Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer. It currently ranks as one of the most aggressive and deadly cancers worldwide, with an increasing mortality rate and limited treatment options. An important hallmark of liver pathologies, such as liver fibrosis and HCC, is the accumulation of misfolded and unfolded proteins in the lumen of the endoplasmic reticulum (ER), which induces ER stress and leads to the activation of the unfolded protein response (UPR). Upon accumulation of misfolded proteins, ER stress is sensed through three transmembrane proteins, IRE1α, PERK, and ATF6, which trigger the UPR to either alleviate ER stress or induce apoptosis. Increased expression of ER stress markers has been widely shown to correlate with fibrosis, inflammation, drug resistance, and overall HCC aggressiveness, as well as poor patient prognosis. While preclinical in vivo cancer models and in vitro approaches have shown promising results by pharmacologically targeting ER stress mediators, the major challenge of this therapeutic strategy lies in specifically and effectively targeting ER stress in HCC. Furthermore, both ER stress inducers and inhibitors have been shown to ameliorate HCC progression, adding to the complexity of targeting ER stress players as an anticancer strategy. More studies are needed to better understand the dual role and molecular background of ER stress in HCC, as well as its therapeutic potential for patients with liver cancer.
multikinase inhibitors sorafenib, lenvatinib, and regorafenib. While these agents prolong patient survival, they are also associated with serious side effects and limited efficacy [3]. Most recently, combination therapy including immune checkpoint inhibitor PD-L1 and a VEGF antagonist has shown promising results in extending HCC survival with less side effects than sorafenib alone [4], encouraging further research on the HCC molecular landscape in an effort to identify new biomarkers and therapeutic targets for patients with liver cancer.

The unfolded protein response (UPR) is a conserved cell survival strategy and stress response, which is initiated when the cell’s need for protein synthesis exceeds the endoplasmic reticulum’s capacity to ensure accurate protein folding. In such cases, the accumulation of misfolded or unfolded proteins, known as endoplasmatic reticulum (ER) stress, is sensed via three ER transmembrane proteins (IRE1α, PERK, and ATF6) and the UPR is activated, with the goal of re-establishing normal ER function [5]. The cytoprotective role of the UPR includes lowering protein synthesis, cell cycle arrest, and upregulation of UPR-responsive genes. However, in cases of irredeemable or chronic ER stress, actors of the UPR activate pro-apoptotic signaling pathways. ER stress is triggered by external stimuli that characterize a solid tumor microenvironment, such as hypoxia, acidosis, and nutrient deficiency; tumor cells have a high metabolic rate and a mutation-driven excessive need for protein synthesis requirements. However, different intrinsic and extrinsic perturbations such as mutations, pathogens, and an increased secretory load can drive the cell’s protein synthesis needs beyond what the capacity of the ER can cope with in order to ensure accurate protein folding [11]. In these cases, three conserved ER transmembrane sensory proteins known as inositol-requiring enzyme 1α (IRE1α) and IRE1β; protein kinase RNA-like ER kinase (PERK); and activating transcription factor 6α (ATF6α) and ATF6β trigger the adaptive mechanism known as the UPR, which aims to restore normal ER function and homeostasis [9]. In nonstressed cells, these three UPR signaling transducers remain inactive and anchored to the ER membrane through binding to BiP (a heat-shock protein HSP70 family chaperone) with their luminal domains. However, upon unfolded protein accumulation in the ER lumen, BiP dissociates from the UPR transducers, releasing their luminal domains and allowing for their subsequent activation [5]. Moreover, IRE1 has been shown to directly bind to unfolded proteins, which can serve as activating ligands that induce IRE1 oligomerization independently of BiP [12]. While the UPR mechanisms will aim to re-establish accurate protein folding without activating cell death pathways, prolonged UPR activity and unresolved ER stress will trigger apoptosis [13].

The most conserved and main pro-adaptive branch of the UPR is mediated by IRE1α. This UPR transducer exerts two enzymatic activities, as its cytosolic region contains both a kinase and an endoribonuclease (RNase) domain. Upon activation, IRE1α undergoes transautophosphorylation of its kinase domain and dimersizes, which triggers its RNase activity and subsequently catalyzes two signaling responses [14]. This includes the nonconventional splicing of X-box binding protein 1 (XBP1) mRNA, whereby the active transcription factor, spliced XBP1 (XBP1s), is produced and goes on to regulate the transcription of a broad range of target genes involved in protein folding, ER-associated degradation (ERAD) and protein secretion [15]. Additionally, the RNase activity of IRE1α degrades a subset of ER-bound mRNAs through a process termed regulated IRE1-dependent decay of mRNA (RIDD), thereby affecting inflammation, angiogenesis, and apoptotic factors [9,13,16].
The role of ER stress in hepatocellular carcinoma

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The kinase domain of IRE1 is also involved in transducing the IRE1-mediated ER stress response, by mediating the JUN N-terminal kinase (JNK)-apoptosis signal-regulating kinase 1 (ASK1) pathway [17] and nuclear factor-xB (NF-xB) signaling [18]. Furthermore, as the IRE1 kinase domain serves as the substrate of IRE1 autophosphorylation and provides ATP-binding sites, it is used as a pharmacological target for modulating IRE1 activity [16]. Considering its roles in both mediating pro-adaptive UPR mechanisms to ameliorate the detrimental effects of ER stress on cellular homeostasis and promoting cell death under prolonged and irredeemable ER stress, IRE1 is considered a crucial regulator of cell fate determination [14]. However, the threshold at which IRE1 favors apoptotic signaling over adaptive responses seems to be cell type, as well as ER stress duration- and stimuli-dependent [13,19].

Upon PERK activation, the ubiquitous translation initiation factor eIF2α is phosphorylated, consequently inhibiting protein synthesis and rapidly reducing the number of proteins entering the ER, thereby adding to the pro-adaptive mechanisms the UPR uses to cope with ER stress. As part of this response, ATF4 translation is induced, whereby the expression of genes involved in antioxidant responses, autophagy, but also apoptosis is regulated. A fine balance between pro-adaptive and apoptotic signaling is likely determined through levels of phosphorylated eIF2α [5]. Another mechanism through which PERK mediates pro-adaptive UPR signaling is via activation of transcription factor Nrf2, which contributes to redox homeostasis maintenance and cell survival following ER stress [20,21].

As a response to ER stress conditions, ATF6 is translocated to the Golgi to be processed by two proteases (site-1 and site-2 protease). This frees ATF6 of its luminal and transmembrane anchor, allowing the ATF6 cytosolic fragment (ATF6f) to move into the nucleus and activate UPR genes encoding ERAD and XBP1 [9].

Overall, the UPR’s response to ER stress can be summarized as a two-wave signaling cascade. An immediate reaction involves PERK-mediated inhibition of mRNA translation and IRE1α-regulated activation of mRNA decay (RIDD) and autophagy, with the goal of reducing the protein synthesis load imposed on the ER. Secondly, a pro-adaptive signaling cascade involving ATF6f, XBPI, and ATF4 aims to restore ER function through triggering a massive gene expression response. These three transcription factors upregulate a wide range of UPR genes in a highly complex and intertwined manner. The specificity of which UPR genes will be targeted depends on the ER stress-inducing stimulus and the cell type affected and possibly on the cross-talk between the transcription factors [13]. As mentioned, unmitigated ER stress triggers pro-apoptotic signaling, for which the B-cell lymphoma 2 protein family is crucial. Prolonged PERK activation induces pro-apoptotic activity through upregulating CHOP and GADD34. Furthermore, IRE1α may promote cell death through binding adaptor proteins such as tumor necrosis factor (TNF) receptor-associated factor 2 (TRAF2), subsequently activating the JNK-ASK1 pathway, and through RIDD, which degrades mRNA transcripts of anti-apoptotic proteins and factors involved in enhancing the ER’s protein folding yield [9].

The UPR’s link to hepatocarcinogenesis

Hepatocytes are structurally rich in ER and maintain a high rate of protein and lipid biosynthesis and secretion in normal physiological conditions. Due to the integral role of the ER in protein folding, lipid synthesis, and calcium signaling, a tightly regulated UPR machinery is crucial for the physiologic function of cell types with high metabolic rates, such as hepatocytes. Indeed, hepatocytes are known to be sensitive to disturbances of normal ER function, which can lead to various metabolic disorders. For instance, loss of BiP is known to result in liver injury, hepatic steatosis, and hepatocyte apoptosis, while XBPI knockout mice have been shown to develop severe hypcholesterolemia and hypotriglyceridemia [22]. However, besides its contribution to metabolic disease, ER stress is also known to induce other liver pathologies [23]. Hallmarks of a solid tumor microenvironment, such as hypoxia, acidosis, nutrient deprivation, increased metabolic rates, and reactive oxygen species (ROS), are known as potent ER stress stimuli [6]. It has been demonstrated that key UPR players are activated in the majority of human HCCs, irrespective of grade or stage [24]. Shuda et al. [25] have reported that elevated mRNA levels of BiP, ATF6, and spliced XBPI significantly correlate with well-differentiated cancerous tissue from HCC patients, compared to healthy tissue. Overexpression of BiP and heat-shock cognate protein was shown to be present in 100% of both HCC and nontumorous cirrhotic livers, suggesting that unresolved ER stress represents a risk factor for HCC in liver cirrhosis [26]. BiP has also been implicated in various mechanisms of hepatocarcinogenesis, including resistance to chemotherapeutic agents [25] and attenuating lipotoxicity in hepatoma cells [27]. A study by Tang et al. [28] showed that cancer biomarker CD147 upregulation
triggers the unfolded protein response and BiP overexpression in human hepatoma tissue and that CD147-induced UPR leads to inhibited cell death and decreased chemosensitivity in a hepatoma mouse model. Protein disulﬁde isomerasers (PDI), a superfamily of oxidoreductases with multiple functions in the ER protein folding machinery, have also been proposed as attractive targets for regulating UPR signaling to combat HCC. Indeed, PDI-targeting compounds such as PACMA 31 and Bacitracin have been shown to increase ER stress to reduce HCC cell viability by improving the efﬁcacy of sorafenib and other antitumoral agents in vivo [29]. Finally, by using the Human Protein Atlas database, we have recently identiﬁed 44 UPR-associated proteins as unfavorable prognostic markers for patients with liver cancer. The expression of these markers was shown to be the highest in disease stages 2 and 3, indicating that chronic ER stress is an important contributor to the progression of hepatocarcinogenesis. Furthermore, through scoring immunohistochemical staining on HCC biopsies, we found that these markers are mainly expressed in the tumoral hepatic tissue [30].

Numerous studies have shown that inhibiting IRE1α activity decreases tumor cell proliferation and increases chemosensitivity in various cancer types, such as breast cancer, colorectal cancer, and HCC, establishing the IRE1-XBP1 signaling branch as a promising anticancer target [15,31,32]. Moreover, increased IRE1-XBP1 activity has been attributed to different liver pathologies that precede HCC initiation. For instance, dysregulated IRE1-XBP1 signaling has been associated with human nonalcoholic steatohepatitis, one of the prime causes of HCC in Western countries [33,34]. Upregulated IRE1 signaling has also been demonstrated as crucial for liver cirrhosis development [35]. A recent study by our group showed that blocking the endoribonuclease activity of IRE1α with small molecule inhibitor 4μ8C, signiﬁcantly decreased tumor burden and ﬁbrosis in a chemically induced mouse model for HCC. We also observed lower levels of spliced XBP1 in 4μ8C-treated mice with HCC, correlating with decreased stellate cell activation, ﬁbrosis, and tumor growth, thereby providing further evidence that the IRE1α-XBP1-axis could be a relevant target in HCC. Moreover, we found that in a co-culture of hepatic stellate cell line LX2 and HCC cell lines, inhibiting the IRE1α-ER stress pathway in stellate cells signiﬁcantly decreases proliferation and migration of HCC cells [7]. In a recent study by Wu et al. [36], IRE1α was shown to play a crucial role in accelerating the malignant progression of hepatocarcinogenesis in a mouse model of HCC on a high-fat diet. However, Li et al. [37] have reported that IRE1α-silencing suppresses ER stress-mediated apoptosis in hepatoma cells, while its overexpression inhibited their proliferation.

The UPR branch mediated by PERK has been shown as essential for tumor cell proliferation and invasiveness, as it enables cancer cells to adapt to hostile microenvironmental conditions by regenerating intracellular antioxidants, as well as by regulating redox homeostasis and DNA damage checkpoint activation [38]. A study on a chemically induced HCC mouse model revealed that, while a peak in IRE1α signaling and modest ATF6 activation was noted in the tumor initiation phase, the PERK-induced ER stress branch was shown to be activated during tumor progression [39]. Furthermore, pharmacological inhibition of PERK reduced the expression of UPR-induced chaperones, which protect tumor cells from UPR-induced cell death in vitro, and led to a signiﬁcant reduction in tumor burden in this HCC mouse model [39]. However, critical issues have been raised regarding the speciﬁcity of certain PERK inhibitors used in preclinical studies, highlighting the need for careful evaluation [40]. Patient-derived HCC specimens have revealed a close correlation between the expression of autophagy-associated marker Beclin1 and PERK, and the combined expression of these two markers was associated with advanced disease stage and a shorter overall survival time. Moreover, inhibition of the PERK-ATF4-Beclin1 pathway with speciﬁc ER stress inhibitors 4-phenylbutyric acid (PBA) and tauroursodeoxycholic acid (TUDCA), as well as melatonin, was shown to increase the sensitivity of HCC cells to sorafenib, a systemic treatment for HCC which frequently leads to resistance in patients with liver cancer [41]. Overall, these ﬁndings emphasize the importance of the pro-adaptive yield of PERK for tumor cell survival and hepatocarcinogenesis, and the therapeutic potential of targeting PERK-mediated pro-adaptive signaling in HCC.

On the other hand, PERK-mediated pro-apoptotic cellular responses have been the focus of a number of reports on HCC drug resistance. A study examining the efﬁcacy of next-generation proteasome inhibitor oprozomib (OZ) and its effect on the UPR found that OZ reduced viability and proliferation of HCC cells and diminished ATF6-mediated cytoprotective signaling, but increased pro-apoptotic UPR-mediated protein levels. Interestingly, additional boosting of the UPR activity improved OZ sensitivity through the PERK-mediated signaling pathway [42]. Another line of evidence for PERK’s implication in HCC drug sensitivity comes from a recent study by Li et al. [43].
where it was shown that lobaplatin promotes $^{125}$I-induced apoptosis and inhibits proliferation of HCC via upregulation of the PERK-eIF2$\alpha$-ATF4-CHOP pro-apoptotic pathway. Chen et al. [44] have reported that the potent antitumoral agent sinulariolide promoted HCC cell death in a PERK-eIF2$\alpha$-ATF4-CHOP pathway-dependent manner. Delanzomib, a second-generation proteasome inhibitor, was shown to exert potent antitumor activity in the same PERK-mediated pathway, thereby significantly suppressing HCC progression in vivo, while also remaining efficient against sorafenib-resistant HCC cells [45]. Pterostilbene (PT), a natural dimethylated analogue of resveratrol known as cytotoxic to HCC cells, was shown to promote HCC cell death and reduce tumor growth in mice with a SK-Hep-1 tumor xenograft in an autophagy (ATF4/LC3 pathway)-dependent manner. A combined use of eIF2$\alpha$ phosphatase inhibitor Salubrinal and PT showed increased HCC cell death and enhanced expression of p-eIF2$\alpha$, ATF4, LC3-II, and cleaved-PARP, highlighting eIF2$\alpha$ phosphorylation as crucial for autophagy-dependent ER stress-induced apoptosis [46]. Therefore, while PERK-mediated UPR effectors are strongly implicated in hepatocarcinogenesis and tumor survival strategies, a growing number of studies suggest that inducing the PERK-regulated pro-apoptotic ER stress activity can be a relevant approach for suppressing HCC proliferation and sensitizing HCC cells to anticancer agents.

Activating transcription factor 6, along with BiP, was shown to be concomitantly upregulated as the histological grade increased in patient-derived HCC tissues [25]. Moreover, in HCC cells overexpressing ATF6$\alpha$, it was shown that ATF6 upregulates proliferation-associated and other functional genes. This was then confirmed in HCC and noncancerous tissue, which showed an upregulation of these genes, albeit not necessarily in a UPR-dependent manner [47]. Pro-apoptotic UPR-regulator CHOP, which can be induced by both the PERK and ATF6 activation, has been shown to play a key role in tumorogenesis in an HCC mouse model, as CHOP knockout resulted in significantly smaller tumors in mice, specifically under the regulation of the ATF6 nuclear fragment. The authors also report CHOP-mediated macrophage recruitment into the tumors and propose CHOP as a potential oncogenic factor in hepatocarcinogenesis [48]. Bu et al. [49] have reported that melatonin selectively inhibits ATF6 and cyclooxygenase-2 expression in an HCC cell line and enhances ER stress-mediated HCC cell death, thereby suggesting ATF6 inhibition as a strategy for sensitizing liver cancer cells to ER stress-induced apoptosis.

As mentioned, both ER stress inhibitors and inducers have been proposed as viable therapeutic strategies in HCC, with the goal of either suppressing tumor proliferation, invasion and survival strategies, sensitizing tumors to anticancer agents, or promoting ER stress-induced apoptosis of HCC cells. This led to a variety of compounds involved in mediating adaptive/ apoptotic UPR functions being tested in preclinical HCC models (summarized in Fig. 1) and showing success in slowing down HCC progression and decreasing chemoresistance, with a number of these agents going through clinical trials (Table 1).

**ER stress contributes to hepatic stellate cell activation and represents a therapeutic target in liver fibrosis**

Hepatic stellate cells (HSC) are key contributors to the tumor–stroma interactions which drive HCC initiation and progression. In normal physiological conditions, these cells serve as quiescent vitamin A reservoirs in the liver, but transition to an activated state during liver damage [50]. They thereby acquire a contractile, myofibroblast-like phenotype which results in excessive ECM deposition and remodeling. Furthermore, stellate cells strongly influence the hepatic microenvironment by secreting growth factors, such as transforming growth factor beta (TGF-Î²) and connective tissue growth factor [1]. Stellate cell activation is therefore known to actively engage in profibrotic and dysregulated wound-healing signaling, and thereby modulate the surrounding stroma to support tumor initiation and growth. Moreover, activated stellate cells are known to directly stimulate tumor cell proliferation and induce a metastatic HCC phenotype [7,51]. Hence, targeting activated HSC through induction of cellular apoptosis or senescence has been of great interest when assessing new potential targets in the treatment of liver cancer [52].

A strong correlation between ER stress and HSC activation has been demonstrated in numerous cancer models, as well as patient studies [7,35,53–55]. Both in vitro and in vivo studies have widely demonstrated that the excessive ECM production following stellate cell activation leads to ER stress and UPR activation, while in turn, actors of the UPR are potent contributors of stellate cell activation and profibrotic gene expression [54]. Hernández-Gea et al. [56] showed that IRE1$\alpha$ regulates the p38/MAPK pathway in primary hepatic stellate cells, which upregulates fibrogenic gene expression through autophagy. Inducing ER stress in rat HSC with brefeldin A also showed that IRE1$\alpha$-dependent p38/MAPK signaling resulted in increased...
collagen type I expression and induced Smad activation [55]. Adding to this, the XBP1-arm of the UPR was shown to promote fibrogenic activation of primary hepatic stellate cells and HSC lines through upregulation of profibrotic genes such as COL1A1 (collagen 1-alpha), ACTA2 (alpha-SMA), PDGFRB, MMP2, and TIMP1 and that autophagy induction is required for this [57]. Liu et al. [54] reported that TGF-beta induces IRE1α signaling through C/EBPβ–p30 to promote stellate cell activation and profibrotic signaling in the hepatic stellate cell line LX2. However, a recent study on HSC isolated from mice with fibrosis demonstrated that UPR actors such as BiP, CHOP, and XBP1s are upregulated in the early stages of stellate cell activation, but that this is not enough to drive the activation process [58]. Nonetheless, IRE1α signaling was found to be critical in fibrosis development in a study on mice with liver and skin fibrosis. Namely, inhibiting the RNase activity of IRE1-α with the specific inhibitor 4μ8C not only reduces fibrosis in vivo, but reverts the fibrotic phenotype of myofibroblasts [35]. The study also showed that IRE1α mediates fibrosis by directly cleaving and inactivating miR-150, which represses the expression of c-Myb and αSMA under nonfibrotic conditions [35]. XBP1 was also found to be crucial in regulating TANGO1-mediated collagen I secretion and fibrogenesis in vivo [59]. In vitro studies exploring the cross-talk between stellate cells and HCC cells revealed that tumor cells promote UPR activation and ER stress in stellate cells, along with their activation. In turn, stellate cells induce proliferation and a more invasive, prometastatic phenotype in tumor cells. However, this effect was ameliorated when the endoribonuclease activity of IRE1α was blocked with selective inhibitor 4μ8C. Furthermore, actors of the IRE1α signaling branch show an upregulated expression in fibrotic HCC patient tissue, compared to nonfibrous HCC tissue, while this

Fig. 1. A summary of ER stress inhibitors and inducers tested in HCC and their effect on disease progression.
increased expression significantly correlates with poor survival in patients with liver cancer [7].

Although little is known about the involvement of PERK and ATF6 in profibrotic signaling, both of these UPR orchestrators have been linked to HSC activation [55]. Levels of phosphorylated PERK were shown to be positively correlated with severe fibrosis in patients, while subsequent studies revealed that PERK-mediated profibrotic signaling involves phosphorylation of heterogeneous nuclear riboprotein A1, which results in increased SMAD 2 expression. [53]. A recent study on rats with bile duct ligated (BDL)-induced liver fibrosis revealed that the protein expression of all three ER stress transducers (PERK, IRE1, and ATF6), along with BiP, was elevated in fibrotic rats, which was accompanied by increased levels of fibrosis markers COL1A1 and alpha-SMA. Treatment of rats with BDL-induced liver fibrosis with natural compound salvianolic acid A (SalA) was shown to diminish BDL-induced liver injury and fibrosis by suppressing ER stress in a SIRT1/HSF1-dependent manner. Namely, SalA was shown to upregulate key fibrosis regulator SIRT1, which in turn increased the expression and deacetylation of HSF1 and consequently suppressed ER stress, stellate cell activation, and fibrosis both in vitro and in vivo [60].

While studies linking the pro-adaptive mechanisms of the UPR with HSC activation, proliferation, and survival bring attention to ER stress as an important hallmark of fibrogenesis (Fig. 2), ER stress-induced HSC apoptosis has been shown to promote recovery from fibrosis [61]. Both in vitro and in vivo studies have revealed that inducers of ER stress-mediated apoptosis of activated HSC contribute to fibrosis resolution, suggesting that triggering HSC apoptosis by inducing ER stress is a relevant antifibrotic strategy, as opposed to inhibiting ER stress in HSC to suppress their activation and consequential contribution to fibrogenesis [61–66]. Anticancer drug etoposide, and compounds such as caffeine, cannabidiol, and quercetin have been shown to induce hepatic stellate cell death through UPR signaling [63,67–69]. Overexpression of ECM-associated CCN proteins has been shown to induce ER stress and UPR activation in stellate cells and hepatocytes both in vitro and in vivo. Consequently, this led to senescence and apoptosis of hepatic stellate cells in later stages of fibrosis. CCN protein-mediated ER stress induction has therefore been proposed as another strategy for fibrosis resolution [70,71].

A large challenge in inducing stellate cell apoptosis for fibrosis resolution, or inhibiting ER stress to block stellate cell activation, lies in the difficulty to specifically and effectively target stellate cells. Additionally, more studies are needed to understand the highly cross-linked prosurvival/apoptotic mechanisms of the UPR in order to determine the therapeutic potential of targeting ER stress in liver fibrosis. The importance of studying the molecular basis of ER stress involvement in hepatic stellate cell activation may be extrapolated to other liver pathologies, such as HCC, considering that underlying fibrosis and stellate cell activation have a central role in HCC initiation, progression, and response to treatment. Finally, the findings revolving around ER stress actors contributing to profibrotic stellate cell signaling underline the relevancy of studying ER stress in stromal biology, particularly for a cancer with an abundant stroma, such as HCC.

**ER stress contributes to hepatocarcinogenesis in the background of chronic HBV/HBC infections**

Hepatocellular carcinoma is an inflammation-driven disease and commonly develops in the background of

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**Table 1. Summary of compounds targeting ER stress tested preclinical HCC models, with an overview of their respective clinical trial status.**

| Compound | Targeted UPR branch | Trial | Phase | Status | References |
|----------|---------------------|-------|-------|--------|------------|
| 4μ8C     | IRE1α               | Not applicable | Preclinical development | Not applicable |  |
| GSK2656157 | PERK               | Not applicable | Preclinical development | Not applicable |  |
| PBA      | All                 | NCT00771901 | Not applicable | Completed | [92] |
| TUDCA    | All                 | NCT00771901 | Not applicable | Completed | [92] |
| Oprozomib | PERK, ATF6       | NCT01416428 | II | Completed | [93] |
| Lobaplatin | PERK            | NCT03210389 | II | Ongoing | [94] |
| Delanizomed | PERK        | NCT01489519 | II | Terminated | [95] |
| Pterostilbene | PERK       | NCT01267227 | III | Completed | [96] |
| Salubrinil | PERK             | Not applicable | Not applicable | Not applicable |  |
| Sinulariolide | PERK         | Not applicable | Not applicable | Not applicable |  |
| Melatonin | PERK, ATF6      | Not applicable | Not applicable | Not applicable |  |
chronic hepatitis B (HBV) and hepatitis C (HCV) viral infections, both of which are important etiological agents of chronic liver disease. The ER plays a key role in viral maturation and replication, while studies have shown that viral infections can induce ER stress due to the excessive amount of unfolded viral proteins produced in infected cells [72]. Both HBV and HCV can trigger ER stress and induce the UPR by altering Ca\textsuperscript{2+} signaling and increasing ROS levels. During viral infection, ER stress becomes chronic and autophagy is initiated to restore normal ER function, promote cell survival, virus replication, and persistence, all of which are known risk factors for HCC [73]. HCV subgenomic replicons have been shown to induce ATF6 activation, ER chaperone transcription, and overall increased protein synthesis [74]. HCV replicons were also found to inhibit XBP1 transcriptomic activity, thereby potentially repressing IRE1-XBP1 signaling, consequently promoting synthesis of viral proteins and thereby contributing to the persistence of the virus in hepatocytes [75]. Furthermore, in vivo hepatic ER stress has been observed in patients with chronic HCV, with all three UPR inducers being activated. In addition, dilated and disorganized hepatocyte ER morphology was noted in patients with mild HCV-related fibrosis [76]. Physical interactions between the virus and the ER are also known to induce ER stress, which then proceeds to have numerous adverse effects on disease progression, as it will stimulate HSC activation, cause accumulation of mutations by generating free radicals, stimulate tumor growth, and promote tumor survival strategies [77,78]. A study examining the effect of HBV regulatory protein (HBx protein) on UPR activation in three human hepatoma cell lines revealed HBx as a potent UPR inducer and activator of the ATF6 and IRE1-XBP1 UPR branches, emphasizing this as a potential new HBV-induced mechanism of HCC development [79]. Studies have also reported on the ER stress-inducing capacity of the HBV pre-S mutant large surface antigen, as it leads to strong oxidative stress and genomic instability, thereby actively fueling hepatocarcinogenesis [80,81].

**The role of ER stress in HCC-associated immunosuppression**

Inflammation-driven HCC development is potently fueled by immune cell infiltration to the liver. Increasing evidence suggests that the UPR can regulate the innate immune response by controlling the release of damage-associated molecular patterns, which act as ‘eat me’ signals or chemoattractants [82]. Furthermore, accumulating evidence suggests the involvement of the UPR in coordinating immunosuppressive signaling,
protumoral functions of myeloid cells, and T-cell exhaustion within the tumor microenvironment (Fig. 2) [83]. A recent study by Dasgupta et al. revealed that the activation of the IRE1-XBP1 UPR branch in hepatocytes increases the expression of serine palmitoyltransferase genes, thereby promoting ceramide biosynthesis and the release of extracellular vesicles (EVs) in mice with diet-induced steatohepatitis. This consequently results in monocyte-derived macrophage recruitment to the liver, which thereby contributes to inflammation and injury in mice with steatohepatitis. Furthermore, patients with nonalcoholic steatohepatitis, one of the leading etiologies for liver disease and HCC, were found to have increased levels of liver-bound XBP1, serine palmitoyltransferase, and plasma EVs [84]. Targeting immune checkpoint inhibitors, such as PD1 and PD-L1, has shown promising results in treatment of advanced HCC. Liu et al. [85] have recently reported that ER stress in tumor cells helps them escape immunosurveillance and demonstrated that ER-stressed HCC cells upregulate PD-L1 expression in macrophages by releasing exosomes, which consequently suppresses T-cell function through an exosome miR-23a-PTEN-AKT pathway. A high density of infiltrated macrophages in the liver is known to correlate with increased tumor aggressiveness and poor prognosis in HCC patients. Blocking ER stress actors such as BiP and PERK in macrophages has been shown to promote an antitumoral immune response and cancer cell clearance [86,87]. IRE1α expression on tumor-associated macrophages (TAMs) in the liver has recently been shown to play a role in macrophage migration and, consequently, cancer progression in HCC-implanted mice. Tan et al. have shown that treatment with anti-inflammatory metabolite genipin inhibited IRE1-α expression and XBP1 splicing on TAMs and slowed down HCC progression in vivo. IRE1-α may therefore attribute to HCC progression through promoting the expression of inflammatory cytokines in macrophages, which consequently primes HCC proliferation [88]. However, recent findings from our group revealed that inhibiting the ADP-binding P2Y12 receptor, which was found to be expressed by Kupffer cells and involved in the pathogenesis of liver cirrhosis and HCC, induces an antitumoral macrophage (M1) phenotype and upregulates ER stress markers such as BiP, PERK, CHOP, and spliced XBP1 in a macrophage cell line. This upregulation of ER stress-associated genes was found to be correlated with the macrophages’ ability to phagocytose tumor cells, as P2Y12 inhibition increased tumor cell clearance by macrophages, while treatment with ER stress inhibitor TUDCA diminished this effect [89]. In line with these findings, a recent study on the effect of fatty acid on macrophage polarization revealed that PERK upregulation promotes with an M1 macrophage phenotype, while inhibiting PERK with PBA, GSK2656157 or by siRNA suppressed the M1 polarization and increased M2 (protumoral) priming [90].

Given the importance of understanding the molecular basis of chronic inflammation and immunosuppressive signaling in HCC pathogenesis, more studies are needed to provide better insight into the involvement of ER stress actors in these facets of HCC development.

Concluding remarks

ER stress has emerged as an important hallmark of various solid tumors, including liver cancer. The challenge in understanding the complex role of ER stress in HCC is highlighted by its dual, adaptive, and apoptotic functions in carcinogenesis. In this review, we provide a state-of-the-art overview of current scientific literature on the role of the unfolded protein response in liver cancer. Numerous studies have reported that pharmacologically targeting UPR actors to inhibit ER stress can ameliorate the adverse effects of tumor–stroma interactions, such as stellate cell activation and immunosuppressive signaling, as well as sensitize tumor cells to targeted therapy in HCC. However, another line of evidence suggests ER stress inducers as promising antifibrotic and antitumoral agents, as promoting the pro-apoptotic ER stress pathways can contribute to fibrosis resolution and increased chemo sensitivity in HCC. The duality of these proposed approaches could in part be explained by the different thresholds at which different cell types experience ER stress, as well as different microenvironmental cues that induce it. When hepatocytes become malignant, they exert an increased metabolic rate and mutation-driven protein synthesis need. This disruption of normal cell metabolism and ER homeostasis is further fueled by hostile microenvironmental conditions in a diseased liver, which consequently increases the threshold at which tumor cells will experience ER stress. Hence, careful consideration of etiological factors, disease stage, and progression, as well as the cell type targeted is needed when assessing the therapeutic strategy for targeting UPR actors in HCC.

Furthermore, despite the success of inhibiting proximal UPR signaling components with small molecules in preclinical cancer models, targeting the actors of the UPR systemically may have undesired long-term side effects for patients. This might increase the need
for targeted nanotherapies that specifically deliver the drugs to the cell-of-interest and thereby decrease off-target side effects [91]. It also remains unsure whether ER stress-targeting agents alone would suffice as effective treatments for HCC. It has been widely demonstrated that targeting UPR signaling in HCC cells sensitizes them to sorafenib-induced cell death, suggesting that ER stress inducers and inhibitors could have potential as adjuvant treatment in combination with standard HCC therapy. All of these factors encourage further research on the role of ER stress in the molecular pathogenesis of HCC and on determining which therapeutic targets and strategies could be utilized to effectively and safely ameliorate the effect ER stress imposes on the development of liver cancer.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

Conceptualization (F.H. and N.P.); resources (F.H.); writing—original draft preparation (N.P. and F.H.); writing—review and editing (N.P. and F.H.), funding acquisition (F.H.).

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