoxicity has been widely reported on the administration of saturated fatty acids (FA), particularly palmitic and stearic acid [125]. While the presence of FA in plasma can be extended beyond these fatty acids, nevertheless, these are the major saturated fatty acids found in plasma and their level correlates with the degree of hepatic damage [126,127]. In addition, FAs, especially palmitic acid (PA)-induced hepatic lipoapoptosis, have been attributed to lysophosphatidylcholine generated by phospholipase A2 (PLA2) [128]. Likewise, subsequent reports have uncovered that ER stress contributes to hepatic toxicity by lysophosphatidylcholine [129]. Thus, establishing a link between FA metabolism and hepatotoxicity with an involvement of ER stress. The ultimate consequence of these is observed in the form of lobular inflammation, hepatocyte ballooning, necrosis and a rise in liver enzymes, including alanine transaminase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALKP) in the serum [130,131]. It is intriguing to think about how a mechanism such as UPR that is primarily associated with the accumulation of misfolded proteins leads to enhanced steatosis. However, it turns out that ER stress responses promote the activation of transcription factors associated with lipogenesis, including the master regulator sterol regulatory element-binding protein (SREBP) and fatty acid synthase (FAS),
which ultimately leads to upregulation in lipids [132]. ER stress can also amplify hepatic inflammation via the regulation of major transcription factors associated with upregulation in inflammatory response, such as nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB) and JNK [133]. It needs to be noted that NFkB can also have a protective role in the liver, but extensive activation of NFkB can shift the response towards adverse effects rather than beneficial [134–137]. In this way, with enhanced lipogenesis and inflammation, ER stress regulates both hepatic steatosis and steatohepatitis well within the models of the 2-hit, parallel-hit or multiple-hit hypotheses of NASH. In fact, cumulative assessment of a predominantly large number of studies indicates the involvement of ER stress in some way in almost all liver pathologies, with a common factor being lipid accumulation and inflammation, although notable exceptions are also reported where ER stress did not contribute in any adverse physiological event [138]. In spite of this, it is quite exciting to assess the fact that since ER stress modulates such a large number of hepatic pathologies, inhibition of ER stress might be a common therapy for predominant hepatic disorders. In line with this hypothesis, there have been some encouraging results, mainly in experimental settings.

In addition to NASH, ER stress is associated with liver diseases primarily driven by abnormal activation of the adaptive immune system, including autoimmune hepatitis and primary biliary sclerosing cholangitis (PBC) [27,139]. These conditions arise because of the abnormal activation of the adaptive immune responses, particularly T and B lymphocytes producing autoantibodies against various hepatic proteins such as anti-asialoglycoprotein receptor (anti-ASGPR), anti-nuclear antibodies (anti-ANA), anti-liver kidney microsomal antibody type 1 (anti-LKM1) [140,141], etc. Although ursodeoxycholic acid (UDCA), which is a popular ER stress inhibitor, has not shown any significant protective effect when administered alone in AIH patients, it has shown remarkable effect as an adjunct agent when administered with prednisolone [142,143]. This suggests that ER stress alone may not be a causative factor in AIH and either collude with other adverse stimuli to take part in AIH or have no direct causative role in AIH. To the contrary, it alone was found effective in reducing IgG, ANA and SMA in Japanese patients with AIH [144]. These seemingly conflicting reports evoke questions about whether AIH simply correlates during ER stress conditions or ER stress, in fact, leads to AIH. Also, is there some genetic predisposition to it? And finally, did UDCA act independently of ER stress? A long time has gone by since these studies, yet these questions seem unanswered.

Since the liver is a major site of drug detoxification, it is affected to a high degree by various drugs and their metabolites. This is often accompanied by a loss in hepatocyte function due to apoptosis or necrosis [145,146]. It has been observed that ER stress contributes substantially to drug-induced liver injury via the induction of these cell death pathways [147,148]. Additionally, alcohol is also reported to exert a significant level of hepatic damage via multiple pathways. It can induce steatosis and also lead to amplification of inflammation [149]. Additionally, the generation of ROS and acetaldehyde following alcohol metabolism by alcohol dehydrogenase and CYP2E1 can incite detrimental effects on liver physiology by damaging membrane proteins and lipids via oxidation along with suppression of antioxidative responses [150]. Incidentally, ER stress has a causative role in all of these hepatic pathophysiology. Similar to that in NASH, ethanol can also induce activation of the major transcription factors associated with hepatic inflammation, such as NFkB, JNK, p38MAPK, etc. [151]. These transcription factors then promote the upregulation of proinflammatory cytokines such as IL-1β, TNFα and interleukin 6 (IL-6), among others, to aggravate hepatic damage [152–154]. Conversely, the inhibition of these pathways can provide hepatoprotection during ethanol administration [155,156].

Multiple lines of evidence indicate that ER stress plays an important role in various stages of HCC [112,157,158]. Additionally, it was also observed that PERK-dependent signaling played a crucial role than other branches of UPR in tumorigenesis, as inhibition of PERK showed a remarkable anti-tumor effect in comparison to inhibition of other arms of UPR [159,160]. Furthermore, it is also a crucial link in the connection between NASH
and its transformation into HCC [24]. Moreover, it contributes substantially to the process of chemoresistance and incites a detrimental role in cancer therapy [161].

Therefore, ER stress can be considered a connecting link in most hepatic disorders presented with excess deposition of lipids, the occurrence of inflammation and oxidative stress with resulting hepatic abnormality as in Table 1. Hence, targeting ER stress could produce a sufficiently beneficial outcome by subsidizing these hepatic-cellular abnormalities.

Table 1. Hepatic disorders associated with ER stress.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Mechanism</th>
<th>Main UPR Protein</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steatosis</td>
<td>Upregulation of lipogenic genes</td>
<td>ATF4</td>
<td>[162]</td>
</tr>
<tr>
<td>Steatohepatitis</td>
<td>Inflammation and steatosis</td>
<td>PERK-CHOP</td>
<td>[58]</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>SMAD2 upregulation in HSC</td>
<td>CHOP</td>
<td>[163]</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>Hepatic apoptosis and inflammation</td>
<td>CHOP</td>
<td>[58]</td>
</tr>
<tr>
<td>AIH</td>
<td>Enhanced IL17A and Treg suppression</td>
<td>ATF6α</td>
<td>[139]</td>
</tr>
<tr>
<td>PBC</td>
<td>Accumulation of hydrophobic bile acids</td>
<td>CHOP, IRE1α</td>
<td>[27]</td>
</tr>
<tr>
<td>DILI</td>
<td>Hepatocyte apoptosis, necrosis</td>
<td>CHOP, ATF6α</td>
<td>[164,165]</td>
</tr>
<tr>
<td>HCC</td>
<td>Metabolic dysfunction and inflammation</td>
<td>IRE1α, XBP1</td>
<td>[166,167]</td>
</tr>
</tbody>
</table>

9. Therapies Targeting ER Stress in Liver Disease

Several compounds have been tested for their effects on the reduction of ER stress and the subsequent mitigation of associated pathologies (Table 2). In one study, asiatic acid was shown to be hepatoprotective via induction of autophagy [168]. Similarly, free fatty acids-induced ER stress and associated hepatic steatosis were inhibited by astragaloside via activation of AMPK [169]. In yet another study, berberine, which is an isoquinoline alkaloid has been reported for its efficacy in limiting ER stress and hepatic damage by free fatty acid and LPS [170]. Reduction in activation of hepatic stellate cells by shikonin via inhibition of autophagy and increase in JNK phosphorylation conferred an anti-fibrotic effect [171]. Dantrolene is another compound known for its effect in inhibition of ER stress and improvement in hepatic steatosis [172], but it has some controversial information because it has been reported as a hepatoprotective agent in some studies while reports accounting for its noxious effects [173,174] are also in existence. In a CCl₄ model of hepatic fibrosis, resveratrol demonstrated a significant anti-fibrotic effect as observed via suppression in hepatic stellate cells-activation characterized by downregulation in CCL₄-induced upregulation of stellate cell activation-markers such as collagen-1 and α-SMA via suppression of ER stress [175]. Salubrinal is another agent that can limit hepatic damage via inhibition of ER stress especially by targeting PERK signaling [176]. Suppression of ER stress by agents such as allin, capsaicin and gingerol prevented hepatic steatosis [177]. The hepatoprotective properties of some of these agents such asiatic acid, baicalein and ginsenoside derivatives [178] lie in the vicinity of ER stress inhibition and the resultant autophagy induction. Autophagy is a complex process which have a dual role in cytoprotection or cytotoxicity in different cell types and experimental conditions, and also the interrelationship between ER stress and autophagy is complicated. For instance, ER stress can also lead to induction of autophagy and not necessarily inhibition of autophagy at all stages and conditions [179], so due considerations should be made in interpretation of these studies along with transformation into a suitable clinical setting.

Although many agents have been reported for their protective effect in hepatic disorders via inhibition of ER stress, they are still in the preliminary stages of transformation.
into any relevant effect in human subjects. On the other hand, UDCA and its taurine conjugate taurosodeoxycholic acid (TUDCA) have shown a remarkable degree of safety and efficacy against a lot of hepatic disorders, including PBC, steatosis, steatohepatitis, fibrosis, etc. [180]. UDCA can bind to the hydrophobic regions of proteins and prevent their subsequent aggregation. The consequence of this could be the stabilization of the misfolded proteins or the facilitation of their degradation through cellular degradation pathways. Furthermore, it has also been approved by the FDA for the treatment of cholestatic liver disease. However, TUDCA is not infallible because a report highlights that it is not effective in preventing adipose tissue IR [181]. This can be of particular relevance because of the close relationship between adipose tissue dysfunction and hepatic pathologies. Nonetheless, in a large number of studies, TUDCA has shown a remarkable level of efficacy in countering ER stress and mitigating adverse hepatic events, so TUDCA-included tailored therapies directed towards a specific condition might also prove to be efficacious, especially as an adjuvant therapeutic. Although a few reports are in line with this hypothesis, more research would be required to delineate if, in fact, this strategy would provide the supposed benefit. This safety and efficacy of UDCA and its derivative is preserving some hope in the management of other hepatic disorders, too. Additionally, another such compound popularly used in the inhibition of ER stress and associated hepatic pathologies is 4-PBA. It is a prodrug and is converted to its active metabolite phenylacetate via β-oxidation, which is involved in ammonia scavenging. The basic mechanism by which it can inhibit ER stress lies in the abrogation of protein synthesis so as to allow some recovery time by preventing incoming protein load into ER [22]. It is found effective in various hepatic disorders, even though it may not fare so well in comparison to TUDCA in certain situations, such as partial hepatectomy [182]. Nonetheless, it has the ability to scavenge ammonia and has been approved by the FDA for the management of urea cycle disorders. For the most part, inhibition of ER stress has provided beneficial effects outweighing the risks. However, it also needs to be noted that ER stress-targeting therapies can have some off-target effects, too. For instance, a compound called GSK 157 was developed as an inhibitor of PERK, and while it can inhibit PERK at concentrations in the nanomolar range, it has an untoward effect at high concentrations by activating integrated stress response in a GCN2-dependent manner [183]. Additionally, there are other inhibitors of PERK too that demonstrated some off-target effects. Specifically, the compounds GSK2606414 and GSK2656157 have been demonstrated to significantly inhibit RIPK and prevent TNF-induced death [184]. While prevention of cell death in healthy cells can be beneficial, these reports indicate a propensity of ER stress inhibitors to exert some off-target effects. Any similar or dissimilar off-target effect could have accounted for some side effects of ER stress-targeting therapies [185]. However, it needs further exploration to be assured of the matter.

Hepatocellular carcinoma is another detrimental consequence of ER stress, and inhibition of ER stress provides a beneficial effect by reducing the growth of tumors in vivo [114]. In addition, spliced XBP-1, which is a product generated by IRE1α under conditions of ER stress, has been found to enhance the metastatic potential of HCC cells [167]. Additionally, IRE1α has also been reported to be implicated in the proliferation of tumor cells [166]. Moreover, the reduction of ER stress by 4µ8c resulted in enhanced sensitivity of doxorubicin against HCC [186]. While some agents inhibit HCC by limiting ER stress, there are also some agents that promote cell death of HCC cells by activating ER stress. For instance, sinulariolide is one such compound that can induce apoptosis of HCC HAA2T cells by activating the PERK-CHOP arm of ER stress [187]. Another agent that can induce apoptosis of HCC cells via activation of lethal ER stress is piperlongumine [188]. A cumulative analysis of multiple reports such as these suggests that the connection between HCC and ER stress is quite complicated and might be influenced by the dual nature of ER stress itself. Also, due considerations must be performed during the interpretation of these data with a special emphasis on whether any effect observed is, in fact, associated with ER stress or is due to the direct effect on affected molecules associated with the UPR pathway.
Furthermore, an interesting phenomenon is shown by a flavonoid called kaempferol, which is reported to induce apoptosis of hepatic cancer cells [189] and also protect the liver against the experimental injury induced by LPS/D-galactosamine [190]. Additionally, these beneficial effects are facilitated by its ability to differentially modulate ER stress, where it induces ER stress, resulting in apoptosis of cancer cells while inhibiting ER stress to exert a protective effect against LPS/D-galactosamine-induced liver injury. Natural compounds that possess such dual properties to protect the normal cells against inflammatory death and also prevent cancer growth would be among the best candidates to achieve maximal therapeutic benefit at a potentially low risk. Hence, further research in these aspects must be duly considered.

Table 2. Therapies that target ER stress to ameliorate liver disease.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Major Mechanism</th>
<th>Target UPR Protein</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asiatic acid</td>
<td>Autophagy induction</td>
<td>All</td>
<td>[168]</td>
</tr>
<tr>
<td>Astragaloside</td>
<td>AMPK activation</td>
<td>PERK-CHOP</td>
<td>[169]</td>
</tr>
<tr>
<td>Berberine</td>
<td>Reduction in inflammation</td>
<td>CHOP, ATF4, XBP1</td>
<td>[170]</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>Inhibit HSC activation</td>
<td>CHOP</td>
<td>[175]</td>
</tr>
<tr>
<td>Salubrinal</td>
<td>Inhibit NFκB activation</td>
<td>PERK</td>
<td>[176]</td>
</tr>
<tr>
<td>Allin, capsaicin</td>
<td>Prevent steatosis</td>
<td>Multiple</td>
<td>[177]</td>
</tr>
<tr>
<td>gingerol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sinularolide</td>
<td>Prevent HCC</td>
<td>PERK-CHOP</td>
<td>[187]</td>
</tr>
<tr>
<td>Piperlongumine</td>
<td>Prevent HCC</td>
<td>CHOP</td>
<td>[188,191]</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>Prevent NASH and HCC</td>
<td>CHOP</td>
<td>[190,192]</td>
</tr>
<tr>
<td>4µ8c</td>
<td>Prevent HCC</td>
<td>XBP1s</td>
<td>[186]</td>
</tr>
<tr>
<td>UDCA, TUDCA,</td>
<td>Multipotent including Steatosis, NASH, PBC, etc.</td>
<td>Multiple</td>
<td>[193]</td>
</tr>
<tr>
<td>4-PBA</td>
<td>Counter urea cycle disorders</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inhibit steatosis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

10. Conclusions

The nucleus is often referred to as the director of the cell, and mitochondria as the powerhouse of the cell, but the endoplasmic reticulum does not have a supposedly cool and catchy phrase associated with it, yet its diligence in maintaining vital cellular functions and homeostasis to make sure the cellular functions run smooth (pun intended) makes it an “unsung hero” of the cell. Specifically, any mechanism, such as ER stress, that challenges and disrupts its homeostasis needs to be effectively countered to sustain cellular function and prevent any hepatic physiological abnormalities. Although there have been multiple successful agents in preventing or limiting ER stress-dependent pathologies, careful insight is required to tailor the regimen so as to enhance a maximal beneficial outcome. In addition, looking at the success of CHOP-inhibition on multiple instances of ER stress-induced physiological disorders, it is highly likely that it could be an attractive target to limit subsequent pathologies that arise due to ER stress. Furthermore, the implication of its dual role as a cytoprotective as well as a cytotoxic agent also needs to be considered and explored in further detail. To sum it up, much information has been obtained regarding the physiological processes that govern ER stress and its implication in various hepatic disorders. Additionally, few agents with extreme safety and efficacy, such as TUDCA, have been discovered, yet the transformation of laboratory findings into clinical settings is incomplete and limited to a few ailments. Hence, further research is required to look at ER stress from multiple aspects, especially in conditions where limiting ER stress did not successfully eliminate cellular and physiological abnormalities. This would be beneficial
on multiple levels; for instance, it might help us understand the fundamental aspects of ER stress and the governing mechanisms, which could hopefully help in designing an appropriate remedy to tackle the respective pathologies.

11. Future Directions

Since a major focus in drug discovery would be the search for an active therapeutic agent with a maximum safety profile and minimal side effects, UDCA, TUDCA and 4-PBA, so far, seem the best candidates for their reasonable degree of safety and efficacy. Therefore, while it is necessary to keep searching for new drug moieties that can target ER stress to resolve hepatic pathologies, due attention must also be given if it is possible to repurpose these to manage several other hepatic ailments in addition to PBC and urea cycle disorders.

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