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Review

The unfolded protein response as a target for cancer therapy

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ABSTRACT

Various physiological and pathological conditions generate an accumulation of misfolded proteins in the endoplasmic reticulum (ER). This results in ER stress followed by a cellular response to cope with this stress and restore homeostasis: the unfolded protein response (UPR). Overall, the UPR leads to general translational arrest and the induction of specific factors to ensure cell survival or to mediate cell death if the stress is too severe. In multiple cancers, components of the UPR are overexpressed, indicating increased dependence on the UPR. In addition, the UPR can confer resistance to anti-cancer treatment. Therefore, modification of the UPR should be explored for its anti-cancer properties. This review discusses factors associated with the UPR that represent potential therapeutic targets.

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1. Introduction

The endoplasmic reticulum (ER) is the site within the cell where proteins, steroids, cholesterol and lipids are synthesized. The ER also functions as an intracellular reservoir for Ca^{2+}. The synthesis of proteins depends heavily on these Ca^{2+}-rich conditions, as well as on the oxidizing environment of the ER. In addition, the proper synthesis of new proteins requires assistance of numerous other factors, including ER-resident chaperones, Ca^{2+}-binding proteins and folding enzymes. PDI (protein disulphide isomerase) is an important folding enzyme that forms disulphide bonds in new proteins. Hereby, PDI is reduced and subsequently oxidized by ERO1 (thiol oxidoreductase). The ultimate electron acceptor is molecular oxygen, which generates H_2O_2 and reduced glutathione [1].

Multiple adverse conditions can induce ER stress. These include hypoxia, nutrient deprivation, acidosis and certain chemicals (see Table 1). A consequence of ER stress is the incorrect folding and improper glycosylation of newly synthesized proteins. The accumulation of such unfinished proteins (proteotoxicity) triggers the unfolded protein response (UPR) [2]. The UPR is an evolutionary conserved cytoprotective

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Table 1
ER stress inducing compounds.

List of ER stress inducing compounds		Ref.
Tunicamycin	Inhibitor of N-linked glycosylation	[23,28,67,93]
Thapsigargin	Inhibitor of Ca^{2+} ATPase (SERCA), promotes Ca^{2+} from ER	[14,16,23,93,109,110]
A23187	Ca^{2+} ionophore, promotes Ca^{2+} from ER	[67,93,109]
Dithiothreitol	Reducing agent, induces protein misfolding	[111,112]
2-Deoxyglucose	Blocks glycolysis, also disrupts N-linked glycosylation.	[67]
Brefeldin A	ER-Golgi transport inhibitor	[93,110]
Proteasome inhibitors (Bortezomib, MG132)	Inhibit proteasomal protein degradation	[22,23]

response that allows cells to adapt to ER stress. This response aims to re-establish homeostasis by arresting both the cell cycle as well as general protein translation. This blocks the synthesis of even more proteins allowing the cell to dispose of the misfolded proteins. However, despite the translational halt, the UPR induces expression of specific factors required to restore homeostasis and regulate feedback mechanisms. For example, ER chaperone proteins and folding enzymes are activated. The UPR also enhances the capacity to degrade unfolded proteins by the proteasome or by autophagy. Failure of proper protein assembly and folding activates ER-associated degradation (ERAD). ERAD is an essential part of the UPR as it stimulates the degradation and removal of unfolded proteins from the ER. Proteins are loaded into the cytosol and degraded by the proteasome. However, ER stress may cause excessive accumulation of substrates for the proteasome, which impair the function of this system [3]. If the stress turns out to be too severe, the UPR can control cell fate by inducing apoptosis (see Fig. 1A).

Especially in cancer cells, stresses that induce the UPR are prevalent: within the tumour microenvironment regions deprived of oxygen and nutrients are common [4]. This leads to increased reliance of cancer cells on the UPR for survival and makes it an excellent therapeutic target. In addition, some anti-cancer therapies applied in the clinic are known to provoke therapy-induced ER stress, which further enhances the dependence of tumour cells on the UPR. In this review we discuss the factors that are involved in the UPR and how they affect tumour cells. Finally, we will highlight the current data on interfering with the UPR for therapeutic benefit in cancer.

2. Transducers of the UPR

The UPR comprises three signalling pathways that operate in parallel. The master regulators of these arms are three resident ER trans-membrane proteins: PERK (PKR-like endoplasmic reticulum kinase), IRE1 (inositol-requiring enzyme 1) and ATF6 (activating transcription factor 6) (see Fig. 2). The cytoplasmic parts of both PERK and IRE1 have kinase activity. PERK and IRE homo-dimerize and are activated by trans-autophosphorylation. In contrast, activation of ATF6 relies on translocation to the Golgi-apparatus, where it is cleaved. Cleaved ATF6 is transported into the nucleus, where it functions as a transcription factor for several genes. Before signalling through the UPR arms can take place, GRP78 (glucose-regulated protein 78) has to dissociate from the ER trans-membrane proteins.

2.1. Activation of the UPR depends on GRP78

In unstressed conditions, the luminal domains of PERK, IRE1 and ATF6 are occupied by GRP78, also known as BiP (binding immunoglobulin protein). This sterically blocks homo-dimerization of PERK and IRE1, and prevents translocation of ATF6. During ER stress, the accumulation of unfolded proteins triggers GRP78 to release PERK, IRE1 and ATF6, resulting in their activation. GRP78 binds the unfolded proteins with higher affinity and functions as an important ER chaperone assisting with proper protein folding or degradation. Recent evidence suggests that GRP78 dissociation from IRE1 is not sufficient for IRE1-activation [5]. Mutation of the luminal domain of IRE1 in yeast, which lacks the binding site for GRP78 but is still able to homo-dimerize, does not lead to permanent activity of IRE1. These data indicate that other factors besides GRP78 dissociation are necessary for the activation of IRE1, and possibly also PERK and ATF6.

Apart from the translocation of GRP78 from PERK, IRE1 and ATF6 to unfolded proteins, ER stress also induces de novo synthesis of GRP78 to assist in protein folding in the ER. Therefore, the induction of GRP78 expression is a well-established hallmark of ER stress and UPR-activation. Nevertheless, GRP78 has other functions besides its task as a chaperone. During ER stress, Ca^{2+} can be released from the ER, increasing the intracellular Ca^{2+}-concentration and potentially triggering cell death. Within the ER, GRP78 can bind Ca^{2+} and overexpression of GRP78 reduces cell death induced by Ca^{2+}-efflux of the ER [6]. In addition, GRP78 has anti-apoptotic properties. It can block caspase-mediated cell death [7] and inhibition of GRP78 expression increases apoptosis [8]. Furthermore, GRP78 inhibition sensitizes cells to hypoxia, oxidative stress and ionophore treatment [9].

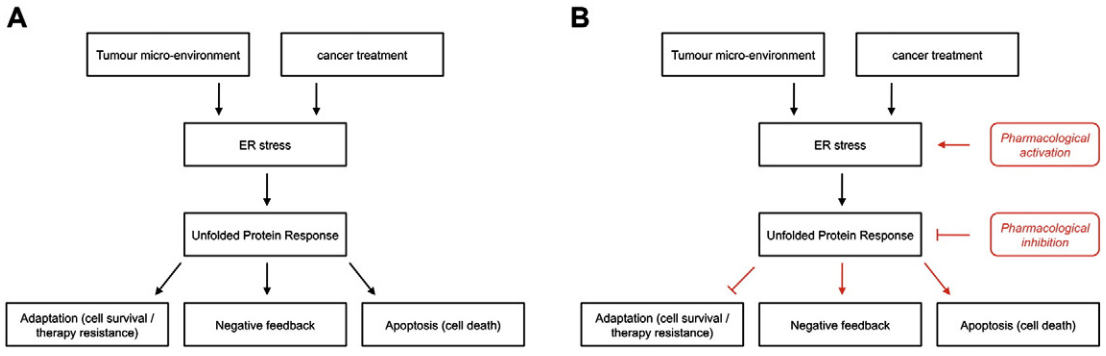

Fig. 1. The UPR induces an adaptive response after induction of ER stress, which can be therapeutically exploited. A. The UPR as a mechanism to deal with ER stress. The tumour microenvironment, as well as several cancer therapies, induces ER stress. In turn, this activates the UPR. The UPR leads to either a cellular response to cope with the stress or to cell death if the stress turns out to be too severe. In addition, a negative feedback mechanism is launched to end the response if homeostasis is restored. B. Modulation of the UPR for therapeutic benefit. Several approaches can be employed to interfere with the UPR. Firstly, ER stress can be pharmacologically activated to hyper-induce the UPR. This leads to an overload of the capacity of the UPR, aimed at the induction of cell death. Second, pharmacological inhibition of the UPR itself leads to removal of the survival benefit the UPR offers during ER stress and therefore to cell death.

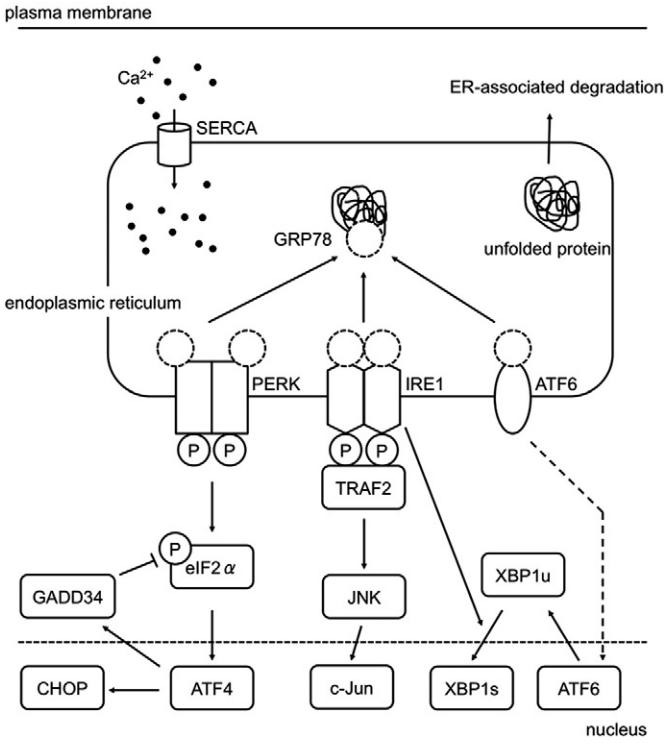

Fig. 2. The molecular pathways of the unfolded protein response. In response to ER stress, GRP78 dissociates from PERK, IRE1 and ATF6 to occupy the generated unfolded proteins. Activated PERK phosphorylates eIF2α, which activates the transcription factor ATF4. ATF4 induces GADD34, which promotes dephosphorylation of eIF2α and impedes signalling through the PERK-arm. IRE1 generates a splice variant of XBP1 and activates the JNK pathway. ATF6 functions as a transcription factor for XBP1.

2.2. The PERK-arm of the UPR induces translational arrest

PERK was first described by Shi et al. [10] and Harding et al. [11], and ever since the importance of PERK-signalling has been highlighted by several studies that have exposed cells with modified PERK-signalling to hypoxia or other ER stressors. After homo-dimerization and trans-autophosphorylation, PERK promotes phosphorylation of eIF2α on serine [10–12]. This causes a general translational arrest, which prevents the formation of more proteins. In addition, by reduced formation of cyclin D1 protein, cell cycle arrest is induced [13]. In fibroblasts and transformed cells hypoxia causes activation of PERK, thereby inducing eIF2α phosphorylation [14]. The phosphorylation of eIF2α occurs rapidly during severe hypoxia (0.05% O_2) or anoxia but is more gradual during moderate hypoxia (1% O_2). Upon reoxygenation eIF2α is swiftly dephosphorylated. Loss of PERK-signalling represses phosphorylation of eIF2α as well as the inhibition of protein synthesis [14,15]. In addition, mutation of PERK or eIF2α reduces cell survival during hypoxia [16]. Xenografted tumours established from these cells are smaller and less tolerant to hypoxia compared to wildtype tumours. With an intact PERK-arm, vast hypoxic areas are present in tumours with few apoptotic cells, whereas defective PERK-signalling leads to less hypoxia, but also more apoptosis [15,16].

Despite the general translational arrest, specific proteins are upregulated after eIF2α phosphorylation. One well-described example is ATF4 (activating transcription factor 4). ATF4 coordinates expression of multiple genes implicated in the response to oxidative stress, amino acid synthesis, differentiation, metastasis and angiogenesis. One target gene of ATF4 is GADD34 (growth arrest and DNA damage inducible protein 34). By promoting the dephosphorylation of eIF2α, GADD34 provides a negative feedback loop to terminate the stress response when homeostasis is restored [17,18]. Pharmacological inhibition of eIF2α dephosphorylation has been shown to prolong the stress response and protect cells from stress-induced apoptosis [19]. CHOP (CCAAT-enhancer-binding protein homologous protein) is also a direct target of ATF4 and represents the pro-apoptotic component of the UPR. After injection with tunicamycin, wildtype mice displayed higher levels of apoptosis in their renal epithelium compared to CHOP knockout mice [20]. Expression of CHOP can be induced by ER stress not only through PERK but also through IRE1 and ATF6, although it appears that ATF4-mediated induction is most important. Knockout of PERK or ATF4 and expression of non-phosphorylatable eIF2α in cells lead to inability to express CHOP during ER stress.

Subsequent to PERK and eIF2α, both ATF4 and CHOP are induced by anoxia in multiple cancer cell types [21]. Indeed, in human tumours ATF4 and CHOP are localized in hypoxic areas [16]. Mouse embryonic fibroblasts lacking ATF4 are more sensitive to hypoxia, even to moderate levels (1% O_2) [16]. The proteasome inhibitors MG132 and bortezomib also increase ATF4 levels in both normoxia and hypoxia, indicating that protein stabilization is an important regulator of ATF4 levels [21,22].

Multiple other factors that are under the control of ATF4 have important functions in cancer cells. Here, we will discuss some examples: LAMP3 (lysosome-associated membrane protein 3) [23], TRB3 (Tribbles homologue 3) [24] and STC2 (Stanniocalcin 2) [25].

LAMP3 is a highly glycosylated protein located in the lysosomal membrane. It is frequently overexpressed in tumour tissue compared to normal tissue [26]. LAMP3 is a stress-induced gene [23] with prognostic value in breast cancer [27] and head and neck cancer [28]. In addition, LAMP3 mediates hypoxia-induced metastasis in both breast and cervix cancer [29,30].

TRB3 is overexpressed in multiple human tumours [31,32]. Its expression is induced by various ER stress-inducing compounds and hypoxia via ATF4 and CHOP [24,31,33,34]. TRB3 has a negative feedback loop on itself by inhibiting ATF4 and CHOP. The precise effect of TRB3 on cell fate after ER stress is controversial, as TRB3 may promote survival but also death [33,35,36].

STC2 is the mammalian homologue of a glycoprotein hormone first identified in bony fish, regulating calcium and phosphate homeostasis. In cells, hypoxia and ER stress-stimulating chemicals induce STC2 expression, which is mediated by PERK and ATF4 [25]. In addition to the UPR, STC2 has also been described as a HIF1 (hypoxia-inducible factor 1) target gene [37]. Silencing of STC2 in cells reduces proliferation, whereas STC2 overexpression increases proliferation [37]. Knockdown of STC2 also sensitizes cells to thapsigargin, whilst overexpression reduces thapsigargin-induced apoptosis [25]. In addition, in neuroblastoma cells, STC2 mediates the invasive potential [38] and in ovarian cancer cells cultured under hypoxia, overexpression of STC2 stimulates epithelial-to-mesenchymal transition, thereby promoting invasion [39]. Indeed, STC2 is associated with lymph node metastasis in esophageal squamous cell carcinoma [40].

ATF4 is not the only factor preferentially stimulated by PERK. PERK also induces phosphorylation and consequential activation of the transcription factor NRF2 (nuclear factor erythroid-derived 2-like 2). This factor regulates the anti-oxidant response, but its activation does not depend on eIF2α [41]. During unstressed conditions, KEAP1 (kelch-like ECH-associated protein 1) functions as a cytoskeletal anchor to retain NRF2 in the cytosol. Activation of PERK leads to the release of NRF2 enabling its nuclear import. Mutation of NRF2 stimulates cell death in response to ER stress, when compared to wildtype cells. In addition, ATF4 and NRF2 have been reported to dimerize and regulate the response to oxidative stress together [42]. Indeed, ATF4 knockout cells display increased sensitivity to oxidative stress [43].

Apart from PERK, other kinases can phosphorylate eIF2α in response to specific stimuli [44]. HRI (hemin-regulated inhibitor) is activated by heme-depletion, but is also stimulated by oxidative stress and heat shock. Protein kinase R (PKR) is associated with the rough ER membrane and is stimulated by double stranded RNA, generated upon viral infection. GCN2 (general control non-de-repressible 2) is activated by uncharged tRNAs that accumulate in response to amino acid starvation

with the purpose of reducing protein translation. However, proteasome inhibition and UV irradiation have also been shown to induce GCN2. During hypoxia, eIF2α is not only phosphorylated by PERK, but also by GCN2, whereas phosphorylation does not depend on PKR [15].

Overall, the PERK-arm of the UPR represents an important stress response at the level of translation, where it halts general protein synthesis, but preferentially increases translation of factors important for the adaptive response.

2.3. The IRE1- and ATF6-arms of the UPR induce expression of specific factors that aid in alleviating ER stress

IRE1 is present in two isoforms: α and β. IRE1α is ubiquitously expressed whereas IRE1β is restricted to gastrointestinal epithelial cells. IRE1 interacts with TRAF2 (tumour necrosis factor-associated factor 2) [45,46], which stimulates phosphorylation of JNK (c-Jun NH_2-terminal kinase) and activates the transcriptional activity of c-Jun. IRE1α knockout fibroblasts display reduced activation of JNK in response to ER stress [46]. JNK-signalling influences differentiation, proliferation and autophagy, but also apoptosis. These diverse effects are illustrated by studies showing that c-$Jun^{-/-}$ mouse fibroblasts are more sensitive to thapsigargin-induced cell death compared to wildtype fibroblasts [47], but are more resistant to cisplatin-induced cell death [48].

ATF6 functions as a transcription factor for several genes, including GRP78 and PDI, but also XBP1 and CHOP. The unspliced XBP1 mRNA can be activated by excision of a 26-nucleotide intron by IRE1. This spliced XBP1 acts as a transcription factor for genes involved in ER protein maturation and ER-associated degradation. Both XBP1 transcription and its splicing are induced by hypoxia [49]. Cells deficient for XBP1 are more sensitive to hypoxia-induced apoptosis and show reduced clonogenic survival. In transplanted tumours, loss of XBP1 suppresses growth, due to decreased hypoxia tolerance but does not significantly alter the secretion of pro-angiogenic factors [49]. XBP1-deficient cells are also more sensitive to oxidative stress induced by H_2O_2, due to a suppressed expression of anti-oxidant enzymes. Conversely, XBP1 overexpression restores expression of these enzymes, hindering H_2O_2-induced ROS-generation. However, this is caused by unspliced XBP1, abrogating the involvement of IRE1 [50].

3. Tumours are addicted to UPR-signalling

In general, tumours display increased activation of the UPR compared to normal tissue (see below). This may be a direct consequence of the tumour microenvironment or a response to ER stress induced by anti-cancer therapy. In the following section, we will describe the importance of this increased UPR activity for tumour behaviour and response to cancer treatment.

3.1. GRP78

Expression of GRP78 is chronically elevated in several types of cancer compared to normal tissues. This has been observed in hepatocellular carcinoma [51], gliomas [52], prostate [53] and gastric cancers [54].

Despite one study in lung cancer showing an association between GRP78 overexpression and better prognosis [55], the overall consensus is that GRP78 expression is linked with increased tumour aggressiveness and worse patient prognosis. For example, high GRP78 levels in gastric tumours correlate with worse patient prognosis and with enhanced lymph node metastasis [54]. These data are corroborated by preclinical work showing that silencing of GRP78 reduces invasion of gastric tumour cells in culture and tumour growth and metastasis in xenografts [54]. Multiple studies have shown that GRP78 is critical for tumour progression [8,56,57]. In prostate tumours, high GRP78 staining intensity correlates with reduced patient survival [53]. This was also observed in breast tumours [58,59], where high GRP78 expression is associated with a shorter time to recurrence [59]. In addition, higher-grade oestrogen-receptor negative tumours more frequently overexpress GRP78 than the lower-grade oestrogen receptor positive tumours [58,60]. Overexpression of GRP78 was also found in oestrogen-receptor positive breast cancer cells and tumour with acquired anti-oestrogen resistance [58]. In contrast, a correlation between GRP78 or XBP1 expression and oestrogen receptor positivity was reported [61]. This study shows that expression of GRP78 and XBP1 can even be stimulated by oestrogen supplementation [61].

GRP78 also contributes significantly to therapy resistance. In a panel of therapy resistant breast cancer cell lines, GRP78 expression is elevated [62]. Inhibition of GRP78 expression increases sensitivity to chemotherapy in glioma cells [52] and breast cancer cells [62], and also re-establishes sensitivity to anti-oestrogens in resistant breast cancer cells [58]. On the other hand, induction or overexpression of GRP78 increases resistance to chemotherapeutics [51,52,61,63] and anti-oestrogens [58]. In breast tumours, GRP78 expression may even predict responsiveness to chemotherapy [59]. Conversely, knockdown of GRP78 enhances resistance to chemotherapy in renal cell carcinoma cells [64], indicating that GRP78-mediated therapy resistance is not a general phenomenon. Nevertheless, GRP78 may be a suitable target for cancer therapy. Chen et al. reported the use of the GRP78-promoter to drive the expression of a suicide gene [65,66]. This gene would be activated in cells experiencing conditions of hypoxia and glucose deprivation, precisely where therapy resistance is at its peak. This approach has been successfully employed to reduce growth of murine mammary carcinoma in immune-competent mice [65,66]. Another approach is to target GRP78 expressing cells directly. Green tea (−)-epigallocatechin gallate (EGCG) binds the ATP-binding domain of GRP78 and blocks GRP78 function. Treatment with EGCG lowers resistance of glioma cells to temozolomide [52]. Alternatively, versipelostatin, a transcriptional inhibitor of GRP78, causes selective death of glucose-deprived cells and inhibits the growth of xenografts in mice [67]. Treatment with this compound represses expression of the UPR targets XPB1 and ATF4, but only during glucose deprivation, not after treatment with tunicamycin or A23187. Combining versipelostatin with cisplatin enhances growth inhibitory effects.

Increasing evidence indicates that in cancer cells GRP78 also functions outside the ER [68]. Not only have GRP78 and related isoforms been detected in the nucleus, the mitochondria, the cytosol and within the ER membrane [63,69,70], GRP78 can also relocalize to the cell surface [71,72]. Here, it functions as a receptor for multiple signalling pathways, thereby expanding its biological effects [68]. Expression of GRP78 on the cell surface may turn out to be of great therapeutic value as it offers the possibility for treatment with therapeutic antibodies. One such example is PAT-SM6, an auto-antibody isolated from a gastric cancer patient [72]. This antibody is directed against a particular isoform of GRP78, which is a tumour-specific membrane molecule. In preclinical studies, PAT-SM6 was found to inhibit growth of gastric carcinoma xenografts [73]. In addition, the antibody also specifically induces apoptosis in multiple myeloma cells, whereas non-malignant cells are left unaffected [74]. In melanoma, a phase I clinical trial for drug safety and tolerability reported encouraging results for the clinical use of PAT-SM6 [75].

3.2. PERK and IRE1

In malignant tissue, expression of ATF4, the downstream factor of PERK, is higher in comparison with normal tissue [21]. In addition, ATF4 plays a significant role in the response against chemotherapy. Expression of ATF4 correlates with cisplatin sensitivity in lung cancer cell lines: resistant cell lines display higher expression [76]. Forced ATF4 overexpression causes multidrug-resistance, reducing sensitivity to cisplatin, doxorubicin, etoposide, irinothecan and vincristine, but not to 5-fluorouracil [76,77]. ATF4 overexpression leads to increased

intracellular glutathione levels, which are involved in the resistance to cisplatin [77]. Conversely, ATF4 knockout cells have a decreased glutathione biosynthesis and display higher sensitivity to anti-cancer treatments.

In addition to chemotherapy, the UPR also has a significant impact on the efficacy of radiotherapy. In breast cancer cells, irradiation induces signalling through the PERK-arm of the UPR, evident by increased protein levels of PERK, phosphorylated eIF2α, ATF4 and LAMP3 [78]. Furthermore, in rat intestinal epithelial cells, irradiation stimulates expression of GRP78, phosphorylation of eIF2α and splicing of XBP1 [79]. Reportedly, this stress response is independent of ATF6 [79]. Sensitivity of breast cancer cells to radiotherapy is enhanced after knockdown of PERK, ATF4 or LAMP3 [78]. Moreover, GADD34 knockdown, which essentially prolongs eIF2α phosphorylation, confers resistance whereas treatment with a pharmacological PERK inhibitor (GSK2606414) leads to radiosensitization [78]. A related PERK inhibitor (GSK2656157) has been reported to suppress growth of xenografted tumours by reducing the vascular density [80,81].

The anti-oestrogen tamoxifen also induces components of the UPR, such as GRP78 and LAMP3 [61,82]. In addition to radiosensitization of breast cancer cells, knockdown of LAMP3 sensitizes cells to tamoxifen [82]. Breast cancer cells cultured tolerant to tamoxifen show increased expression of LAMP3 and correspondingly, LAMP3 silencing decreases their tamoxifen-resistance [82].

Not only the PERK-arm of the UPR is upregulated in anti-oestrogen resistant breast cancer cell lines: XBP1 expression is also increased [83]. In breast tumours XBP1 is co-expressed with the oestrogen receptor. Oestrogen receptor positive cells can grow independent of oestrogens when spliced XBP1 is overexpressed. In addition, this makes cells more resistant to the anti-oestrogens tamoxifen and faslodex [83]. Furthermore, higher levels of spliced XBP1 correlate with more aggressive, ER-negative breast tumours and a poorer survival [84]. ER-positive tumours also had a significantly poorer outcome with elevated XBP1 splicing [84].

3.3. ER stress-inducing agents as anti-cancer therapies

There are several pharmacological ER stress-inducing agents that have potential as anti-cancer therapies. The small molecule eeyarestatin 1 induces ER stress by impeding ER-associated degradation [85]. Eeyarestatin 1 synergizes with bortezomib and has preferential cytotoxicity against cancer cells.

Furthermore, tunicamycin-induced ER stress increases sensitivity of breast cancer cells to radiotherapy [86] as well as sensitivity of ovarian cancer cells to cisplatin and carboplatin [87]. In addition to these promising data, other reports have shown quite the opposite, where resistance to chemotherapy is induced by ER-stress induction [88–90]. In hepatocellular carcinoma cells, apoptosis induced by chemotherapy is significantly reduced by tunicamycin through induction of GRP78 [91]. Indeed, silencing of GRP78 reduces sensitivity to the effects of tunicamycin.

Overall, pharmacological induction of ER stress can promote both sensitivity and resistance to anti-cancer therapies, depending on the treatment given. Important in this respect is a study by Ledoux et al., which shows that via the UPR, glucose withdrawal stimulates the expression of P-glycoprotein in hepatoma cells [92]. P-glycoprotein is involved in multidrug resistance, by enhancing the efflux of chemotherapeutic drugs.

3.4. UPR-induced autophagy helps to survive ER stress

Autophagy, the cellular degradation process in which cells consume parts of their own cytoplasm in order to generate energy in times of stress, is increasingly recognized as an important mechanism to cope with ER stress. Indeed, ER stress-inducing compounds (A23187, tunicamycin, thapsigargin and brefeldin A) and conditions (hypoxia and detachment from the extracellular matrix) have been shown to not only induce the UPR, but also autophagy [93–97]. Here, autophagy represents a way to alleviate ER stress and allow for cell survival [93,94,98]. Interestingly, this effect was found only in cancer cells, not in non-transformed cells [93]. Kouroku et al. showed that polyglutamine aggregates induce ER stress and stimulate autophagy as a degradation mechanism [99]. Under these circumstances, the PERK-arm of the UPR is responsible for the activation of autophagy. Mutated non-phosphorylatable eIF2α and dominant-negative PERK prevent the conversion of LC3-I to LC3-II, whereas phosphorylation of eIF2α stimulates ATG12 expression [99].

For tumours, ER stress-induced autophagy also leads to resistance against cancer therapies. Pharmacological induction of ER stress prior to treatment with cisplatin was found to stimulate autophagy and to confer resistance to cisplatin-induced apoptosis [100]. In breast cancer cells, treatment with radiotherapy induces PERK-dependent autophagy [86,101]. These cells can be sensitized to radiotherapy by both pharmacological inhibition of autophagy and silencing of the PERK-arm [78,101]. In addition, treatment of breast cancer cells with tamoxifen induces autophagy, which is, at least partly, mediated by ATF4-induced LAMP3 [82,102] or by GRP78-dependent inhibition of mTOR [58]. Treatment of breast cancer cells with bortezomib leads to an ATF4-dependent increase in LC3B and autophagy, protecting against cell death and inducing bortezomib-resistance [22].

Other reports have indicated that not the PERK-arm, but the IRE1-arm is responsible for UPR-mediated autophagy. In neuroblastoma cells, ER stress induced by tunicamycin, thapsigargin or amino acid starvation stimulates autophagy [98]. Interestingly, this induction can be inhibited by IRE1 silencing or treatment with a pharmacological JNK inhibitor and is therefore IRE1-dependent. Indeed, cells deficient for PERK or ATF6 induce autophagy similar to wildtype cells, indicating that autophagy is stimulated independent of these transducers.

UPR-independent mechanisms may also trigger autophagy in response to ER stress. ER stress induced by thapsigargin or tunicamycin induces autophagy via protein kinase Cθ (PKCθ) [103]. PKCθ activation occurs independent of the UPR sensors. PKCθ silencing or its pharmacological inhibition inhibits ER stress-activated autophagy. ER stress-induced autophagy and activation of PKCθ can also be prevented by chelating intracellular Ca^{2+}, whereas PKCθ is not responsive to amino acid starvation. In addition, in response to increased cytosolic Ca^{2+}, ER stress-induced autophagy is triggered by CAMKK-β (Ca^{2+}/calmodulin-dependent protein kinase kinase β)-mediated induction of AMPK (AMP-activated protein kinase) to inhibit mTOR (mechanistic target of rapamycin) [103].

4. Conclusion and future perspectives

The UPR represents an essential cytoprotective mechanism in the response to ER stress. Normal cells rarely encounter such conditions, which abolishes the need to utilize the UPR. As a result of the tumour microenvironment, tumour cells critically depend on UPR-signalling to survive these adverse circumstances. Targeting the UPR may therefore be advantageous to specifically eliminate cancer cells. An activated UPR implies that tumour cells are already struggling to survive. Taking this survival advantage away by pharmacological inhibition of the UPR may result in increased cell death. Conversely, pharmacological agents to hyperactivate a UPR that is already activated by the tumour microenvironment likely surpasses the threshold and leads to cell death as well (see Fig. 1B).

The UPR has also emerged as a mechanism for therapy resistance, reducing the efficacy of anti-cancer treatment. Combining inhibitors of the UPR with conventional anti-cancer treatment may reduce therapy resistance and increase sensitivity. As both the proteasome and autophagy are important in the response to ER stress, it may be beneficial, or perhaps even necessary to target these pathways in parallel.

Screens for small molecule inhibitors of the UPR are ongoing [81,104], but show great promise for therapeutic applications [78,80,81]. A subject that needs to be addressed is how to inhibit the UPR: via PERK, IRE1 or ATF6? Or interfere with all three arms by targeting GRP78? Despite some redundancy between arms, for example in the induction of CHOP and GRP78, the UPR-arms have unique features. For example, prolonged PERK-signalling is lethal, whereas IRE1-signalling is not [105]. Furthermore, even though hypoxia stimulates both PERK- and IRE1-signalling, pharmacological PERK inhibition increases sensitivity to chemical ER stressors and hypoxia, whereas IRE1 inhibition does not [106].

The tolerability of UPR inhibition in patients remains to be examined. IRE1α and PERK have their highest expression in the pancreas. Pancreatic secretory cells rely heavily on their ER. In these cells, the UPR appears to be chronically activated to prevent ER-overload by the massive protein production. Inactivation of PERK in mice leads to pancreatic failure as a result of apoptosis of secretory cells [107,108]. The extent of toxicity of pharmacological inhibition of the UPR in patients remains to be elucidated.

Finally, not in all tumour types the UPR is similarly regulated. Recently, we have shown that in head and neck squamous cell carcinomas, the PERK-arm of the UPR is not universally induced by hypoxia [28]. This indicates that apart from the expected variability in response between tumours of the same type, we should also be aware that UPR modification may not be universally applicable to all cancers.

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