Targeting the unfolded protein response in disease

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Abstract | Stress induced by the accumulation of unfolded proteins in the endoplasmic reticulum (ER) is a feature of specialized secretory cells and is also observed in many diseases, including cancer, diabetes, autoimmune conditions, liver disorders, obesity and neurodegenerative disorders. Cellular adaptation to ER stress is achieved by the activation of the unfolded protein response, which is an integrated signal transduction pathway that modulates many aspects of ER physiology. When these mechanisms of adaptation are insufficient to handle the unfolded protein load, cells undergo apoptosis. Here, we discuss recent advances in the design of novel compounds and therapeutic strategies to manipulate levels of ER stress in disease.

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The endoplasmic reticulum (ER) is the main subcellular compartment involved in protein folding and maturation, and around one-third of the total proteome is synthesized in the ER. Many different perturbations can alter the function of this organelle, leading to the accumulation of unfolded or misfolded proteins inside the ER, a cellular condition referred to as ER stress. ER stress initiates a series of adaptive mechanisms that together are known as the unfolded protein response (UPR)[1]. Activation of the UPR affects many aspects of the secretory pathway to restore protein-folding homeostasis. Conversely, if cell damage is sufficiently severe, UPR signalling results in cell death by apoptosis[2].

The UPR is classically linked to the maintenance of cellular homeostasis in specialized secretory cells, including plasma B cells, salivary glands and pancreatic β cells, in which the high demand for protein synthesis and secretion constitutes a constant source of stress. In addition, recently developed mouse models in which essential UPR components are knocked out have revealed unexpected functions of the UPR in many physiological processes that are not directly linked to protein folding, including lipid and cholesterol metabolism, energy control, inflammation and cell differentiation[3,4].

The broad spectrum of activities of the UPR in organ homeostasis is also reflected in the role of ER stress in the progression of diseases such as cancer, diabetes and neurodegenerative disorders. Thus, there is a rationale for investigating approaches to therapeutically target the UPR in such diseases, and several pharmaceutical companies and academic laboratories are currently developing screening strategies to identify molecules that selectively modulate discrete UPR signalling modules. In addition, gene therapy using RNA interference technology or recombinant viral vectors is becoming an attractive approach to selectively modulate UPR function in affected tissues. The effects of manipulations of ER stress levels using these various strategies in preclinical models of disease have provided promising indications of their therapeutic potential. In this article, we review recent advances in targeting the UPR and ER homeostasis with pharmacological and gene therapy approaches, highlighting the application and limitations of the currently available agents.

Key players in UPR signalling

A ground-breaking study almost 25 years ago revealed the existence of a homeostatic pathway in mammalian cells that orchestrates adaptation to protein-folding stress through the transcriptional upregulation of key ER chaperone proteins[5]. In mammals, there are three classes of sensors of ER stress: inositol-requiring enzyme 1α (IRE1α) and IRE1β; protein kinase RNA-like ER kinase (PERK); and activating transcription factor 6 (ATF6; both α and β isoforms)[1] (FIG. 1).

Activation of IRE1α involves its dimerization, oligomerization and *trans*-autophosphorylation, which leads to a conformational change that activates the RNase domain. Activated IRE1α excises a 26-nucleotide intron of the mRNA that encodes the transcription factor X-box binding protein 1 (XBP1), which shifts the coding reading frame and leads to the expression of a more stable and active form known as XBP1s (for the spliced form)[6–8]. XBP1s transactivates a subset of target genes

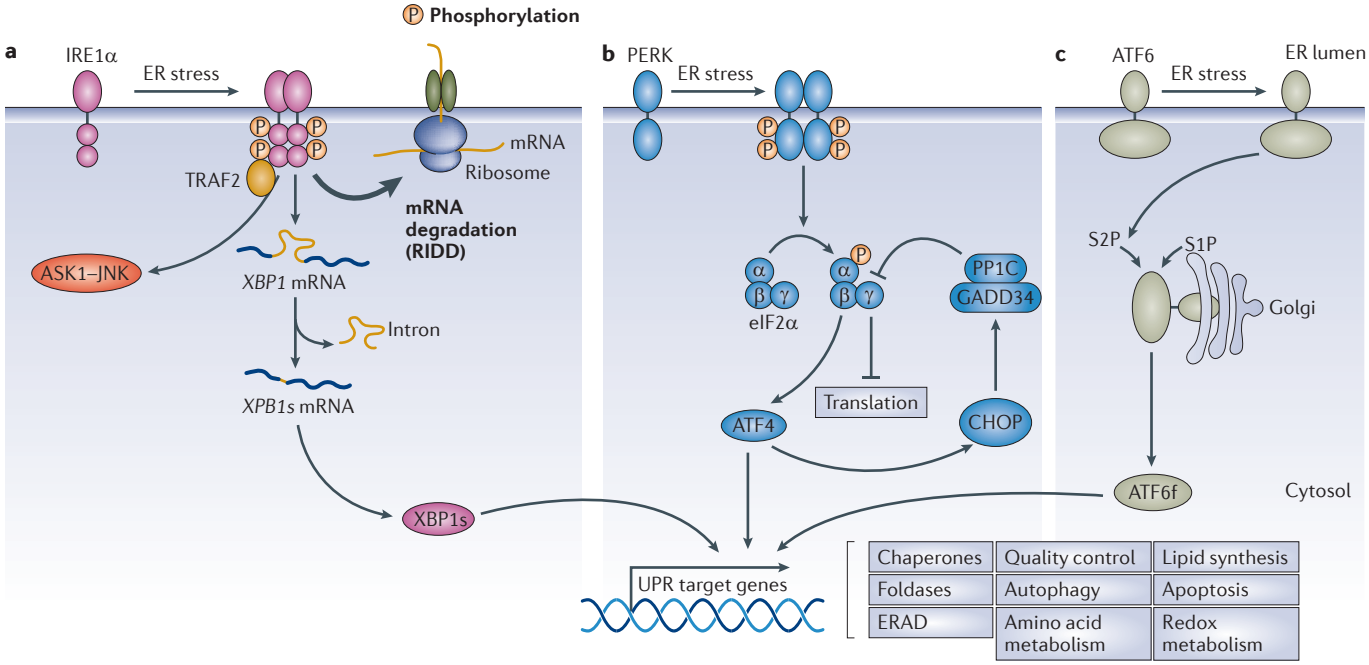


Figure 1 | **The unfolded protein response.** Endoplasmic reticulum (ER) stress induces an adaptive response known as the unfolded protein response (UPR). Three major stress sensors control UPR-dependent responses: inositol-requiring enzyme 1α (IRE1α), protein kinase RNA-like ER kinase (PERK) and activating transcription factor 6 (ATF6). These ER transmembrane proteins transduce signals to the cytosol and nucleus to restore protein-folding capacity through various pathways. **a** | IRE1α RNase activity processes the mRNA encoding the transcription factor X-box binding protein 1 (XBP1). This leads to the expression of an active transcription factor (XBP1s) that upregulates a subset of UPR target genes related to protein folding, ER-associated protein degradation (ERAD), protein quality control, and organelle biogenesis. IRE1α also degrades select mRNAs through a process called regulated IRE1-dependent decay (RIDD). In addition, IRE1α activates the JUN N-terminal kinase (JNK)– apoptosis signal-regulating kinase 1 (ASK1) pathway through the binding to adaptor proteins, such as tumour necrosis factor (TNF) receptor-associated factor 2 (TRAF2). **b** | Activation of PERK attenuates general protein synthesis through phosphorylation of the eukaryotic translation initiator factor 2α (eIF2α). EIF2α phosphorylation allows the selective translation of the *ATF4* mRNA, which encodes a transcription factor that induces the expression of genes involved in antioxidant responses, amino acid metabolism. autophagy and apoptosis. ATF4 controls the expression of the pro-apoptotic components GADD34 (growth arrest and DNA damage-inducible 34) and C/EBP-homologous protein (CHOP). GADD34 also binds protein phosphatase 1C (PP1C) to dephosphorylate eIF2α. **c** | ATF6 is localized at the ER in basal conditions and encodes a bZIP transcription factor in its cytosolic domain. In cells undergoing ER stress, ATF6 translocates to the Golgi apparatus where it is processed by a site 1 protease (S1P) and site 2 protease (S2P) releasing its cytosolic domain (ATF6f). ATF6f controls the upregulation of select UPR target genes.

Secretory pathway
The cell infrastructure dedicated to the folding, maturation and trafficking of transmembrane and secreted proteins.

ER-associated protein degradation
(ERAD). A mechanism to eliminate misfolded proteins generated at the endoplasmic reticulum (ER) through their retrotranslocation to the cytosol and further degradation by the proteasome.

UPRosome
A signalling platform assembled at the level of IRE1 that modulates it activity and controls downstream effector responses.

that are involved in protein folding, ER-associated protein degradation (ERAD), protein translocation to the ER, and protein secretion[9,10]. In addition to controlling gene expression upon ER stress, IRE1α signals through other complementary mechanisms mediated by the assembly of a protein platform — termed the UPRosome — that comprises many adaptor proteins and regulators[11,12]. The oligomerization of IRE1α into large clusters is also proposed to dynamically modulate its own signalling[13].

IRE1α interacts with the adaptor protein tumour necrosis factor (TNF) receptor-associated factor 2 (TRAF2) to instigate the downstream activation of apoptosis signal-regulating kinase 1 (ASK1) and JUN N-terminal kinase (JNK)[14,15]. IRE1α RNase activity also degrades a subset of mRNAs through a process known as regulated IRE1-dependent decay (RIDD) of mRNA[16–18]. Remarkably, the pool of mRNAs degraded by RIDD activity depends on the cell type and, in general, is specific for mRNAs that encode proteins of the secretory pathway. This selective degradation of mRNAs by IRE1α may depend on the tendency of the encoded protein to misfold and on the presence of a conserved nucleotide sequence accompanied by a defined secondary structure[16–19]. In addition, IRE1α can cleave premature microRNAs, which affects the regulation of apoptosis[20]. Recently, the regulation of expression levels of IRE1α by microRNAs was shown to affect its biological functions[21–23].

The activation of PERK is similar to IRE1α, in that it also involves dimerization and *trans*-autophosphorylation and the formation of large clusters[24,25] (FIG. 1). Activated PERK phosphorylates eukaryotic translation initiator factor 2α (eIF2α), which leads to the inhibition of protein synthesis. This rapidly reduces the number of

proteins entering the ER, and therefore has an important pro-survival effect on the cell[26]. Phosphorylation of eIF2α also allows the translation of mRNAs containing short open reading frames in their 5′-untranslated regions, such as activating transcription factor 4 (ATF4). ATF4 controls the expression of genes that encode proteins involved in redox processes and amino acid metabolism, as well as ER chaperones and foldases[27,28]. ATF4 also regulates the expression of important genes involved in apoptosis, including the transcription factor C/EBP-homologous protein (CHOP) and growth arrest and DNA damage-inducible 34 (GADD34). GADD34 participates in a feedback loop to dephosphorylate eIF2α by interacting with protein phosphatase 1C (PP1C), which restores protein synthesis[29].

ATF6α is a type II ER located protein that contains a bZIP transcription factor in its cytosolic domain. Upon ER stress, ATF6α translocates to the Golgi complex where it is processed to release a cytosolic fragment, ATF6f[30,31] (FIG. 1c). ATF6f is a transcription factor that regulates the expression of genes of the ERAD pathway, among other target genes[7,32]. The exclusive or combined action of ATF6f and XBP1s may also have a differential effect on gene expression[33].

In general, the understanding of the mechanism of activation of UPR sensors in mammals is limited[24]. In yeast, a direct recognition mechanism of IRE1 has been demonstrated, in which the binding of misfolded proteins to a major histocompatibility complex class I-like structure is involved in the oligomerization and further activation of this sensor protein[34,35]. However, *in vitro* and structural evidence suggests that a direct recognition mechanism may not operate for IRE1α activation in mammalian cells[24]. Nevertheless, a recent report suggests that IRE1β may directly bind misfolded proteins, similarly to yeast IRE1 (REF. 36). Moreover, it is proposed that the binding and dissociation of BiP (an ER chaperone) to the ER luminal domains of UPR stress sensors upon ER stress operates as an activating signal (reviewed in REF. 24).

Many components of the UPR trigger apoptosis under chronic ER stress conditions (reviewed in REFS 2,37). For example, the induction of ATF4 and its downstream target CHOP leads to apoptosis[38], possibly through the transcriptional upregulation of several pro-apoptotic proteins of the BCL-2 family, known as BH3-only proteins (such as BIM)[39] and the downregulation of BCL-2 (REF. 40). Expression of GADD34 may also sensitize cells to cell death, as GADD34 resumes protein synthesis in stressed cells[41]. IRE1α activation also contributes to cell death through two distinct mechanisms: activation of ASK1 and JNK[14,42], and sustained RIDD[16]. Excessive generation of reactive oxygen species at the ER and the release of calcium from this compartment are also emerging as factors that lead to the elimination of irreversibly damaged cells[2]. Many other components of the ER stress–apoptosis machinery have been described and reviewed elsewhere[2,37,43].

One of the mechanisms underlying the shift of UPR signalling from adaptive to pro-apoptotic responses involves differential kinetics of activation and attenuation of stress sensor proteins. For example, in certain systems, the IRE1α–XBP1 pathway is turned off in cells undergoing prolonged ER stress, whereas PERK signalling is sustained[11,44,45]. This striking difference in signalling behaviour may ablate the protective effects of XBP1 expression, and also enhance the expression of downstream apoptosis targets of ATF4 and CHOP. In summary, growing evidence indicates that UPR signalling integrates information about the intensity and the duration of the stress stimuli to promote cell adaptation or cell death (FIG. 1).

ER stress and disease

The UPR is becoming an attractive pathway to target for drug discovery because of emerging evidence from animal models indicating its contribution to diverse diseases, including cancer, metabolic diseases, diabetes, neurodegenerative disorders, inflammation, liver dysfunction, and brain and heart ischaemia.

Initial attention in the field was focused on the relationship between protein-misfolding disorders (PMDs) and ER stress because many genetic alterations lead to the expression of mutant proteins that accumulate in the ER[46]. However, studies in genetically modified mice in which essential UPR components were altered have shown that the UPR pathway is relevant to many physiological processes and diseases (see REFS 4,47 for reviews).

In this section, we summarize a selection of the most relevant functional studies that have validated the role of ER stress in disease, focusing on *in vivo* models that have genetically manipulated the UPR (TABLES 1,2). We also relate this information to possible functions of the UPR pathway in the affected tissue.

Neurodegenerative diseases. Analysis of brain tissue from mouse PMD models and from patients affected with PMDs have shown a direct correlation between the accumulation of abnormal protein aggregates and the upregulation of ER stress markers (reviewed in REF. 48). Interestingly, genetic manipulation of the UPR has distinct effects on disease progression and histopathological features, with contrasting results in different models. Moreover, specific signalling branches of the UPR may have distinct and sometimes opposing effects in PMDs.

For example, several studies indicate that ER stress is an early pathological process mediating neurodegeneration in Parkinson's disease (reviewed in REFS 49,50). Ablation of ATF6α enhances the susceptibility of dopaminergic neurons to neurotoxins that induce Parkinson's disease[51,52], whereas CHOP deficiency has the opposite effect[53]. Experiments in mouse models of amyotrophic lateral sclerosis (ALS) indicate that activation of the PERK pathway promotes survival[54], whereas XBP1 and ATF4 may have the opposite effect by modulating autophagy levels[55] or apoptosis gene expression[56], respectively. These results suggest that inhibiting particular UPR signalling modules may have contrasting effects on ALS pathogenesis. Similarly, genetic manipulation of the UPR in mouse models of Huntington's disease indicate that XBP1 deficiency also delays progression of the disease, which is associated with the degradation of the

Table 1 | **Examples of therapeutic effects of targeting the UPR in disease models *in vivo***

Organ	Disease	Agent	Effects	Refs
Pharmacological approaches				
CNS	Amyotrophic lateral sclerosis	Salubrinal	Extends lifespan of mutant *SOD1* transgenic mice	147
	Parkinson's disease	Salubrinal	Extends lifespan and increases neuronal survival of α-synuclein transgenic mice	146
	Ischaemia	BIX	Reduces infarct volume	161,162
	Excitotoxicity	Salubrinal	Improves neuronal survival	145
	Parkinson's disease	Tunicamycin	Protects neurons against 6-hydroxydopamine-induced toxicity	167
Gene therapy				
CNS	Retinitis pigmentosa	AAV-BiP	Restores visual function in mutant rhodopsin transgenic rats	169
	Retinal degeneration	AAV-XBP1s	Reduces axonal degeneration	170
	Glaucoma	AAV-XBP1s	Increases neuronal survival	170
	Spinal cord injury	AAV-XBP1s	Improves locomotor recovery	58
	Huntington's disease	AAV-XBP1s	Reduces aggregation of mutant huntingtin	171
	Parkinson's disease	AAV-BiP	Reduces toxicity and aggregation of α-synuclein	172
	Parkinson's disease	AV-XBP1s	Protects neurons against MPTP-induced toxicity	173
	Prion-related disorder	LV-GADD34	Reduces neuronal degeneration	60
Heart	Ischaemia	AV-PDIA1	Improves survival of myocardial cells	174
Liver	Diabetes	AAV-XBP1s	Improves glucose metabolism and insulin resistance	78
	Obesity	AAV-BiP	Reduces liver steatosis in obese mice	175

AAV, adeno-associated virus; AV, adenovirus; BIX, BiP inducer X; CNS, central nervous system; GADD34, growth arrest and DNA damage-inducible 34; LV, lentiviral vector; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PDIA1, protein disulphide isomerase A1; *SOD1*, superoxide dismutase 1; XBP1s, X-box binding protein 1 (spliced form).

mutant huntingtin protein by autophagy[57]. By contrast, targeting ATF4 does not have any effect on huntingtin protein levels[57]. Thus, predicting the effects of the UPR in PMDs affecting the nervous system is complex, which is possibly due to the different cellular effects induced by distinct UPR signalling modules in specific diseases.

Both the adaptive phases of the UPR (assessed using mice with a genetic deficiency in XBP1 or ATF4) and the pro-apoptotic phases of the UPR (assessed using mice with a genetic deficiency in CHOP) have been linked to tissue damage in spinal cord injury[58,59]. For prion-related disorders, although ER stress is observed in mouse models of the disease, the PERK pathway[60] and not the IRE1α–XBP1 pathway[61] affects disease progression. There is also functional evidence linking ER stress to ischaemia–reperfusion and to several myelin-related diseases, including multiple sclerosis (reviewed in REFS 50,62).

From all these studies, it is apparent that the contribution of the UPR to neurodegenerative diseases depends on the disease context. These examples highlight the need for a systematic assessment of the specific contribution of distinct UPR signalling components to particular neurodegenerative diseases to better define optimal drug targets for intervention.

Inflammatory diseases. One of the best illustrations of the involvement of ER stress signalling in inflammatory diseases is provided by inflammatory bowel diseases[63–65]. In Crohn's disease and ulcerative colitis, IRE1β[66], XBP1 (REF. 67) or PERK[68] play a key role in disease progression. The UPR is also activated in innate immune responses. As such, Toll-like receptor 2 (TLR2) and TLR4 trigger the activation of IRE1α and the subsequent splicing of *XBP1* mRNA[69]. The splicing of *XBP1* mRNA is required for maximal production of pro-inflammatory cytokines, such as interleukin-6 in macrophages. In contrast, TLR signals attenuate ATF6 and PERK activity and the downstream effector ATF4 in macrophages[70,71]. The UPR also has an important function in other immune cells, including B cells and dendritic cells, where it modulates cell differentiation and protein secretion (reviewed in REFS 72,73).

Metabolic disorders. ER stress enhances various inflammatory and stress signalling pathways to aggravate metabolic dysfunction, contributing to obesity, insulin resistance, fatty liver and dyslipidaemia (reviewed in REFS 74,75). ER stress is functionally linked with hepatic steatosis, which is due to either enhanced lipogenesis or decreased hepatic lipoprotein secretion. ER stress also inhibits hepatic lipoprotein secretion[76]. XBP1s regulates

Table 2 | **Targeting endoplasmic reticulum proteostasis in cancer**

Molecule	Target (effect)	Indication	Phase	Readout	Refs
Sunitinib*	Multiple kinases (inhibits tumour proliferation)	Multiple myeloma	Phase II; FDA approved for renal cell carcinoma	Inhibition of IRE1 activity	111
Sorafenib*	Multiple kinases (cytotoxic)	Various cancers	FDA approved for renal carcinoma and hepatocellular carcinoma	Induction of VCP phosphorylation, activation of UPR	185
STF-083010	IRE1 RNase domain (cytotoxic)	Multiple myeloma	Preclinical	Inhibition of *XBP1* mRNA splicing	104
4μ8C	IRE1 RNase domain	Multiple myeloma	Preclinical	Inhibition of *XBP1* mRNA splicing	102
MKC-3946	IRE1 RNase domain (sensitization to bortezomib)	Multiple myeloma	Preclinical	Inhibition of *XBP1* mRNA splicing	10
Toyocamycin	IRE1 (cytotoxic)	Various cancers	Preclinical	Inhibition of *XBP1* mRNA splicing	108
GSK2656157	PERK (anti-angiogenic)	Multiple myeloma, pancreatic cancer	Preclinical	Inhibition of PERK and eIF2α phosphorylation, ATF4 translation and *CHOP* mRNA expression	114
Bortezomib (PS341)	26S proteasome (cytotoxic, apoptotic)	Various cancers	Preclinical; FDA approved for multiple myeloma	Activation of PERK, ATF4 or CHOP activity	103, 123–126
MG-132	26S proteasome (apoptotic)	Various cancers	Preclinical	Activation of UPR	123–125
Eeyarestatin	VCP (sensitization to bortezomib, inhibits tumour proliferation)	Cervical cancer, non-small cell lung cancer	Preclinical	Induction of transcription of UPR genes, accumulation of ubiquitylated proteins	117
ML240	VCP (inhibits tumour proliferation)	Various cancers	Preclinical	Accumulation of ubiquitylated proteins and LC3-II	119
DBeQ	VCP (inhibits tumour proliferation)	Various cancers	Preclinical	Accumulation of ubiquitylated proteins and LC3-II	118
17-AAG	HSP90 (apoptotic)	Various cancers	Phase III	Activation of UPR	135
Radicicol	HSP90 (apoptotic)	Various cancers	Preclinical	Activation of UPR	134,135
MAL3-101	HSP70 (inhibits tumour proliferation, apoptotic)	Multiple myeloma	Preclinical	Induction of *XBP1* mRNA splicing	134

ATF4, activating transcription factor 4; CHOP, C/EBP-homologous protein; eIF2α, eukaryotic translation initiator factor 2α; GADD34, growth arrest and DNA damage-inducible 34; HSP, heat shock protein; IRE1, inositol-requiring enzyme 1; LC3-II, microtubule-associated protein 1 light chain 3 (phosphatidylethanolamine conjugate); PERK, protein kinase RNA-like ER kinase; XBP1, X-box binding protein 1; UPR, unfolded protein response; VCP, valosin-containing protein.*Note that sunitinib and sorafenib were approved by the US Food and Drug Administration (FDA) on the basis of receptor tyrosine kinase signalling inhibition and not on their capacity to modulate the UPR.

fatty acid synthesis by inducing the expression of crucial lipogenic enzymes, such as stearoyl-CoA desaturase 1 (REF. 77). Interestingly, XBP1s interacts with the forkhead box O1 (FOXO1) transcription factor[78] and the regulatory subunits of phosphoinositide 3-kinase (PI3K), decreasing hepatic gluconeogenesis[79,80]. Cyclic AMP-responsive element-binding protein 3-like protein 3 (CREB3L3; also known as CREBH), a liver-specific membrane-anchored transcription factor intermediate of the UPR[81], controls iron metabolism and inflammation[81,82]. CREBH also regulates hepatic lipogenesis, fatty acid oxidation and lipolysis under conditions of metabolic stress[83]. Together, these studies show that the UPR is involved in several pathological conditions associated with metabolic alterations.

Cancer. A role for ER stress signalling in cancer was initially proposed in 2004, introducing the concept that it could either be beneficial for tumour growth or play a guardian role to prevent cell transformation[84] (BOX 1). In recent years, the involvement of the PERK and IRE1 arms of the UPR in tumour growth has been demonstrated (reviewed in REF. 85). As most of the available molecules intended to pharmacologically target the UPR have been tested in cancer models, in this section we expand the discussion to describe the specific involvement of ER stress in different aspects of cancer biology.

Several genomic screens have revealed that IRE1α is commonly mutated in human cancers[86–88]. Cells deficient for XBP1 or PERK have a large reduction in their ability to form solid tumours in nude mice[89,90]. The activation of the UPR in cancer has been attributed to the hypoxic condition of the tumour environment[91]. In addition, ER stress signalling was shown to represent a barrier against melanocyte transformation[92]. The IRE1α arm of the UPR is directly involved in tumour development in glioblastoma through the regulation of the expression of pro-inflammatory cytokines and pro-angiogenic mechanisms[93,94]. Moreover, gene expression profiling showed that loss of functional IRE1α signalling

Box 1 | **The unfolded protein response and cancer**

Roles for the unfolded protein response (UPR) in cancer have been suggested since the mid-1990s and were formally proposed in 2004 by Ma and Hendershot[84]. UPR signalling is primarily initiated by three endoplasmic reticulum (ER) stress sensors: protein kinase RNA-like ER kinase (PERK), activating transcription factor 6 (ATF6; both α and β isoforms) and inositol-requiring enzyme 1α (IRE1α) and IREβ. These sensors are activated by the accumulation of misfolded proteins in the ER lumen, which then triggers downstream cascades, leading to an enhancement of protein folding, increased efficiency of quality control mechanisms, the degradation of misfolded proteins and reduced translation, among other effects that attenuate ER stress[1].

The UPR mediates adaptation of cells to stress to restore ER homeostasis or to induce cell death of irreversibly damaged cells[1]. Tumour cells are often subjected to major molecular changes due either to transformation-dependent metabolic demand or to stressful environments, including hypoxia, nutritional stress or pH stress[85]. In such conditions, ER stress signalling represents an important constituent of tumour progression and survival[85]. IRE1α also enhances angiogenesis and may alter cell adhesion and migration through regulated IRE1-dependent decay (RIDD).

Pro- or anti-oncogenic role of the UPR? There is evidence indicating that the activation of ER stress signalling pathways promotes anti-oncogenic or pro-oncogenic activities. Both PERK and IRE1α arms of the UPR have important roles in cancer, possibly in cell transformation and tumour progression[92,93,96]. XBP1s (a more stable and active form of XBP1) has also been suggested to mediate multiple myeloma cell differentiation[184]. By contrast, the ATF6 arm was reported to contribute to tumour cell dormancy[100]. It is currently unclear which signalling outputs of the UPR are most important in oncogenesis.

results in the upregulation of genes that encode extracellular matrix proteins for which the mRNAs were direct targets of RIDD[95]. This highlights the role of IRE1α signalling in tumour growth, infiltration and invasion and extends the paradigm of secretome control in tumour microenvironment conditioning[95].

More recently, the role of ER stress in promoting oncogene-driven cell transformation was also proposed[96]. Indeed, ER stress-mediated autophagy induction was demonstrated to favour cell transformation induced by MYC[97]. Indeed, c-MYC and N-MYC activate the PERK–ATF4 arm of the UPR, leading to increased cell survival through the induction of cytoprotective autophagy[98].

Results documenting the role of ATF6α in cancer are sparse. It has been suggested that ATF6α is involved in hepatocarcinogenesis[99], but the mechanism underlying this is unclear. ATF6α has also been implicated in the regulation of tumour cell dormancy, in which it acts as a pivotal survival factor for quiescent but not proliferative squamous carcinoma cells[100]. ATF6α is essential for the adaptation of dormant cells to chemotherapy, nutritional stress, and, most importantly, the *in vivo* microenvironment[100].

Expression of components of the ER protein-folding machinery, such as BiP, has also been suggested to promote tumour progression, cell survival, metastasis and resistance to chemotherapy (reviewed in REF. 101). BiP has even been proposed as a biomarker of cancer progression.

Thus, accumulating evidence indicates that all branches of the UPR contribute to the development of cancer, affecting diverse aspects of the disease including angiogenesis, cell differentiation, cell migration, tumour growth and the inflammatory microenvironment.

Chemical probes for modulating UPR components

As detailed above, the activation of UPR signalling can engage both pro-survival and pro-apoptotic cellular programmes. Thus, modulating UPR signalling components has the potential to either stimulate an increased capacity to alleviate protein misfolding, which could have therapeutic effects in PMDs, or to promote apoptosis, which could be used as an anticancer strategy. The available chemical probes for manipulating these pathways are summarized below, with an emphasis on the most recently identified classes of molecules and on those with potential uses in the diseases listed in TABLE 1 and TABLE 2. We define two major classes of compounds: those that inhibit UPR pro-survival effects and those that promote adaptation to stress.

Inhibiting UPR pro-survival effects

Screening studies have identified several compounds that can inhibit key mediators of ER stress signalling.

IRE1α inhibitors. Compounds that target IRE1α interact with one of two sites on this sensor: the catalytic core of the RNase domain or the ATP-binding pocket of the kinase domain (FIG. 2a). Molecules that interact with the catalytic core of the IRE1α RNase domain include salicylaldehydes (typified by 3-methoxy-6-bromosalicylaldehyde), 4μ8C, MKC-3946 and STF-083010 (FIG. 2b), which were identified in high-throughput screens for IRE1α RNase activity[102–105]. The salicylaldehydes, 4μ8C and MKC-3946 were identified using recombinant IRE1α and FRET (fluorescence resonance energy transfer)-based *XBP1* mRNA cleavage assays *in vitro*, whereas STF-083010 was identified using a reporter gene assay in cells[102–105].

4μ8C targets the critical lysine 907 residue in the catalytic core of the RNase domain, forming a stable imine that blocks cleavage of *XBP1* mRNA and RIDD (FIG. 2c). Other inhibitors in this group may have a similar mode of action to 4μ8C given their structural similarity or ability to competitively block 4μ8C binding to the lysine 907 residue in IRE1α[102,104].

In *in vitro* studies, 3-methoxy-6-bromosalicylaldehyde blocked XBP1 splicing and RIDD activity, and specifically bound to IRE1α in a reversible manner[105]. This compound (at a dose of 50 mg per kg) inhibited *XBP1* mRNA splicing in the kidney, liver and spleen in animals injected with tunicamycin, an ER stress inducer[105].

In mice bearing human multiple myeloma xenografts, treatment with STF-083010 (at a dose of 30 mg per mg per week) significantly inhibited growth of tumours[106]. STF-083010 also showed preferential cytotoxicity for multiple myeloma cells compared with cells obtained from healthy donors[104,105].

MKC-3946 alone showed little toxicity against multiple myeloma cells. More importantly, MKC-3946 inhibited the formation of tumours *in vivo* in a xenograft model of multiple myeloma, and also showed synergistic effects in combination with the proteasome inhibitor bortezomib[103]. In this context, it seems that bortezomib induces ER stress, which is increased by the inhibition of *XBP1* mRNA splicing by MKC-3946. These effects correlated with attenuated *XBP1* mRNA splicing in the tumour and enhanced ER stress.

4μ8C has not been tested *in vivo*, probably because of its unfavourable pharmacokinetics. However, it was characterized in cell culture. Interestingly, although 4μ8C blocked *XBP1* mRNA splicing and RIDD, it did not affect the survival of cells under acute ER stress[102]. By contrast, 4μ8C had a clear effect on the expansion of the secretory pathway in a cellular model of exocrine pancreatic cells.

Another screen for small molecule modulators of IRE1α RNase activity (using an XBP1 luciferase reporter assay) identified toyocamycin as a potent inhibitor[107]. This compound, produced by an *Actinomycete* strain, did not affect IRE1α phosphorylation *in vitro*, but specifically blocked its endoribonuclease activity. Toyocamycin also did not affect ATF6α or PERK signalling. Similar to other IRE1α inhibitors, toyocamycin showed synergistic activity with bortezomib to induce apoptosis of multiple myeloma cells at nanomolar concentrations, and retarded the growth of xenografts in a mouse model of the disease (at a dose of 0.5 mg per kg twice a week)[107]. In the same screen, the authors previously identified trierixin and quinotrierixin as possible repressors of *XBP1* mRNA splicing; however, detailed mechanistic insights about these drugs are not available[108,109]. Thus, available compounds to inhibit IRE1α activity have demonstrated potential for the treatment of cancer, and may be used in combination with other chemotherapeutic agents to increase their anticancer efficacy.

IRE1α modulators that interact with the hinge region of the ATP-binding pocket and stabilize an active kinase domain conformation include sunitinib and APY29 (also classed as type I ATP competitive broad kinase inhibitors). Sunitinib and APY29 block IRE1α activity yet allosterically activate the IRE1α RNase domain *in vitro* and possibly in cells; however, there are mixed reports as to whether sunitinib is inhibitory or stimulatory to *XBP1* mRNA splicing[110]. APY29 was not tested in animal models of ER stress, but showed potent effects on IRE1α signalling in cell culture[110]. In addition, a peptide modulator derived from IRE1α was shown to modulate its oligomerization and consequently affect signalling outcomes by enhancing *XBP1* mRNA splicing[111]. Unexpectedly, this peptide prevented JNK activation and RIDD activity[111].

Finally, IRE1α modulators that compete with ATP binding and stabilize an inactive conformation of IRE1α include compound 3 (which is also classed as a type II kinase inhibitor)[112] (FIG. 2d,e). Compound 3 was able to prevent kinase activity, oligomerization and RNase activity[112]. Compound 3 was not tested in animal models of ER stress, but showed potent effects on IRE1α signalling in cell culture[112].

PERK inhibitors. Structure-guided optimization of a screening hit identified compound 38 (also known as GSK2606414), a small molecule that inhibits PERK phosphorylation[113] (FIG. 3). GSK2606414 (at a dose of 50–150 mg per kg per day) was orally active and decreased tumour growth in a xenograft model of pancreatic cancer[113]. A related optimized PERK inhibitor, GSK2656157 (at a dose of 50–150 mg per kg twice a day) also inhibited tumour growth in several mouse xenograft models[114]. In this study[114], *in vivo* testing revealed that GSK2656157 affects PERK autophosphorylation in the pancreas, and several physiological aspects were correlated with the anticancer activity of GSK2656157, including altered amino acid metabolism, decreased blood vessel density, and vascular perfusion[114].

A small-molecule screen using an ATF4 luciferase reporter identified ISRIB (FIG. 3) as a potent inhibitor of eIF2α phosphorylation that does not affect PERK activation but impairs adaptation to ER stress in cell culture[115]. ISRIB had favourable pharmacokinetic properties and no overall toxicity in mice. The authors reported promising effects of ISRIB in improving learning and memory *in vivo* based on previous reports indicating a negative role of eIF2α phosphorylation and ATF4 expression in this cognitive process[116].

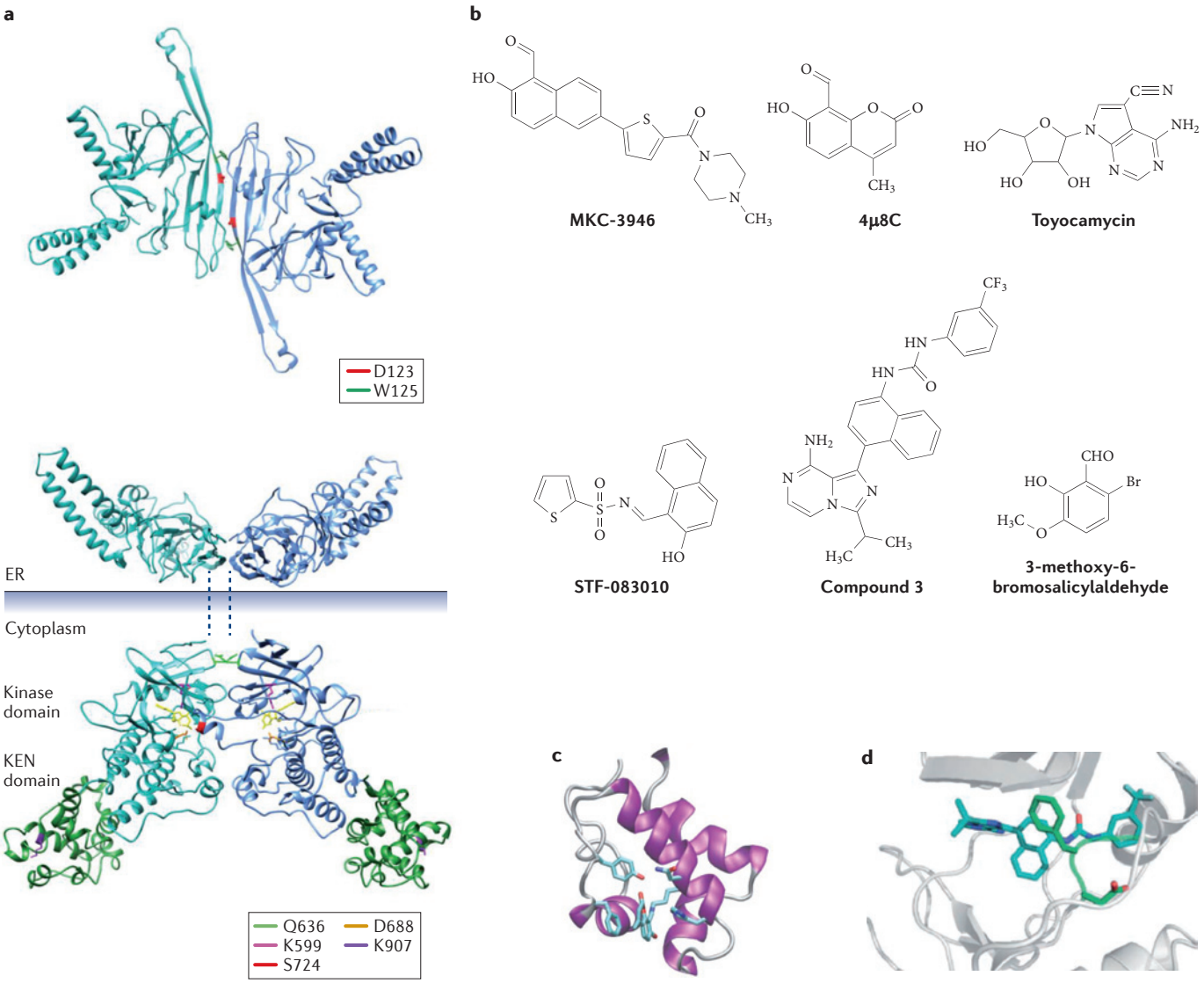
In summary, inhibition of PERK signalling has promising applications for the treatment of cancer and also cognitive deficits.

ERAD modulators. Inhibition of protein clearance pathways that are involved in the degradation of misfolded proteins generated at the ER is predicted to trigger a strong stress response, and to decrease the survival of UPR-dependent tumours. Small-molecule-mediated inhibition of ERAD can be achieved either by directly targeting the proteasome or by blocking ER client dislocation (ERAD). Proteasome inhibitors include bortezomib and MG132. Inhibitors of ERAD include valosin-containing protein (VCP; also known as p97) ATPase inhibitors such as alkylsulphanyl-1,2,4-triazoles, DBeQ and its derivatives ML240 and ML241, and eeyarestatin[117–121,185] (FIG. 4).

Multiple second-generation proteasome inhibitors have been developed following the success of bortezomib in treating myeloma[122–124]. The cause of cell death induced by bortezomib requires further study but may involve several mechanisms, including the induction of ER stress[125,126]. The AAA+ ATPase VCP, which is required for retrotranslocation of ERAD substrates, can be inhibited by the first-generation inhibitor eeyarestatin or the more recently identified reversible quinazoline inhibitors DBeQ, ML240 and ML241, that have greater potency and specificity against VCP over other ATPases[117–121]. Thus, generic alteration of ER proteostasis by inhibiting ERAD may have applications for the treatment of UPR-dependent cancer.

Modulators of chaperone activity and quality control. Negative regulation of chaperone activity has been investigated as an anticancer strategy as 'chaperone addiction' is a common feature driving cell transformation. BiP is an essential ER chaperone with key roles in cell survival. Strategies to downregulate BiP in cell culture models of cancer or through the use of BiP ATP-binding domain inhibitors have great cytotoxic potential (reviewed in REFS 127,128). For example, honokiol, a *Magnolia grandiflora* derivative, is a BiP inhibitor and was shown to induce apoptosis in brain tumours[129]. Other indirect approaches evaluated also suggest that the negative regulation of BiP, either at the transcriptional or post-translational levels, could have anticancer effects[130–133].

Chaperone addiction
The dependency of a tumour cell on high chaperone levels and activities to cope with metabolic and environmental demands. This provides the tumour cells with a survival advantage which, when inhibited, might become toxic.



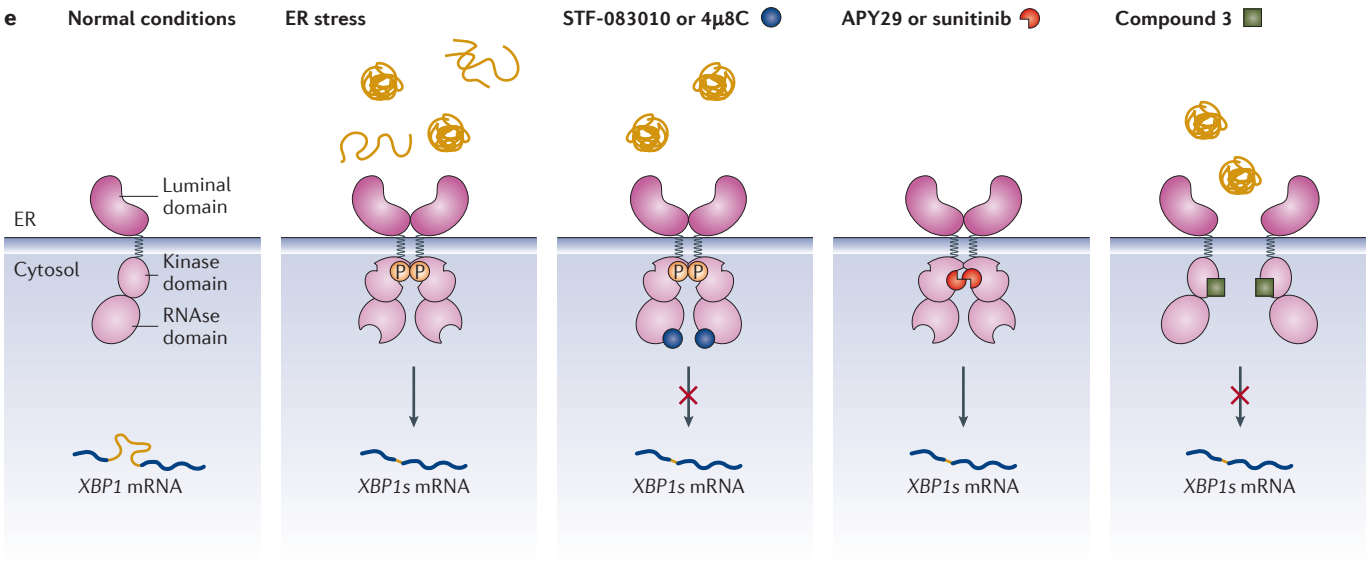


Figure 2 | **Pharmacological modulation of IRE1. a** | A three-dimensional structure of human inositol-requiring enzyme 1α (IRE1α) highlighting the endoplasmic reticulum (ER) luminal domain (Protein Data Bank (PDB) code: 2HZ6) and the cytosolic domain (PDB code: 3P23). The upper image depicts the structure of the ER luminal domain of IRE1α and highlights the dimer interface. The amino acid residues D123 and W125 are indicated, which are important for dimerization and activation. Each monomer is indicated with a different colour. The lower image depicts the cytosolic portion of human IRE1α and highlights the kinase domain and the RNase and kinase extension nuclease (KEN) domain. The ATP binding site is indicated where ATP is shown in yellow. In the kinase domain the autophosphorylation amino acid residue (S724), the ATP binding site (K599), active site (protein acceptor, D688) are indicated. In addition, the RNase active site (K907) is indicated, in addition to Q636, which is a key residue for dimerization. **b** | Structures of small molecules that target IRE1α. **c** | Model of the IRE1α RNase domain covalently bound to 4μ8C through the K907 residue via an imine[102–105]. **d** | A molecular model of compound 3 (an IRE1α kinase inhibitor) interacting with the ATP binding site of IRE1α. This model is in the DFG-out inactive conformation[112]. **e** | Schematic representations of the mechanism of action of several compounds that modulate IRE1α activity. Under ER stress conditions, IRE1α dimerizes and *trans*-autophosphorylates, leading to a conformational change of the RNase domain and inducing its activation, which then mediates the splicing of the X-box binding protein 1 (*XBP1*) mRNA to generate active XBP1s. STF-083010 and 4μ8C inhibit the RNase activity of IRE1α by directly binding to its active site. Although STF-083010 inhibits the RNase activity of IRE1α, it does not affect the kinase activity or the overall oligomerization state of IRE1α. APY29 or sunitinib (both type I kinase inhibitors) inhibit IRE1α *trans*-autophosphorylation but promote oligomerization and activate the RNase domain. Compound 3 (a type II kinase inhibitor) inhibits both the kinase and RNase domains of IRE1α and stabilizes the monomeric form of IRE1α. Figure 2c is modified, with permission, from REF. 112 © (2012) Macmillan Publishers Ltd. All rights reserved. Figure 2d is reproduced from REF. 102.

Other agents that modulate chaperone activity include heat shock protein 90 (HSP90) inhibitors such as 17-AAG (a less toxic derivative of the benzoquinone ansamycin antibiotic geldanamycin) and radicicol. These inhibitors bind to the amino-terminal ATP-binding domain of their targets and result in cell death[134–136]. GRP94, the ER resident HSP90 homologue, is also a target of geldanamycin[137], and inhibition of GRP94 induced apoptosis in B chronic lymphocytic leukaemia cells[138].

Protein disulphide isomerases (PDIs) are becoming interesting targets for drug discovery owing to their emerging role in several human diseases (FIGS 4,5; reviewed in REFS 139,140). PDIA1 inhibitors have strong anticancer activity in models of melanoma and malignant glioma[141,142], possibly due to the inhibition of the pro-survival effects of the UPR in cancer. Unexpectedly, a screen for molecules that inhibit mutant huntingtin and amyloid-β toxicity also identified PDIA1 inhibitors as potent neuroprotective compounds[143]. Although ER chaperones are emerging as interesting targets to modify ER proteostasis, there is a need for the development of more specific compounds and to define in detail the ER folding networks.

Attenuators of ER stress levels

Several strategies have been developed to enhance the adaptive capability of ER stressed cells or to reduce protein misfolding inside the ER. In this section, we discuss the most relevant approaches available to ameliorate the detrimental effects of chronic ER stress observed in several human diseases.

Enhancers of eIF2α phosphorylation. Cell-based assays conducted to search for compounds that promote survival under stress identified inhibitors of eIF2α phosphatases (FIG. 3). Salubrinal indirectly inactivates the eIF2α phosphatase complexes GADD34–PP1C and constitutive regulator of eIF2α phosphorylation (CReP)–PP1C, possibly by promoting the disassembly of the complexes[144]. This effect increases the levels of eIF2α phosphorylation, reduces translation rates and activates downstream ATF4 signalling[144]. Salubrinal can reduce neuronal death after excitotoxicity in the hippocampus[145], and also alleviate degeneration in models of Parkinson's disease[146] and ALS[147]. By contrast, salubrinal had detrimental effects in a prion disease model in that it accelerated disease progression[60].

Guanabenz directly binds to GADD34 but not CReP, preventing GADD34–PP1C assembly and thereby increasing eIF2α phosphorylation and downstream signalling only under stress conditions. Guanabenz is also a clinically approved α_2-adrenergic receptor agonist used to treat hypertension, and so off-target effects must be considered when interpreting results[148]. However, the selectivity of guanabenz on ER stress-induced eIF2α phosphorylation means that it could have higher potential than salubrinal for targeting this pathway in a disease context, as salubrinal might induce undesirable effects in the long-term because of uncontrolled translational inhibition (FIG. 3c). This concept is supported by genetic evidence, as GADD34 knockout mice develop normally[41], whereas knockout of both CReP and GADD34 is embryonically lethal[149], suggesting that persistent inhibition of the two eIF2α phosphatases may have negative effects.

Taken together, inducing a general decrease in protein synthesis through enhanced phosphorylation of eIF2α and/or the induction of ATF4 have promising potential to reduce ER stress levels in several diseases.

Chemical chaperones. Chemical chaperones are a group of low-molecular mass compounds that stabilize the folding of proteins and buffer abnormal protein aggregation (FIGS 4,5). Chemical chaperones have been shown to improve ER function, possibly by attenuating protein misfolding and consequently reducing ER stress[150].

The most studied chemical chaperones in a disease context *in vivo* are 4-phenylbutyrate (4-PBA) and tauroursodeoxycholic acid (TUDCA), which have been approved by regulatory authorities for primary biliary cirrhosis (4-BPA) and urea cycle disorders (TUDCA). Another chemical chaperone, trehalose, is currently used as a preservative in the food industry. These compounds have good safety profiles in humans.

Chemical chaperones reduced ER stress in the liver of mouse models of obesity, improved insulin sensitivity and glucose homeostasis[151], and reversed leptin resistance[152]. Treatment with 4-PBA also improved glucose tolerance in patients with insulin-resistance[153] and TUDCA partially restored insulin sensitivity in liver and muscle, but not adipose tissue in patients with obesity[154]. Administration of chemical chaperones to an animal model of brain ischaemia–reperfusion alleviated ER stress, correlating

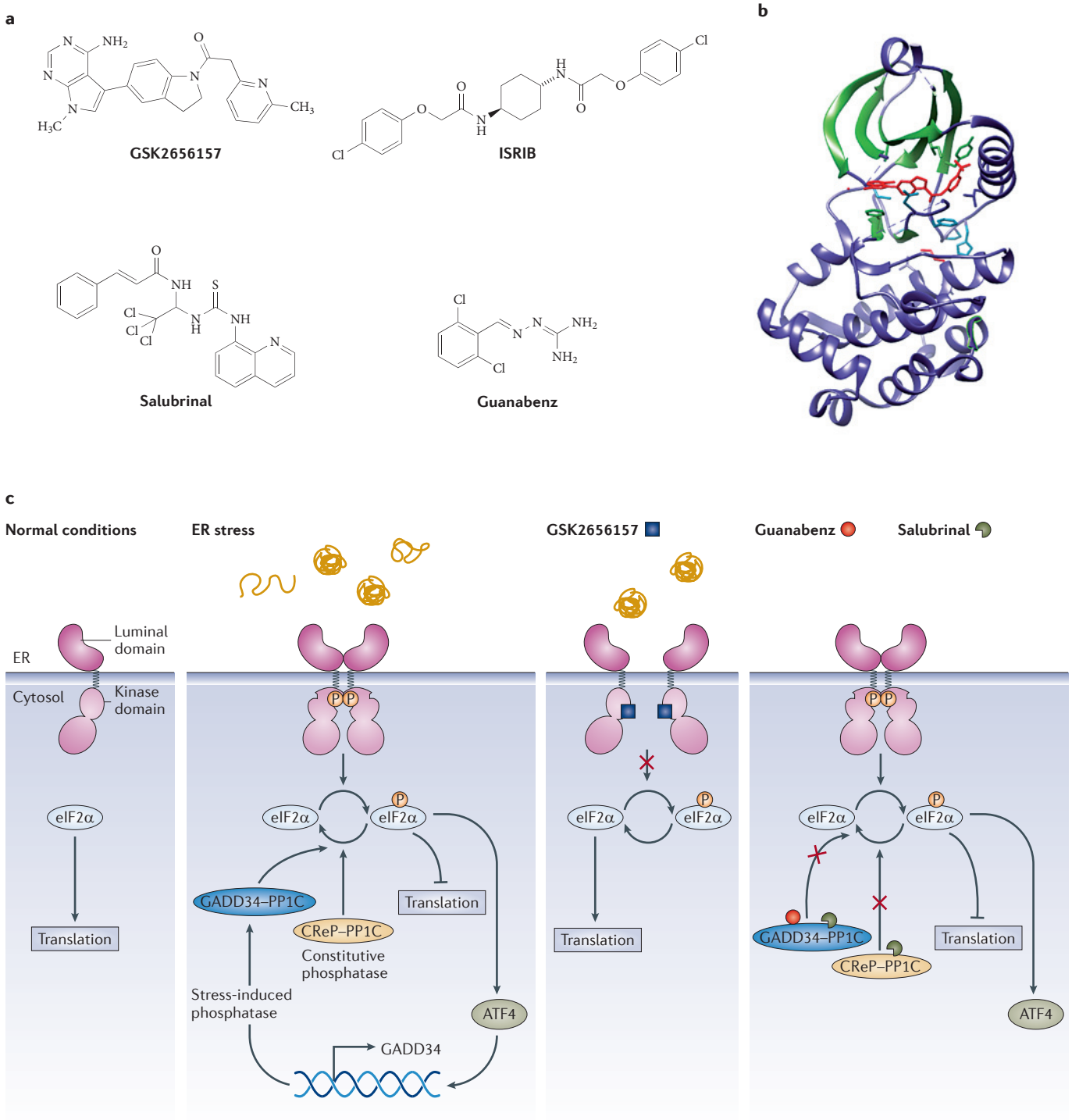


Figure 3 | **Pharmacological modulation of PERK signalling.** **a** | Structures of small molecules that target protein kinase RNA-like ER kinase (PERK) and eukaryotic translation initiator factor 2α (eIF2α). **b** | Crystal structure of compound 38 (also known as GSK2606414)[113] bound to the active site of the PERK kinase domain (Protein Data Bank code: 4G31). Protein residues mediating the interaction are indicated by showing the lateral residues. **c** | Schematic representation of PERK signalling indicating the effects on translational control in response to endoplasmic reticulum (ER) stress mediated by eIF2α phosphorylation, the downstream effects on activating transcription factor 4 (ATF4) expression, and the feedback loop triggering eIF2α phosphatases. Pharmacological manipulation of PERK or eIF2α phosphorylation with GSK2656157, guanabenz and salubrinal is shown. Guanabenz selectively inhibits the stress-induced eIF2α phosphatase, whereas salubrinal induces eIF2α phosphorylation in both unstressed and in stressed cells. CreP, constitutive regulator of eIF2α; phosphorylation GADD34, growth arrest and DNA damage-inducible 34; PP1C, protein phosphatase 1. Figure 3b courtesy of J. Axten, GlaxoSmithKline, Collegeville, Pennsylvania, USA.

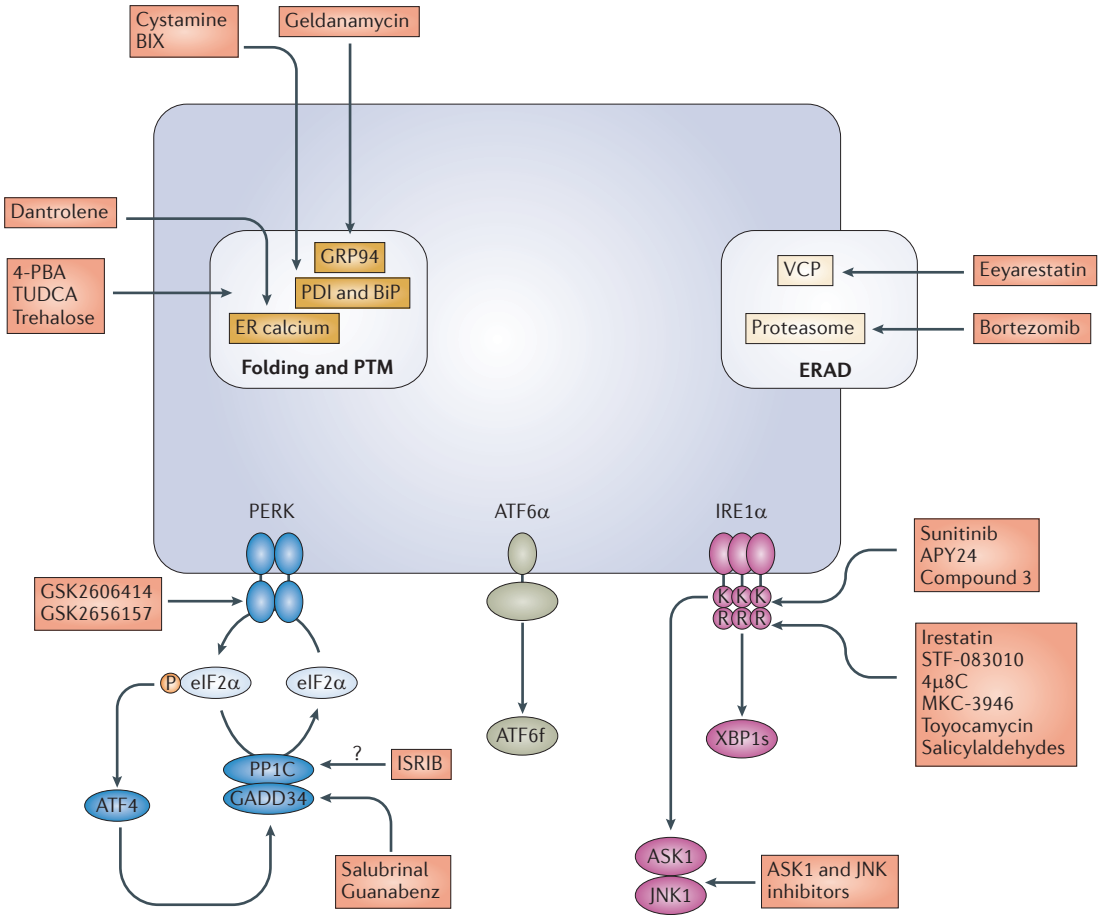


Figure 4 | **Therapeutic molecules to target ER proteostasis.** Specific molecules targeting different aspects of the unfolded protein response and endoplasmic reticulum (ER) physiology are indicated. 4-PBA, 4 phenylbutyrate; ASK1, apoptosis signal-regulating kinase 1; ATF, activating transcription factor; BIX, BiP inducer; X; eIF2α, eukaryotic translation initiator factor 2α; ERAD, ER-associated protein degradation; GADD34, growth arrest and DNA damage-inducible 34; IRE1α, inositol-requiring enzyme 1α; JNK1, JUN N-terminal kinase 1; PDI, protein disulphide isomerase; PERK, protein kinase RNA-like ER kinase; PP1C, protein phosphatase 1C; PTM, post-translational modification; TUDCA, tauroursodeoxycholic acid; VCP, valosin-containing protein; XBP1s, X-box binding protein 1 (spliced form).

with neuroprotective effects[155]. Similar results were also reported in models of spinal cord injury and photoreceptor pathology[156]. Chemical chaperones also protected the liver from steatosis[157] and ischaemia, which was associated with ER stress mitigation[158]. Thus, chemical chaperones offer a therapeutic opportunity to reduce global pathological ER stress in a broad range of diseases.

Other targets. Several molecules are available to improve ER folding capacity by altering general processes (FIGS 4,5), including enhancers of BiP expression, autophagy activators, antioxidants, and drugs that affect ER calcium homeostasis (reviewed in REFS 159,160), and we highlight a few here. BiP inducer X (BIX) was identified in a screen for compounds that induce BiP expression[161]. Treatment of mice with BIX reduced the infarct volume in models of middle cerebral artery occlusion[161,162] and protected photoreceptors against light-induced cell death[163]. Moreover, post-treatment of animals after severe artery occlusion provided strong neuroprotective effects[164]. Similar results were reported in a kidney ischaemia model[165].

Other small molecules that can alleviate ER stress *in vivo* include flavonoids, which have a broad impact on UPR gene expression[160]. The ryanodine receptor antagonist dantrolene increases ER calcium content, and was reported to decrease ER stress levels in animal models of brain ischaemia[166]. Finally, a recent study described the unexpected finding that treatment of mouse and fly models of Parkinson's disease with sublethal doses of the ER stress agent tunicamycin provided protection against neuronal degeneration, possibly due to a preconditioning effect[167]. These studies highlight the concept that there are multiple ways of targeting ER physiology that may have therapeutic potential to treat diseases related to abnormal ER stress.

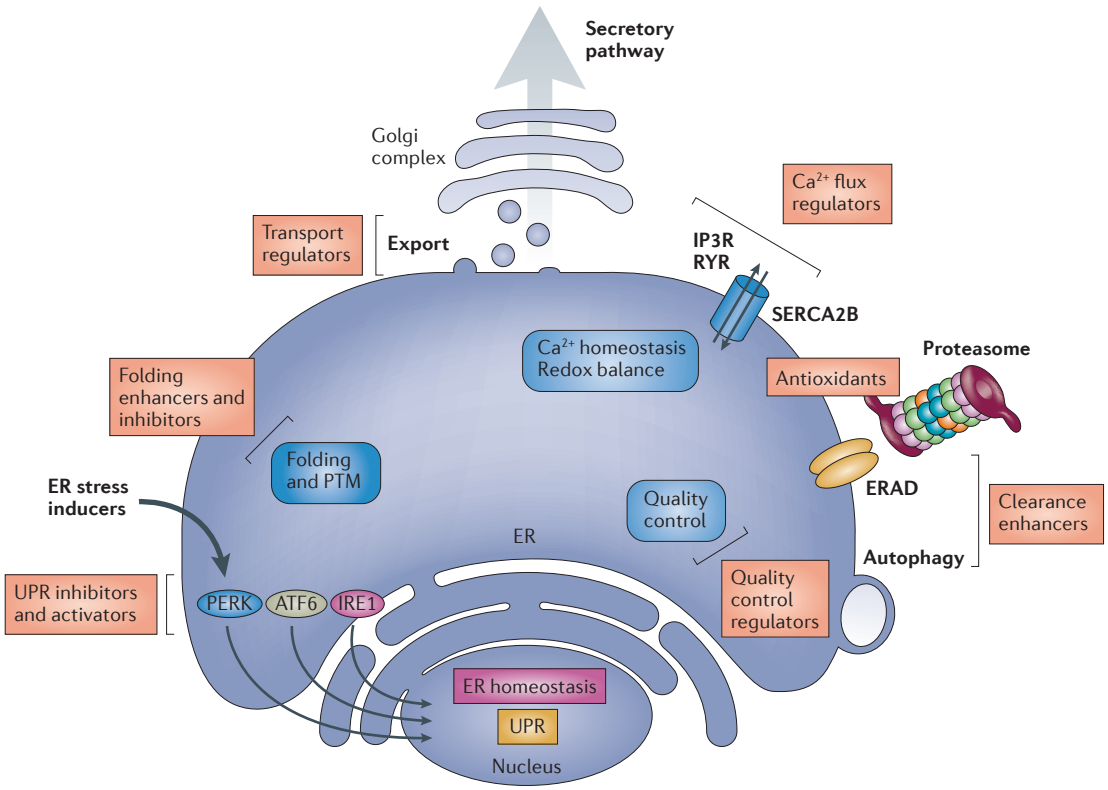


Figure 5 | **Sites of action of therapeutic molecules to target ER proteostasis.** Numerous points of intervention are possible to attenuate endoplasmic reticulum (ER) stress in a disease context. The unfolded protein load could be reduced through specific manipulation of signalling components of the unfolded protein response (UPR), including inhibitors of eukaryotic translation initiator factor 2α (eIF2α) phosphorylation or activators of inositol-requiring enzyme 1 (IRE1). The protein folding capacity can be improved at different levels through various mechanisms, including the stabilization of protein structures using chemical chaperones, the expression of folding components or the enhancement of quality control mechanisms. The degradation of abnormally folded proteins could be improved with strategies that upregulate macroautophagy or enhance the efficiency of the ER-associated protein degradation (ERAD) pathway. As the efficiency of the protein folding status at the ER depends on specific redox conditions and calcium concentration in its lumen, modulators of these two components with calcium flux modulators or antioxidants can improve protein folding. Finally, regulators of ER to Golgi trafficking or enhancers of protein secretion may reduce the unfolded protein load at the ER. ATF6, activating transcription factor 6; IP3R, inositol trisphosphate receptor; PERK, protein kinase RNA-like ER kinase; PTM, post-translational modification; RYR, ryanodine receptor; SERCA2B, sarco/endoplasmic reticulum Ca^{2+}-ATPase 2B.

Gene therapy to modulate UPR signalling

Gene therapy using recombinant viruses is becoming an attractive strategy to deliver active UPR components to specific tissues. This method may also avoid the pleiotropic effects of systemic and chronic administration of ER stress-targeting compounds. In addition, for central nervous system diseases, gene therapy might be a way to circumvent issues that small-molecule drugs may have, such as limited penetration across the blood–brain barrier, although gene therapy presents its own range of delivery challenges.

Adeno-associated viruses (AAVs) are the current choice to deliver therapeutic genes into the brain because of their safely profile, as demonstrated in pilot clinical trials (see REF. 168 for a review). The latest generation of AAVs is episomal (thereby avoiding possible mutagenesis effects), they do not trigger significant immunological reactions, their production can be scaled up to large volumes for human use, and the expression of the transgene is sustained for many years after one injection[168]. Moreover, there is a large spectrum of serotypes available with selective tropism for specific neuronal and glial populations.

A few recent studies have shown positive effects of modulating ER stress in neurodegenerative conditions using gene therapy. For example, the AAV-mediated subretinal delivery of BiP decreased ER stress levels and restored visual function in a rat model of retinitis pigmentosa[169]. Enforced expression of XBP1s using an AAV also reduced retinal ganglion cell loss induced by retinal axon degeneration or glaucoma[170]. This strategy also improved motor recovery and oligodendrocyte survival in spinal cord injury models[58], and decreased mutant huntingtin aggregation *in vivo*[171].

Strong therapeutic effects using an injected AAV to deliver BiP into the substantia nigra were demonstrated in a Parkinson's disease model based on α-synuclein expression[172]. Using adenoviruses, XBP1s was also directly expressed into the substantia nigra of mice, which reduced the loss of dopaminergic neurons after the exposure of animals to a Parkinson's disease-inducing neurotoxin[173]. In addition, delivery of the expression vectors for ER foldase PDIA1 into the heart using adenoviruses yielded protection against acute myocardial infarction[174]. As mentioned, in other diseases such as prion-related disorders, sustained eIF2α phosphorylation may underlie the pathology. Ectopic expression of GADD34 into the brain using lentiviral vectors reduced neurodegeneration of prion-infected mice *in vivo*[60].

Injection with adenoviruses to express XBP1s in the liver led to strong effects in mouse models of diabetes[78]. AAV-mediated delivery of BiP to the liver of *ob*/*ob* mice (a model of type 2 diabetes) or in wild-type mice exposed to high fat diet led to the decrease of steatosis through attenuation of UPR signalling[175]. In the context of cancer, it remains to be determined whether knocking down important UPR components (that is, PERK and IRE1α) in solid tumours using the local delivery of gene therapy viruses may also have therapeutic effects.

All these studies indicate that gene therapy approaches to deliver active UPR components or downstream effectors are effective in reducing ER stress levels *in vivo*. Such therapies can be locally applied in the affected tissue without compromising animal survival or other tissues. The field is at an early stage, however, and more studies are needed to assess the efficacy of targeting other important UPR mediators *in vivo* using gene therapy.

Perspectives

ER dysfunction is considered an important factor in a wide range of diseases, and because the type, intensity and temporality of ER stress stimuli determines UPR responses, the pathway offers interesting targets to therapeutically modulate both cell survival and cell death mechanisms. As discussed here, genetic evidence from mouse models indicates that depending on the disease context, therapeutic strategies intended to promote cell survival may aim to enhance protective UPR signalling responses involved in adaptation to stress, attenuate ER stress levels, or inactivate UPR pro-apoptotic components.

With regard to enhancing the protective effects of the UPR, salubrinal and guanabenz are so far the only small molecules identified that can selectively enhance the activity of a particular signalling branch of the UPR pathway (that is, eIF2α–ATF4). Most of the studies using salubrinal have focused on brain disorders because chronic ER stress is well validated as a pathological mechanism driving neurodegeneration. In this context, activating the pro-survival or adaptive effectors of the UPR is anticipated to have a substantial impact in PMDs. There is still a need to identify novel compounds that enhance UPR signalling, and hopefully provide selective activation or enhancement of IRE1α–XBP1 or ATF6 signalling. The main disadvantage with this approach is the fact the ATF4 is a major component in the transition between adaptive to cell death programmes, and sustained ATF4 expression can result in apoptosis. In addition, ATF4 is involved in bone physiology[176] and in learning and memory processes[177]. Indeed, salubrinal can decrease the learning and memory capacity of mice[177]. Calibrating drug concentrations and regimens for chronic use of these compounds is a challenging issue for future clinical trials, which would also benefit from the development of compounds that have greater specificity and potency.

The use of chemical chaperones has illustrated the possible consequences of attenuating ER stress in disease. However, it is important to note that most of the compounds tested as chemical chaperones remain poorly characterized in terms of their mode of action, they have poor selectivity and require high doses and chronic treatment regimens. Inhibition of UPR pro-apoptotic components is also an attractive strategy to mitigate cell death in conditions such as stroke or spinal cord injury; however, this strategy in the long term does not restore ER homeostasis.

IRE1α and PERK have conserved kinase domains, and so these stress sensors offer particularly promising opportunities for future drug development. Primarily, as discussed above, inhibitors of these kinases could have anticancer activity, based on pro-apoptotic effects described *in vivo*. However, it is necessary to characterize in more detail the selectivity of the available IRE1α and PERK inhibitors, and the possible side effects of the chronic administration of such compounds has not been established. Based on the robust literature delineating the fundamental role of XBP1 and IRE1α in the function of highly specialized secretory cells, we predict that although IRE1 inhibition has great potential for the treatment of cancer, it may have considerable long-term side effects on the function of pancreas, the immune system and the liver. The same is predicted for the use of PERK inhibitors. Hepatotoxicity should be explored carefully as IRE1α and XBP1 have important roles in several key functions of the liver, including cholesterol and lipid metabolism[178,179], in addition to drug detoxification[180] mechanisms.

As RIDD activity is becoming recognized as an important effector of the UPR, with implications in diabetes, lipid synthesis[178,179], acetaminophen toxicity[180], and cell migration[95], new screening efforts are needed to identify compounds that selectively target RIDD or *XBP1* mRNA splicing. Finally, the demonstration that a type I kinase inhibitor can activate IRE1α RNase function[107] may stimulate a search for a more potent and specific activator that could potentially be used to promote XBP1s expression and downstream activation of cytoprotective mechanisms.

Because XBP1, ATF4 and ATF6f are transcription factors, and their structures are not available, it is currently difficult to rationally design specific compounds that enhance or inhibit their activities. Interestingly, data indicate that XBP1s function can be modulated by several post-translational modifications including

phosphorylation, sumoylation, ubiquitylation and acetylation[11], suggesting that, in theory, XBP1 activity or stability could be modulated using pharmacological approaches. Similarly, the activity of ATF4 is tuned by post-translational modification and through the association with other cofactors[181]. The activation of ATF6 is also affected by the formation of disulphide bonds and glycosylation[182,183], which could be explored as a target to alter its activity.

We believe that gene therapy will emerge as an alternative strategy to enhance selective UPR responses, and available data indicate therapeutic effects from delivering active UPR components locally into the affected tissue in different disease models. However, so far, no reports have explored the possible detrimental long-term effects of the chronic expression of active UPR components. Gene therapy approaches are designed to deliver and sustain expression of the transgenes for several months and even years, and the possible effects of this strategy on cell differentiation, immunogenicity and cell transformation should be explored.

Besides the known role of the UPR in protein folding stress responses and PMDs, it is becoming clear that the pathway represents a broader stress response with important physiological functions in diverse organs. These include activities of the UPR in innate and adaptive immunity, cell differentiation, angiogenesis, cholesterol and lipid metabolism, insulin signalling, glucose homeostasis, synaptic function and ageing[3]. Novel signalling crosstalk may underlie these emerging functions of UPR signalling modules beyond protein folding stress. Thus, agents that affect specific UPR signalling components may have applications in a wide range of diseases, although for the same reason, the side effects of UPR-targeting drugs may be complex to predict.

In the context of cancer treatment, transient inhibition of the UPR may avoid off-target effects on organ function, and low doses of the compounds could be used in combination with other chemotherapy agents. In other disease contexts, therapeutic strategies may require partial modulation of UPR activity and not full inhibition. As UPR stress sensors are modulated by positive and negative regulators through a physical association[11], we propose that targeting UPRosome composition (that is, binding to cofactors) has considerable potential as a strategy to adjust ER proteostasis in the long-term, possibly with less side effects than full inhibition of the UPR. More studies are needed to identify allosteric sites on UPR components and the mechanisms that fine-tune the UPR. More sophisticated assays for drug discovery and systematically defining the effects of compounds on the pathway as a global network are also major needs. Finally, predicting and defining the possible side effects of manipulating the UPR at the systemic level remains an important subject for the validation of the pathway as a true drug target and the progression of UPR modulators into clinical trials.

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Competing interests statement
The authors declare no competing financial interests.

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