Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer, accounting for 85% to 90% of all cases of liver cancer. It is a hepatocyte-derived primary tumor that causes 550,000 deaths per year worldwide, making it the second cause of cancer-related deaths. The incidence of HCC is currently increasing worldwide but varies considerably per region due to differences in the prevalence of underlying risk factors. Risk factors for HCC include: hepatitis B or C virus infections; metabolic disorders such as obesity, type 2 diabetes, and nonalcoholic fatty liver disease; and alcohol abuse or aflatoxin B1 exposure. Most of these risk factors result in chronic liver injury, which leads to fibrosis and cirrhosis, thereby creating a favorable environment for the development of HCC. The main risk factor for HCC in Eastern Asia is hepatitis B virus. However, in the western society, it is currently shifting toward obesity, type 2 diabetes, and nonalcoholic fatty liver disease. HCC remains a major health problem, with a 5-year survival of only 20% due to late detection and limited treatment options. This highlights the need for a better understanding of the pathogenesis of HCC to pave the way for new treatments and improve survival of patients with HCC.

Studies over the last decade indicate that endoplasmic reticulum (ER) stress plays an important role in liver cancer. ER stress is the accumulation of misfolded or unfolded proteins in the ER lumen, which occurs when the capacity of the ER to correctly fold proteins is exceeded. The presence of these proteins in the ER lumen activates the unfolded protein response (UPR), which functions to restore protein homeostasis by slowing down protein translation, increasing the protein folding machinery, and...
up-regulating the degradation of unfolded proteins. However, in case of persistent or excessive ER stress, the UPR can induce pathways leading to cell death. The proposed key role of the UPR in cancer is to protect tumor cells from apoptosis and support their uncontrolled proliferation. Therefore, ER stress and the UPR may have a relevant role in HCC pathogenesis, which could reveal new targets for HCC treatment and diagnosis.

Metabolism has recently gained importance in the pathogenesis of HCC due to the pivotal role of the liver in different metabolic processes. Risk factors for HCC include aberrant metabolism disorders such as nonalcoholic fatty liver disease, obesity, and type 2 diabetes. In addition, the deregulation or reprogramming of glucose metabolism is considered a hallmark of solid tumors, as it helps to fuel tumorigenesis and allows cells to thrive under hypoxic circumstances. Lastly, most of the metabolic processes in the liver are performed by hepatocytes, which comprise 85% of the total mass of this organ. Thus, any transformation of hepatocytes during HCC, such as mutations, increased proliferation, or replicative immortality, leads to metabolic alterations. Interestingly, ER stress and metabolic deregulation are functionally intertwined, and both are considered contributing factors to the pathogenesis of HCC (Figure 1).

The aim of the current review is to summarize the available knowledge that links ER stress and metabolism in HCC. It first provides an overview on the UPR and its involvement in HCC. Next, the role of the liver in the different metabolic pathways and the alterations found in HCC are presented. Finally, the connections between the disrupted metabolism and ER stress in HCC are disclosed.

**Unfolded Protein Response**

ER stress is the physiological condition characterized by an accumulation of misfolded or unfolded proteins in the lumen of the ER. This can be triggered by several stimuli, including a high rate of cell proliferation, hypoxia, nutrient deficiency, and abnormal redox homeostasis. The presence of misfolded or unfolded proteins in the ER lumen is sensed by three transmembrane proteins, namely activating transcription factor 6 (ATF6), inositol-requiring enzyme 1 alpha (IRE1α), and protein kinase RNA-like endoplasmic reticulum kinase (PERK). These proteins each activate their own signaling cascade to induce the UPR. During protein homeostasis, binding immunoglobulin protein (BiP), alias Grp78 or heat shock 70 kDa protein 5, is bound to ATF6, IRE1α, and PERK, keeping them in their inactive conformation. Upon ER stress, BiP preferentially binds to unfolded or misfolded proteins in the ER lumen, thereby dissociating from the ER stress sensors and exposing their interactions.
luminal domains. This facilitates their activation and triggers the UPR, orchestrating a complex signaling network aiming to reduce protein translation, increase the folding capacity of the ER, or, in case of severe or prolonged ER stress, activate proapoptotic pathways.

Inositol-Requiring Transmembrane Kinase Endoribonuclease-Alpha

The most prominent and evolutionarily conserved UPR signal transducer is IRE1α, which gets activated as BiP dissociates from its binding site on the receptor. This action results in homodimerization and subsequent autophosphorylation of its kinase domain. Activated IRE1α also exerts an endonuclease function, which enables splicing of X-box binding protein 1 (XBP1) messenger RNA (mRNA) into its active form, called spliced XBP1 (sXBP1). sXBP1 is then able to act as a transcription factor, which induces transcription of a range of proadaptive genes, including ER chaperones and genes involved in ER stress-associated protein degradation. Misfolded or unfolded proteins in ER stress-associated protein degradation are transported to the cytoplasm for ubiquitination and degradation by the proteasome. In addition, the endonuclease activity of IRE1α regulates IRE1-dependent decay, a process in which several ER-bound mRNAs or precursor microRNAs are spliced. These two processes reduce the number of misfolded or unfolded proteins and mRNA, decreasing the protein-folding load of the ER and hence ER stress. In addition to its primary proadaptive role, IRE1α induces proapoptotic pathways during prolonged ER stress. This is initiated by the assembly of IRE1α with tumor necrosis factor receptor-associated factor 2 and apoptosis signal-regulating kinase 1. Together, they form a signaling complex, which further activates downstream signaling pathways leading to apoptosis. Additionally, regulated IRE1-dependent decay can contribute to apoptosis in case of severe ER stress. Therefore, IRE1α can activate both proadaptive and proapoptotic pathways, depending on the level and duration of ER stress.

Protein Kinase RNA-like ER Kinase

The second transmembrane ER stress protein is PERK, which becomes activated by dimerization and subsequent auto-phosphorylation upon dissociation of BiP during ER stress. After activation, PERK phosphorylates the alpha subunit of eukaryotic initiation factor 2 (eIF2α), resulting in a general suppression of protein translation. The cell cycle regulator cyclin D1 is one of the many proteins that are down-regulated; this action causes the cell cycle to arrest and offers the cell some time to restore protein homeostasis. Simultaneously, phosphorylated eIF2α enhances translation of a specific set of mRNAs, including activating transcription factor 4 (ATF4). This protein can induce transcription of various genes involved in the adaptation to

ER stress, such as genes involved in autophagy and reactive oxygen species protection. In contrast, ATF4 can induce transcription of CCAAT-enhancer-binding protein homologous protein (CHOP), also known as growth arrest, and DNA damage inducible gene (GADD153), which stimulates expression of proapoptotic genes. Hence, as with IRE1α, PERK induces both proadaptive and proapoptotic pathways.

Activating Transcription Factor

The last transmembrane ER stress protein is ATF6. It is translocated to the Golgi apparatus upon dissociation of BiP, where it is cleaved into its active form by site-1 and site-2 proteases. Active ATF6 acts as a transcription factor and stimulates the production of ER chaperones, including BiP, which aid in the folding of proteins and hence reduce ER stress. In addition, it induces the production of XBP1 mRNA that can subsequently be spliced by IRE1α, leading to the initiation of downstream signaling pathways. ATF6 is one of the first branches to be activated, creating a time frame for the cell to restore protein homeostasis solely by an increase in ER chaperones before activation of other pathways. This process highlights the importance of ATF6 in the initial stages of ER stress.

UPR in HCC

Several characteristics of solid tumors are known inducers of ER stress, making ER stress a novel hallmark of cancer. For example, a high rate of cell proliferation, hypoxia, nutrient deficiency, and abnormal redox homeostasis can all trigger the UPR. In addition, tumor cells possess numerous mutations that can induce the production of misfolded proteins. Furthermore, ER stress can also be induced by viral infections such as hepatitis B or C virus, which are both known risk factors for HCC. As a result, an increased expression of ATF6, BiP, and sXBP1 has been seen in HCC patients with HCC of different etiologies, thus indicating an up-regulation of the ATF6 and IRE1α branches of the UPR in HCC.

The up-regulation of the UPR can contribute to the survival of tumor cells, leading to the development and progression of cancer. Studies have shown that the PERK branch of the UPR is crucial for inducing tumor cell proliferation and growth by altering redox homeostasis. This process prevents the activation of oxidative DNA checkpoints, allowing tumor cells to survive in hostile tumor microenvironments. Studies have shown that pharmacologically targeting different arms of the UPR can form a promising target to slow down tumor growth. For instance, inhibition of IRE1α reduces the development of HCC in a diet-induced model for obesity in vivo. Tumor cell proliferation and metastasis is reduced in a chemically induced HCC mouse model when IRE1α endonuclease activity is pharmacologically inhibited. Similarly, a study...
by Vandewynckel et al.\textsuperscript{25} attempted to investigate pharmacologic inhibition of PERK by GSK2656157, which suggested reductions in the expression of ER chaperones and tumor growth in an HCC mouse model. However, a more recent study showed that GSK2656157 and GSK2606414, which are commonly used with the purpose of inhibiting PERK, are in fact not specific for PERK, thereby questioning the accuracy of previous studies using these inhibitors.\textsuperscript{26} Lastly, not much is known about the effect of inhibition of the ATF6 branch on HCC pathogenesis, which could be interesting for further research due to its suggested up-regulation and/or missense polymorphisms in HCC.\textsuperscript{27,28} For instance, there have been cases in which a missense single-nucleotide polymorphism can significantly increase the expression levels of ATF6 mRNA and/or ATF6-regulated genes, such as \textit{GRP78}, \textit{CHOP}, and \textit{XBP1}, contributing to HCC susceptibility. This missense mutation is strongly associated with hepatitis B virus—related HCC, with a reported frequency of 28\% of HCC patients carrying this mutation.\textsuperscript{28}

**Carbohydrate Metabolism**

The liver is responsible for maintaining glucose homeostasis by continuously regulating glucose production and storage, and ER stress pathways interfere with these processes (Figure 2). To maintain glucose homeostasis, hepatocytes perform four metabolic reactions: glycolysis, glycogenolysis, and glucocorticoid synthesis. Glycolysis is a catabolic pathway of enzyme-catalyzed reactions that break down glucose to generate energy. In normal conditions, cells catabolize glucose to pyruvate and later to acetyl coenzyme A through the tricarboxylic acid cycle to finally enter the mitochondrial matrix, converted to acetyl coenzyme A (Acetyl-CoA) by pyruvate dehydrogenase complex, and enter the tricarboxylic acid cycle along with oxaloacetate, generating NADH and FADH2. Forkhead box O1 (FoxO1) is a key transcription factor in energy metabolism that controls hepatic gluconeogenesis via the transcriptional regulation of key genes, such as glucose-6-phosphatase. Loss of glucose homeostasis is a hallmark of cancer cells, and it remains unknown whether inhibition of UPR-associated and metabolism-associated modulators could be used to limit tumor proliferation. BiP, binding immunoglobulin protein; GLUT, glucose transporter; HK2, hexokinase 2; TCA, tricarboxylic acid; TPI, triosephosphate isomerase 1. Figure created with BioRender.com (Toronto, ON, Canada).
produce large amounts of adenosine triphosphate via oxidative phosphorylation. However, in hypoxic or anaerobic conditions, cells follow glycolysis instead, and metabolize glucose to lactate, producing smaller amounts of energy per glucose molecule.

In the 1920s, Otto Warburg reported that tumorigenic cells tend to shift their main energy source from oxidative phosphorylation to glycolysis, even under normal oxygen conditions. During this metabolic change, known as the Warburg effect, tumor cells consume larger amounts of glucose and produce energy in a faster manner, thus stimulating proliferation. Therefore, inhibition of enzymes involved in these pathways could potentially limit tumor proliferation. Down-regulation of triosephosphate isomerase 1 (TPI1), which is involved in the glycolytic and gluconeogenic pathway, reportedly inhibits HCC cell growth, migration, invasion, and survival. Moreover, TPI1 expression is significantly decreased in HCC tissues and is correlated with HCC progression and survival, suggesting that TPI1 might serve as a tumor suppressor. ATF4 knockdown reduces the expression of TPI1 in *Drosophila*, which suggests a role of the UPR in the regulation of this enzyme.

Another key glycolytic enzyme involved in tumor proliferation is hexokinase and, more specifically, one of its four isoenzymes, hexokinase 2 (HKx2). HCC exhibits aberrant expression of HKx2, contributing to early recurrence and poorer prognosis of surgically resected HCC. DeWaal et al. also observed that HCC cells express HKx2. Inhibition of HKx2 with 3-bromopyruvate promotes tumor cell death by inducing ER stress in human HCC cell lines (Huh-7 and SNU-761) and improves efficacy of sorafenib in *in vivo* models of HCC. Similarly, PERK silencing blocks the translocation of HKx2 to mitochondria via inhibition of p-Akt in glioma cells, which inhibits tumor growth. This suggests PERK-dependent regulation of HKx2 in tumor cells, linking ER stress to glycolysis. In addition, depletion of HKx2 results in inhibition of glycolysis and induction of oxidative phosphorylation, opposite to what tumor cells usually experience during the Warburg effect. This also sensitizes HCC cells to metformin, a compound broadly used in patients with HCC, which seems to regulate ER stress response in HCC cells, endothelial cells, and patients with type 2 diabetes. More specifically, metformin reportedly reduces activation of the PERK pathway, thus preventing activation of the proapoptotic arm of ER stress signaling.

The liver can produce glucose from glycogen (glycogenolysis) or from other molecules such as amino acids, lactate, pyruvate, and glycerol (gluconeogenesis). Phosphoenolpyruvate carboxykinase 1 (PCK1), the key enzyme of gluconeogenesis, plays a prosurvival role during the ER stress response and is involved in tumor cell adaptation to nutrient availability. Down-regulation of PCK1 has been shown to reduce tumor growth and promote oxidative stress and apoptosis in the malignant cells. Conversely, elevated blood glucose levels (hyperglycemia) activate glucose conversion and storage into glycogen in the liver via the process of glycogenesis. In 2018, Jin et al. reported that HCC progression can be reduced by targeting the phosphoglucomutase-1 enzyme. This enzyme promotes glucose flow to glycogenesis instead of glycolysis in several *in vivo* and *in vitro* models for HCC. Therefore, up-regulation of phosphoglucomutase-1 enzyme could potentially slow down cellular adenosine triphosphate production and HCC progression by regulating glucose trafficking. Expression of this gene has also been shown to be part of a hypoxia-related gene signature that could serve as an independent prognostic factor for HCC.

Other proteins and processes related to glucose metabolism might also be involved in the pathologic process of HCC. For instance, glucose metabolism is highly intertwined with ER stress and the UPR (*Figure 2*). ER stress impairs the systemic glucose metabolism in chronic metabolic diseases such as obesity, insulin resistance, and type 2 diabetes. In fact, a reduction in ER stress attenuates glucose metabolism disorders in livers of mice with type 2 diabetes. While ER stress has been shown to mediate the expression of glucose transporters (*GLUT1* and *GLUT2*) in diabetes mellitus and insulin resistance, specifically through the PERK/ATF4 branch, its role in tumorigenesis and HCC remains largely unknown. *GLUT1* and *GLUT2* are up-regulated in liver cancers to favor glucose uptake as an energy source, and inhibitors for these transporters have been described as potential antitumoral drugs.

ER stress suppresses insulin receptor signaling through the IRE1α pathway. This UPR pathway activates c-Jun N-terminal kinase, which phosphorylates the serine residues of insulin receptor substrate-1, leading to the inhibition of insulin signaling and impairment of glucose metabolism. The UPR mediator sXBPI interacts with Forkhead box O1, a transcription factor that enhances the expression of genes involved in gluconeogenesis such as phosphoenolpyruvate carboxykinase and glucose-6-phosphatase. The interaction between sXBPI and Forkhead box O1 results in proteasomal degradation of Forkhead box O1, and thereby down-regulation of the gluconeogenesis pathway, which is known to drive HCC. Other studies have found that IRE1α can act as a sensor of glucose metabolism and regulate glucagon signaling via activation by protein kinase A. The PERK/ATF4 branch of UPR also controls glucose levels, as inhibition of the PERK-eIF2α-ATF4 pathway enhances glucose metabolism in diabetic livers. Lastly, the ATF6 arm of the UPR is associated with impaired glucose homeostasis in type 2 diabetes. Together, these studies implicate a main role of ER stress and the UPR in the regulation of carbohydrate metabolism and clearly show their importance in the context of diabetes. However, how and whether this affects the development of HCC remain largely unknown and warrant further research.
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Lipid Metabolism

Deregulation of hepatic lipid metabolism, including synthesis, storage, and break down of lipids, is a driving force of liver cancer.\(^{57,58}\) During synthesis of lipids (lipogenesis), fatty acids are generated from acetyl coenzyme A molecules due to an excess of carbohydrates and proteins. Gene expression of enzymes and transporters involved in lipogenesis are up-regulated in HCC, including fatty acid synthesis, adenosine triphosphate/citrate lyase, or the sterol regulatory element-binding protein 1.\(^{57}\) The degradation of lipids (lipolysis) allows for the production of energy or ketone bodies via the tricarboxylic acid cycle or \(\beta\)-oxidation, respectively. Lipolytic enzymes, such as adipose triacylglyceride lipase, are also highly expressed in HCC, increasing lipid degradation and energy production.\(^{59}\) One of the genes responsible for altered lipid metabolism is liver kinase B1 (LKB1), which is mutated in 22% of HCC cases. Its allelic loss leads to abnormal expression and activation of multiple molecules related to lipid metabolism.\(^{60}\) Furthermore, mutations in feedback regulators of the Wnt pathways, such as Ring Finger Protein (RNF43) and Zinc And Ring Finger 3 (ZNRF3), have been shown to increase hepatic lipid accumulation and enhance tumorigenesis after liver injury, which could imply that RNF43/ZNRF3-mutated individuals might be at higher risk of developing HCC in a background of fatty liver disease.\(^{61}\) Nearly 20% of patients with HCC carry an activating mutation in the CTNNB1 gene, which is responsible for encoding beta-catenin in Wnt pathways. Beta-catenin—activated HCCs are characterized by enhanced fatty acid oxidation, reduced glycolysis, and up-regulated expression of peroxisome-related genes.\(^{57}\) Various peroxisome-related genes are also associated with aberrant tumoral metabolic signaling and act as key regulators in tumorigenesis and tumor progression, by affecting lipid synthesis and utilization.\(^{62}\) Recent studies have shown that alterations in lipid metabolism contribute to an increased energy production, thereby facilitating epithelial—mesenchymal transition and proliferation in HCC cells.\(^{63}\) Therefore, regulation of pathways involved in lipid metabolism might improve HCC prognosis.\(^{58}\)

Lipid metabolism is strongly intertwined with ER stress and the UPR. The ER is the main site of lipid production, and many enzymes involved in lipid metabolism are produced in the ER.\(^{64}\) In addition, the three UPR branches regulate lipid homeostasis by activating transcription factors and pathways that modulate lipid metabolism. For instance, IRE1z controls lipid metabolism by inducing the degradation of mRNAs encoding for lipid metabolism regulators through regulated IRE1-dependent decay,\(^{65}\) which has been specifically shown to be relevant in cancer.\(^{66}\) Moreover, the IRE1z/XBP1 branch is involved in the regulation of hepatic very-low-density lipoprotein production,\(^{67}\) and up-regulates peroxisome proliferator—activated receptor alpha (PPARA) expression during starvation, hence stimulating \(\beta\)-oxidation and ketogenesis.\(^{58}\) Studies on Drosophila have shown that IRE1z deficiency leads to an increased mobilization of lipids and sensitizes flies to starvation, thus indicating that IRE1z is a catabolic sensor acting through the XPB1s—FoxO axis.\(^{69}\) Studies with PERK/eIF2z have shown that this arm of the UPR can promote lipogenesis via different downstream effectors. PERK activates sterol regulatory element—binding protein 1 and 2 pathways, thereby increasing cholesterol, fatty acid, triacylglycerol, and phospholipid synthesis, thus leading to increased lipid accumulation in HCC cells.\(^{70}\) Lastly, ATF6 antagonizes sterol regulatory element—binding protein 2 to regulate the homeostasis of lipids and glucose but also increases fatty acid oxidation by controlling the activity of peroxisome proliferator—activated receptor alpha. Because the different arms of the UPR are suggested to participate in several processes involved in lipid metabolism, the relation between ER stress and lipid metabolism may be bidirectional. For example, triglycerides are shown to induce the expression of ER stress markers such as BiP, IRE1z, XBP1, p-eIF2z, CHOP, and p-JNK.\(^{71}\) Some studies suggest that ER stress mediates lipotoxicity by inducing apoptosis or modulating the membrane composition of phospholipids, but the specifics are still not completely understood.\(^{25,72}\) A widely understood hallmark of the UPR is an increase in de novo lipogenesis to allow the expansion of lipids into the ER. However, this phenomenon has mostly been studied in cell cultures, and a very recent in vivo study by Ward et al\(^{73}\) found that hepatic de novo lipogenesis and cholesterol-genesis might actually be reduced after tunicamycin-induced ER stress. Several chemical molecules, including cinnamaldehyde,\(^{74}\) Asiatic acid,\(^{75}\) and Schisandra chinensis extract,\(^{76}\) have been shown to improve chronic liver disease in vivo by regulating ER stress and lipid accumulation and metabolism. Therefore, targeting this interaction between ER stress and lipid metabolism may contribute to new potential therapies to treat liver diseases, including HCC.

Metabolism of Proteins

The excess of amino acids obtained from diet are also catabolized in the liver. In liver cancer, tumor cells can use the degradation of certain amino acids such as glutamine or leucine as an alternative energy source.\(^{77}\) Dysregulation of glutaminase and glutamine synthase is a hallmark of several cancers, including HCC. This prompted testing of several glutamine analogues and glutaminase inhibitors in preclinical and clinical studies, with variable success.\(^{25}\) Zhou et al\(^{79}\) found that HCC cells can survive and maintain tumor progression in low glucose concentrations by activating glutaminolysis through the up-regulation of glutamate dehydrogenase 1, a key enzyme in the degradation pathway of glutamine. Herein, glutaminase inhibitors can be used as an adjuvant treatment in cancer, reducing glutamine conversion...
to glutamate. Because glucose deprivation is a strong inducer of ER stress, glutamine treatment can reduce ER stress signaling in these glucose-deprived states. Glutamine synthetase (GS) is also increased in the majority of HCC tissues. Because GS can synthesize glutamine from glutamate, it allows cells to survive in glutamine-depleted conditions, thereby supporting an anaplerotic flux for glutamate, which feeds the tricarboxylic acid cycle. In a zebrafish model for HCC, GS can increase glutamine levels for nucleotide biosynthesis and support growth of liver cancer cells in a Yes-associated protein 1–dependent manner. Furthermore, clinical data have shown that patients with CTNNB1 mutations exhibit a 6– to 45-fold increase in mRNA expression of Glutamate-Ammonia Ligase, the gene encoding for GS. Because all of these tumors with increased GS expression also showed an increase in mammalian target of rapamycin signaling, this led to testing the combination treatment of mammalian target of rapamycin inhibition with rapamycin signaling, this led to testing the combination treatment of mammalian target of rapamycin inhibition with rapamycin in combination with a GS/β-catenin inhibitor CG1 (sobetirome). This combined treatment synergistically reduced HCC burden. These results indicate the intricate complexity and warrant the need for further studies to unravel the role of glutamine and GS in the pathophysiology of HCC.

Protein metabolism generates ammonia, a potentially toxic amino group, which is eliminated in the liver via the urea cycle. Genes of the urea cycle [carbamoyl phosphate synthetase-1 (CPS1), ornithine carbamoyl-l transferase, arginase, argininosuccinate synthase, and lyase], and its derived metabolites (citrulline, arginine, and ornithine) are decreased in HCC. A large-scale gene expression data analysis study has shown that dysregulation of genes involved in the urea cycle, and specifically down-regulation of CPS1, are characteristic of patients with HCC. Furthermore, CPS1-deficient HCC cells have a distinctive metabolic phenotype characterized by a deceleration of the tricarboxylic acid cycle, increased adenosine triphosphate levels, and higher dependency on fatty acid oxidation rather than glucose or glutamine.

In addition to amino acid and protein metabolism, the liver is responsible for >80% of the protein synthesis. Albumin, growth factors, and many other functionally important peptides and nonessential amino acids are produced by hepatocytes. Tumor cells usually increase their protein production to promote tumor growth and proliferation. The PERK branch induces biosynthesis of specific amino acids and up-regulation of their corresponding tRNA synthetases, suggesting that amino acid metabolism might be governed by protein synthesis demands during ER stress. Envelope protein E2 of hepatitis C virus, a risk factor for HCC, can modulate protein synthesis and ER stress through PERK activation. Furthermore, deprivation of various amino acids, such as glutamine, arginine, and leucine, seems to induce ER stress. Particularly in the liver, intake of l-leucine can reduce ER stress in the fatty liver by down-regulating splicing of XBP1-mRNA. All of these findings highlight the close relation between ER stress and protein metabolism in HCC and the possibilities for finding new therapeutic targets.

**Vitamin Metabolism**

The synthesis and storage of many vitamins, such as vitamins D and K, is another major function of the liver. Prior studies have reported reduced production of vitamins in several types of cancers, including HCC. Vitamin D has antitumor, antiproliferative, antiangiogenic, and proapoptotic activities, suggesting potential therapeutic benefits in HCC via the use of complementary vitamin D–based treatments. This has been further supported in anti-inflammatory and antifibrotic activities in HCC cell and mice models. Riek et al showed that vitamin D is a natural macrophage ER stress reliever, which could potentially further improve anticancer treatment, as it was previously shown that ER stress can promote a protumoral phenotype in macrophages. Vitamin K primarily participates in bone metabolism, but it is also responsible for the regulation of clotting factor production. As with vitamin D, supplementation with vitamin K appears to reduce HCC proliferation and invasion in HCC cells, improving upon the current treatments. Its role in ER stress is still unclear, although a recent study has shown that vitamin K can modulate organelle damage and decrease the expression of ER stress markers such as CHOP and IRE1α/XBP1. However, additional research is needed to establish its role in HCC.

**Nucleic Acid Biosynthesis and the Pentose Phosphate Pathway**

Nucleic acid metabolism is the final and most critical process for tumor cell replication and fast proliferation. Consequently, the synthesis of nucleic acids is also up-regulated in HCC. Genes involved in pyrimidine biosynthesis, such as carbamoyl phosphate synthetase 2, aspartate transcarbamylase, or dihydroorotase, are increased in HCC. This up-regulation has been associated with tumor cell stemness and poor prognosis. Similarly, the pentose phosphate pathway, which is a source of glycolytic metabolites for nucleotide metabolism, is highly active in HCC tumor cells. However, metabolite levels from this pathway are often reduced, suggesting a fast usage of intermediates for nucleotide biosynthesis. In addition, some upstream signaling pathways that regulate pentose phosphate pathway enzymes contribute to cancer initiation and progression, opening up the possibility of using them as targets in cancer therapy. Lastly, the nucleic acid metabolism is also related to ER stress as inhibition of both the pyrimidine metabolic pathway and the pentose phosphate pathway induces ER stress.
Detoxification and Drug Metabolism

Elimination and detoxification of drugs from the blood are mainly conducted by the liver. As a result, liver function, and particularly cytochrome activity in ER lumen, is crucial for the response to anticancer therapies and drug response. Down-regulation of cytochromes such as cytochrome P450 3A4 (CYP3A4) and UDP-glucuronosyltransferase 1A9 has been found in microsomes of patients with HCC; this action seems to decrease the metabolism of sorafenib, a drug widely used in HCC.\textsuperscript{98} In addition, several drugs, including anticancer compounds such as tunicamycin, doxorubicin, or erlotinib, can induce ER stress and drug-induced liver injury.\textsuperscript{99} These lead to hepatic lesions, steatosis, steatohepatitis, hepatocellular adenoma, and cirrhosis, among others, which occur prior to HCC and contribute to the development of an already existent tumor.\textsuperscript{100} In addition, hepatotoxicity commonly leads to reduction in drug dosing or even early termination of treatment, thus drastically decreasing the patient’s survival rate.\textsuperscript{101} Doxorubicin causes heart failure in some cancer patients through activation of the ATF6, PERK, and IRE1 pathways, which is reduced by combinational treatment with ER stress inhibitors such as alginolate oligosaccharide, sodium hydrosulfide, and sacubitril.\textsuperscript{102,103} Therefore, combination treatment of ER stress inhibitors with chemotherapeutics could potentially decrease adverse effects and improve therapeutic outcomes in several types of cancer (Table 1).

Table 1 Overview of Drugs and Toxins Influencing ER Stress Pathways and Potential Combinational Therapies

<table>
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<th>Compound</th>
<th>Affected ER stress pathways</th>
<th>Combinational treatments</th>
<th>Pathology</th>
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<tr>
<td>Tunicamycin</td>
<td>ATF6, PERK, and IRE1</td>
<td>Chemotherapeutics</td>
<td>Cancer</td>
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<td>Doxorubicin</td>
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<td>Alginolate oligosaccharide (antioxidant), sodium</td>
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<td>hydrosulfide (hydrogen sulfide donor), sacubitril</td>
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<td>Magnesium isoglycyrrhizinate (anti-inflammatory),</td>
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<td>kinsenoside (antioxidant) and geniposide (antioxidant)</td>
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<td>Erlotinib</td>
<td>PERK and XBP1</td>
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<td>Cancer</td>
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<td>Remdesivir</td>
<td>IRE1 and CHOP</td>
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<td>Coronavirus disease 2019</td>
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<tr>
<td>Alcohol</td>
<td>ATF6, PERK, and IRE1</td>
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<td>Alcoholic liver disease, HCC</td>
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ATF6, activating transcription factor 6; CHOP, CCAAT-enhancer-binding protein homologous protein; ER, endoplasmic reticulum; HCC, hepatocellular carcinoma; IRE1, inositol-requiring enzyme 1; PERK, protein kinase RNA-like endoplasmic reticulum kinase.

During the coronavirus disease 2019 pandemic, several drugs, including dexamethasone, hydroxychloroquine, and remdesivir, were repurposed for treating patients with coronavirus disease 2019 (Table 1). A recent study showed that both dexamethasone and remdesivir induced ER stress through IRE1\textsubscript{\alpha} and CHOP in HCC cell lines, as well as in healthy hepatocyte cell cultures, suggesting a possible hepatotoxic effect.\textsuperscript{104} The effect was exacerbated by pre-treatment with alcohol, thus further emphasizing the importance of factoring in drug—drug or alcohol—drug combinations on cellular stress responses, particularly in the context of liver injury.

The liver is crucial for the metabolism and detoxification of alcohol. When alcohol reaches the liver through the bloodstream, it is metabolized by alcohol dehydrogenase, catalase, or cytochrome P450 2E1 into acetaldehyde. This highly reactive metabolite is known to cause protein adduct formation, which results in mutations, conformational changes of proteins, and abnormal protein degradation.\textsuperscript{105} These alterations can cause ER stress and contribute to the development of alcoholic liver disease, thereby increasing the risk for HCC. Both acute and chronic alcohol exposure increases the expression of ATF6, CHOP, and BiP, and enhances the phosphorylation of PERK, eIF2\textsubscript{\alpha}, and IRE1\textsubscript{\alpha}.\textsuperscript{105,106} In mice subjected to chronic and binge ethanol feeding, levels of ERdj5, an ER-resident chaperone protein, are markedly increased, correlating with XBP1s mRNA levels, further supporting the direct effect of alcohol on ER stress.\textsuperscript{105} Loss of ERdj5 can aggravate alcoholic liver disease through inhibition of Nrf2, thus suggesting a protective role of ERdj5 in this pathology. In addition, CYP2E1-overexpressing primary human hepatocytes and HepG2 cells treated with alcohol exhibit an up-regulation in ATF4,\textsuperscript{106} suggesting that excessive alcohol intake increases the expression of ATF4. Together, this indicates that alcohol induces ER stress, resulting in activation of all arms of the UPR. Several pharmacologic approaches to reduce ER stress during alcoholic liver injury have been tested preclinically, including use of magnesium isoglycyrrhizinate,\textsuperscript{106} kinsenoside,\textsuperscript{107} and geniposide\textsuperscript{108} (Table 1). Whether these drugs are also effective in preventing the development of HCC in a background of alcoholic liver disease remains to be elucidated.

Conclusions

HCC is associated with increased ER stress and up-regulation of the UPR, which contribute to the development and
progression of HCC. In addition to ER stress, metabolism is highly altered in tumor cells to facilitate proliferation and tumorigenesis. This includes up- or down-regulation of enzymes in metabolic pathways, different enzymatic isoforms, and altered metabolite flow. However, the causes of the metabolic alterations in HCC and their regulation at distinct levels (eg, genes, RNA expression, protein expression) are partially unclear. ER stress is involved in the regulation of different metabolic processes, but the exact mechanisms that link these processes together are largely unknown. Therefore, further research is needed to understand ER stress, liver metabolism, and their interaction in HCC better and to improve therapeutic outcomes.

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