Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer, accounting for 85% to 90% of all liver cancer cases. It is a hepatocyte-derived primary tumor, causing 550,000 deaths per year, ranking it as one of the most common cancers worldwide. The liver is a highly metabolic organ with multiple functions, including digestion, detoxification, breakdown of fats, and production of bile and cholesterol, in addition to storage of vitamins, glycogen, and minerals, and synthesizing plasma proteins and clotting factors. Due to these fundamental and diverse functions, the malignant transformation of hepatic cells can have a severe impact on the liver’s metabolism. Furthermore, tumorigenesis is often accompanied by activation of the endoplasmic reticulum (ER) stress pathways, which are known to be highly intertwined with several metabolic pathways. Because HCC is characterized by changes in the metabolome and by an aberrant activation of the ER stress pathways, the aim of this review was to summarize the available knowledge that links ER stress and metabolism in HCC, thereby focusing on potential therapeutic targets. (Am J Pathol 2022, 190: 1–12; https://doi.org/10.1016/j.amjpath.2022.09.012)
aims to restore protein homeostasis by slowing down protein translation, increasing the protein folding machinery, and up-regulating the degradation of unfolded proteins.\(^5\) However, in case of persistent or excessive ER stress, the UPR can induce pathways leading to cell death.\(^5,6\) In cancer, it has been proposed that the key role of the UPR is to protect tumor cells from apoptosis and support their uncontrolled proliferation.\(^7\) 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 metabolic disorders such as nonalcoholic fatty liver disease, obesity, and type 2 diabetes, which are characterized by an aberrant metabolism. 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.\(^8\) 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, it has been established that 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 was to summarize the available knowledge that links ER stress and metabolism in HCC. We first provide an overview on the UPR and its involvement in HCC. Next, the role of the liver in 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.\(^5\) 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 (IRE1z), and protein kinase RNA-like endoplasmic reticulum kinase (PERK).\(^5\) These proteins each activate their own signaling cascade to induce the UPR. During protein homeostasis, binding immunoglobulin protein (BiP), also known as Grp78 or heat shock 70 kDa protein 5, is bound to ATF6, IRE1z, and PERK, keeping them in their inactive

![Figure 1](https://string-db.org, last day of accession: August 8, 2022). As shown in the network, the nodes correspond to the proteins, and the edges represent the interactions. **Colored nodes** represent the query proteins and first shell of interactors. The **blue edges** represent interactions from curated databases, and the **pink edges** represent experimentally determined interactions. The **green**, **red**, and **blue edges** correspond to predicted interactions based on gene neighborhood, gene fusions, and gene co-occurrence, respectively.
conformation. Upon ER stress, BiP preferentially binds to unfolded or misfolded proteins in the ER lumen, hence dissociating from the ER stress sensors and exposing their luminal domains. 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 is used for regulated IRE1-dependent decay, a process in which several ER-bound mRNAs or precursor microRNAs are spliced. These two processes reduce the amount of misfolded or unfolded proteins and mRNA, decreasing the protein-folding load of the ER and hence ER stress. In addition to the main proadaptive roles of IRE1α, it is suggested to induce 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. It is further suggested that 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 is known to induce 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 then 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. It has been suggested that ATF6 is one of the first branches that is 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 indicators of ER stress; therefore, ER stress has been suggested as 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 therefore shown that pharmacologically targeting different arms of the UPR can form a promising target to slow down tumor growth.
For instance, inhibition of IRE1α reduced the development of HCC in a diet-induced model for obesity in vivo.\textsuperscript{20} Likewise, a decrease in tumor cell proliferation and metastasis was seen in a chemically induced HCC mouse model when IRE1α endonuclease activity was pharmacologically inhibited.\textsuperscript{24} 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 GRP78, CHOP, and 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 are known to interfere with these various processes (Figure 2). To maintain glucose homeostasis, hepatocytes perform four metabolic reactions:

![Figure 2](https://example.com/figure2.png)

**Figure 2**  Functional connection between endoplasmic reticulum stress and glucose metabolism. The three major unfolded protein response (UPR) signaling pathways [activating transcription factor 6 (ATF6), protein kinase RNA-like endoplasmic reticulum kinase (PERK), and inositol-requiring enzyme 1 (IRE1)], attenuate proteostatic burden, either by endorsing the transcription of folding chaperones or by inhibiting protein translation. The UPR interacts with key transcription factors that regulate glucose homeostasis by controlling metabolic reactions such as glycogenesis, glycogenolysis, glycolysis, and gluconeogenesis. Although the catabolism of glucose into pyruvate is the main pathway of adenosine triphosphate generation, cytosolic pyruvate can be incorporated into 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 glucose homeostasis 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, Ontario, Canada.
glycolysis, glycogenesis, gluconeogenesis, and glyco-
genesis. Glycolysis is a catabolic pathway of enzyme-
catalyzed reactions that break down glucose to generate en-
ergy. In normal conditions, cells catabolize glucose to pyruvate and later to acetyl coenzyme A through the tricarboxylic acid cycle to finally 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 (TP1), which is involved in the glycolytic and gluco-
genesis pathway, reportedly inhibits HCC cell growth, migration, invasion, and survival. Moreover, TP1 expression is significantly decreased in HCC tissues and is correlated with HCC progression and survival, suggesting that TP1 might serve as a tumor suppressor. It has also been shown that ATF4 knockdown reduces the expression of TP1 in Drosophila, which might suggest a role of the UPR in the regulation of this enzyme.

Another glycolytic key enzyme involved in tumor prolif-
eration is hexokinase and, more specifically, one of its four isoenzymes, hexokinase 2 (HXK2). HCC exhibits aberrant expression of HXK2, contributing to early recurrence and poorer prognosis of surgically resected HCC. In another study, DeWaal et al. observed that HCC cells express a different isof orm of the hexokinase enzyme, HXK2. Inhibition of HXK2 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 HXK2 to mitochondria via inhibition of p-Akt in glioma cells, which inhibits tumor growth. This suggests PERK-dependent regulation of HXK2 in tumor cells, linking ER stress to glycolysis. In addition, depletion of HXK2 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.

When increases in blood glucose levels are needed, 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 main control point of gluconeogenesis, seems to play 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 seemed to promote 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 meta-
bolism might also be involved in the pathologic process of HCC. For instance, glucose metabolism is highly inter-
twined with ER stress and the UPR (Figure 2). It has been shown that 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. In addition, 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, although its role in tumorigenesis and HCC remains largely unknown. However, 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.

It has also been suggested that ER stress suppresses in-
sulin 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 inhibition of insulin signaling and impairment of glucose metabolism. The UPR mediator sXBP1 is suggested to interact 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 sXBP1 and Forkhead box O1 results in proteasomal degradation of Forkhead box O1, and thus down-regulation of the gluconeogenesis pathway, which is known to drive HCC. Furthermore, 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 is also suggested to control glucose levels, as inhibition of the PERK-eIF2α-ATF4 pathway enhances glucose metabolism in
diabetic livers.\textsuperscript{35} Lastly, the ATF6 arm of the UPR seems to be associated with impaired glucose homeostasis in type 2 diabetes.\textsuperscript{36} 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 if this is affecting the development of HCC remain largely unknown and warrant further research.

**Lipid Metabolism**

Deregulation of hepatic lipid metabolism, including synthesis, storage, and break down of lipids, is considered a driving force of liver cancer.\textsuperscript{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 synthase, adenosine triphosphate/citrate lyase, or the sterol regulatory element-binding protein 1.\textsuperscript{59} On the contrary, the degradation of lipids (lipolysis) allows production of energy or ketone bodies via the tricarboxylic acid cycle or \( \beta \)-oxidation, respectively. Lipolytic enzymes, such as adipose triglyceride lipase, are also highly expressed in HCC, increasing lipid degradation and energy production.\textsuperscript{60} One of the genes responsible for altered lipid metabolism is liver kinase B1 (\( \text{LKB1} \)), which is mutated in 22\% of HCC cases, and its allelic loss leads to abnormal expression and activation of multiple molecules related to lipid metabolism.\textsuperscript{60} Furthermore, mutations in feedback regulators of the Wnt pathways, such as Ring Finger Protein (\( \text{RNF43} \)) and Zinc And Ring Finger 3 (\( \text{ZNRF3} \)), have been shown to increase hepatic lipid accumulation and enhance tumorigenesis after liver injury, which could imply that \( \text{RNF43/ZNRF3} \)-mutated individuals might be at higher risk of developing HCC in a background of fatty liver disease.\textsuperscript{61} Nearly 20\% of patients with HCC carry an activating mutation in the \( \text{CTNNB1} \) gene, which is responsible for encoding beta-catenin in Wnt pathways. It has been shown that beta-catenin-activated HCCs are characterized by enhanced fatty acid oxidation, reduced glycolysis, and up-regulated expression of peroxisome-related genes.\textsuperscript{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.\textsuperscript{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.\textsuperscript{63} Therefore, regulation of pathways involved in lipid metabolism might improve HCC prognosis.\textsuperscript{58}

It is known that lipid metabolism is strongly intertwined with ER stress and the UPR. First, the ER is the main site of lipid production, and many enzymes involved in lipid metabolism are produced in the ER.\textsuperscript{64} In addition, the three UPR branches regulate lipid homeostasis by activating transcription factors and pathways that modulate lipid metabolism. For instance, IRE1\( \alpha \) controls lipid metabolism by inducing the degradation of mRNAs encoding for lipid metabolism regulators through regulated IRE1-dependent decay,\textsuperscript{65} which has been specifically shown to be relevant in cancer.\textsuperscript{66} Moreover, the IRE1\( \alpha \)/XBPI branch is involved in the regulation of hepatic \textit{very-low-density lipoprotein} production,\textsuperscript{67} and up-regulates peroxisome proliferator–activated receptor alpha (\( \text{PPAR}\alpha \)) expression during starvation, hence stimulating \( \beta \)-oxidation and ketogenesis.\textsuperscript{68} Studies on \textit{Drosophila} have shown that IRE1\( \alpha \) deficiency leads to an increased mobilization of lipids and sensitizes flies to starvation, thus showing that IRE1\( \alpha \) is a catabolic sensor acting through the XBPIs–FoxO axis.\textsuperscript{69} Regarding PERK/eIF2\( \alpha \), different studies have reported that this arm of the UPR could promote lipogenesis via different downstream effectors. As an example, PERK seems to activate 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.\textsuperscript{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.\textsuperscript{71} 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 \( \text{BiP}, \text{IRE1}\alpha, \text{XBPI, p-eIF2}\alpha, \text{CHOP, and p-JNK} \). Some studies suggest that ER stress mediates lipotoxicity by inducing apoptosis or modulating the membrane composition of phospholipids, but it is still not completely understood how this happens.\textsuperscript{25,72} A widely understood hallmark of the UPR is an increase in \textit{de novo} lipogenesis to allow the expansion of lipids into the ER. However, this phenomenon has mostly been studied on cell cultures, and a very recent \textit{in vivo} study by Ward et al\textsuperscript{73} found that hepatic \textit{de novo} lipogenesis and cholesterolgenesis might actually be reduced after tunicamycin-induced ER stress. Concerning pharmacologic intervention, several chemical molecules, including cinna.

**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. Dysregulation of glutaminase and glutamine synthase is a hallmark of several cancers, including HCC, leading to several glutamine analogues and glutaminase inhibitors having been tested in preclinical and clinical studies, with variable success. Zhou et al. 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. In this condition, glutaminase inhibitors could be used as an adjuvant treatment in cancer, reducing glutamine conversion to glutamate. Specifically, because glucose deprivation is a strong inducer of ER stress, glutamine treatment can reduce ER stress signaling in these glucose-deprived conditions. However, it is well established that 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, thus supporting an anaplerotic flux for glutamine, which feeds the tricarboxylic acid cycle. In a zebrafish model for HCC, it has been shown that 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 in combination with a GS/β-catenin inhibitor CG1 (sobetirome). Successfully, this combined treatment synergistically reduced HCC burden. These results warrant the intricate complexity and need for further studies to further 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-1 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 for 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. Moreover, envelope protein E2 of hepatitis C virus, a risk factor for HCC, can modulate protein synthesis and ER stress through PERK activation. Furthermore, depletion 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 again 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. Previous studies have reported reduced production of vitamins in several types of cancers, including HCC. In the case of vitamin D, antitumor, antiproliferative, antiangiogenic, and proapoptotic activities have been found for this molecule, suggesting potential therapeutic benefits in HCC via the use of complementary vitamin D-based treatments. This has been further supported once anti-inflammatory and antifibrotic activities in HCC cell models and mice were reported. Moreover, Riek et al. showed that vitamin D is a natural macrophage ER stress reliever, which would potentially further improve anticancer treatment, as it was previously shown that ER stress can promote a protumoral phenotype in macrophages. Vitamin K mainly participates in bone metabolism, but it is also responsible for the regulation of clotting factor production. As with vitamin D, supplementation with vitamin K seems to reduce HCC proliferation and invasion in HCC cells, improving the current treatments. The role of this regarding 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, more research is necessary to establish whether this is relevant in the context of 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. Furthermore, this up-regulation has been associated...
with tumor cell stemness and poor prognosis. Likewise, 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 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 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 is crucial for the response to anticancer therapies and drug response. Particularly, this activity is developed by some cytochromes in the ER lumen. 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. In addition, several drugs, including anticancer compounds such as tunicamycin, doxorubicin, or erlotinib, can induce ER stress and drug-induced liver injury. These lead to hepatic lesions, steatosis, steatohepatitis, hepatocellular adenoma, and cirrhosis, among others, which are previous steps of HCC and contribute to the development of an already existing tumor. In addition, hepatotoxicity commonly leads to reduction in drug dosing or even early termination of treatment, thus drastically decreasing the patient’s survival rates. Moreover, doxorubicin is known to cause heart failure in some cancer patients through activation of the ATF6, PERK, and IRE1 pathways, which has been shown to be reduced by combinational treatment with ER stress inhibitors, including alginate oligosaccharide, sodium hydrosulfide, and sacubitril. 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).

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 IRE1α and CHOP in HCC cell lines, as well as in healthy hepatocyte cell cultures, suggesting a possible hepatotoxic effect. The effect was exacerbated by pretreatment 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.

Moreover, 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. 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, eIF2α, and IRE1α. 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. However, loss of ERdj5 can aggravate alcoholic liver disease through inhibition of Nrf2, thus suggesting a protective role of ERdj5 in this

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Affected ER stress pathways</th>
<th>Combinational treatments</th>
<th>Pathology</th>
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<tbody>
<tr>
<td>Tunicamycin</td>
<td>ATF6, PERK, and IRE1</td>
<td>Chemotherapeutics</td>
<td>Cancer Cardiomyopathy</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>ATF6, PERK, and IRE1</td>
<td>Alginate oligosaccharide</td>
<td>Sodium hydrosulfide (hydrogen sulfide donor), sacubitril (antihypertensive)</td>
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<tr>
<td>Erlotinib</td>
<td>PERK and XBP1</td>
<td>Magnesium isoglycyrrhizinate</td>
<td>Anti-inflammatory, kinsenoside (antioxidant) and geniposide (antioxidant)</td>
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<tr>
<td>Remdesivir</td>
<td>IRE1 and CHOP</td>
<td></td>
<td>Cancer Coronavirus disease 2019</td>
</tr>
<tr>
<td>Alcohol</td>
<td>ATF6, PERK, and IRE1</td>
<td></td>
<td>Alcoholic liver disease, HCC</td>
</tr>
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ATF6, activating transcription factor 6; ER, endoplasmic reticulum; HCC, hepatocellular carcinoma; IRE1, inositol-requiring enzyme 1; PERK, protein kinase RNA-like endoplasmic reticulum kinase.
pathology. In addition, CYP2E1-overexpressing primary human hepatocytes and HepG2 cells that were treated with alcohol exhibited an up-regulation in ATF4, suggesting that excessive alcohol intake also increases the expression of ATF4. Together, this suggests 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, 

kininonoside, and geniposide (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 isomers, and altered metabolite flow. However, the causes of the metabolic alterations in HCC and their regulation at distinct levels (e.g., genes, RNA expression, protein expression) are partially unclear. In addition, ER stress seems to be involved in the regulation of different metabolic processes, but the exact mechanisms that link these processes together are largely unknown. Therefore, more research is needed to increase knowledge regarding ER stress, liver metabolism, and their interaction in HCC to target these processes and improve therapeutic outcomes.

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