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Review
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Endoplasmic Reticulum Stress in Malignancy

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The combination of relative nutrient deprivation and dysregulation of protein synthesis make malignant cells especially prone to protein misfolding. Endoplasmic reticulum stress, which results from protein misfolding within the secretory pathway, has a profound effect on cancer cell proliferation and survival. In this review, we examine the evidence implicating endoplasmic reticulum dysfunction in the pathology of cancer and discuss how recent findings may help to identify novel therapeutic targets.

In the crowded molecular environment of the endoplasmic reticulum (ER), protein maturation requires the coordinated activity of many chaperones and folding enzymes. BiP is an abundant ER HSP70 chaperone that binds to exposed stretches of hydrophobic residues of immature polypeptide chains, while GRP94 is an HSP90 chaperone involved in subsequent folding steps for a subset of ER client proteins. When the efficiency of secretory protein folding is threatened, the cell is said to experience "ER stress" and elicits a homeostatic "unfolded protein response" (UPR) (Figure 1) (Walter and Ron, 2011). The diverse substrate repertoire of BiP enables it to function as a master regulator of the UPR by binding to and inactivating the three ER stress sensors, PERK, IRE1, and ATF6. During ER stress, increased levels of unfolded substrates lead to the sequestration of BiP, freeing the sensors to initiate UPR signaling. PERK ameliorates ER stress through phosphorylation of the translation initiation factor eIF2α. This causes generalized attenuation of protein synthesis while also promoting the translation of a subset of UPR target proteins, including the transcription factor ATF4. ATF4 induces expression of the transcription factor CHOP and, subsequently, the phosphatase subunit GADD34, which specifically dephosphorylates eIF2α, enabling the recovery of protein translation (Marciniak et al., 2004). The induction of ER oxidase 1α (ERO1α) by CHOP promotes oxidative protein folding in the ER, but this increased formation of disulphide bonds can contribute to worsening cellular stress through the generation of reactive oxygen species (ROS). However, additional targets of ATF4 include enzymes necessary to withstand oxidative stress, which tend to limit this toxicity. Additional targets of ATF4 promote amino acid import and synthesis, thus playing a cytoprotective role during a variety of stressful insults. Because other eIF2α kinases responding to different stresses can trigger this pathway—for example, GCN2 responds to amino acid deprivation—it has been named the integrated stress response (ISR; Figure 2) (Harding et al., 2003).

Tumor Growth

As solid cancers grow, their nutrient requirements eventually exceed the capacity of the existing vascular bed. Although many cancers adapt by triggering angiogenesis, inevitably the cores of most tumors become hypoxic and nutrient depleted. Impaired generation of ATP compromises ER protein folding, thus leading to activation of the UPR and ISR, while amino acid starvation further contributes to ISR activation. Indeed, phosphorylation of eIF2α by PERK has been shown to be necessary for the growth of larger solid tumors (Bi et al., 2005).

During hypoxia, generation of ROS increases both in mitochondria (Brunelle et al., 2005) and the ER, partly through UPR-mediated induction of ERO1α (Marciniak et al., 2004; Song et al., 2008). Accordingly, a key function of the ISR is to defend against oxidative stress, primarily by increasing biosynthesis of the antioxidant glutathione (Harding et al., 2003). The resulting increased capacity for oxidative protein folding is beneficial for tumor growth. Levels of ERO1α correlate with a worse prognosis in breast cancer (Kutomi et al., 2013), and depleting breast carcinoma cells of PERK increases ROS production and impairs cell growth (Bobrovnikova-Marjon et al., 2010). Moreover, the loss of PERK promotes G2/M cell cycle delay due to oxidative damage of DNA (Bobrovnikova-Marjon et al., 2010). This PERK-mediated resistance to oxidative stress is also implicated in resistance to radiotherapy (Rouschop et al., 2013; Rouschop et al., 2010), an effect of tumor adaptation to preconditioned ER stress.

In addition to the ATF4-dependent antioxidant response, cells can induce antioxidant pathways via Nrf2. This transcription factor is normally held inactive within the cytosol through binding to Keap1, which promotes its ubiquitination by Cul3 and subsequent proteasomal degradation. Upon oxidative stress, Keap1 releases Nrf2 to transactivate target genes within the nucleus. It has been suggested that this interaction is modulated by PERK. Two early reports suggested that Nrf2 could be phosphorylated by PERK during ER stress, triggering dissociation from Keap1 and induction of antioxidant genes (Cullinan and Diehl, 2004; Cullinan et al., 2003). Indeed, Nrf2 appears to be beneficial during ER stress-induced oxidative stress. However, activation of PERK's kinase domain in the absence of ER stress leads to induction of ISR target genes in a manner that is entirely dependent on phosphorylation of eIF2α (Lu et al., 2004). This suggests either that phosphorylation of Nrf2 plays a minor role in the transcriptional response to ER stress or that it is important only when additional arms of the UPR are active.

Recent observations suggest that activation of the UPR in hypoxic tumors leads to increased autophagy (Rouschop et al., 2010). Autophagy is cytoprotective during stress by liberating amino acids from long-lived proteins and the removal of damaged organelles. Accordingly, hypoxic regions of human

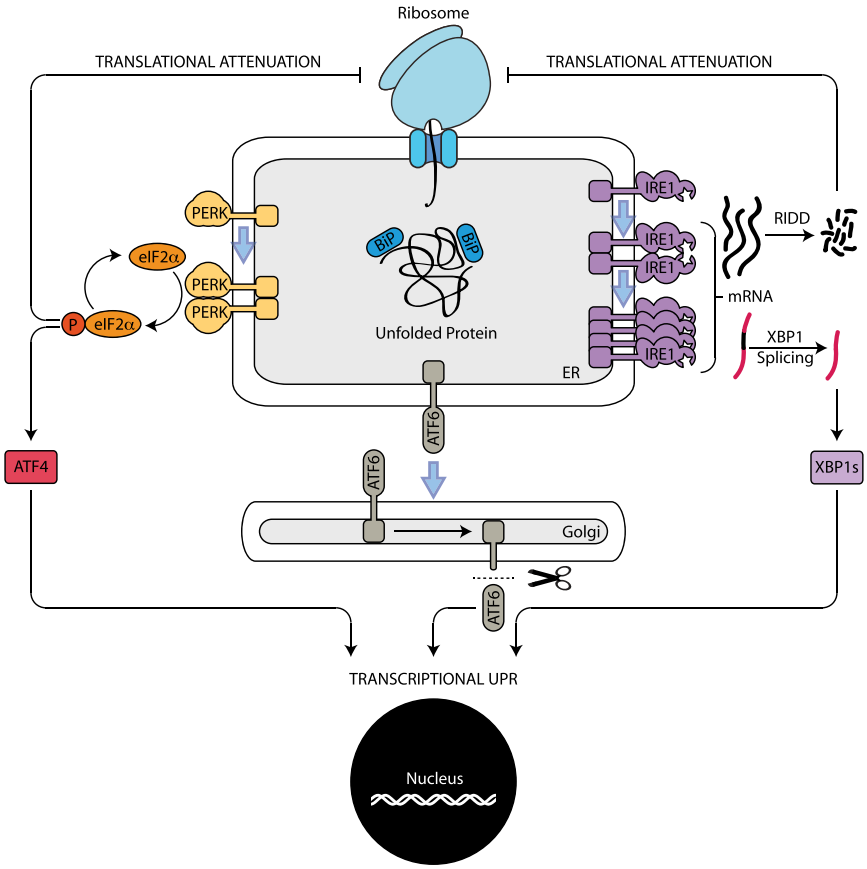

Figure 1. The UPR

Nascent secretory proteins synthesized by ER-bound ribosomes enter the ER lumen. When the load of unfolded protein threatens to exceed the capacity of the organelle to fold them, the cell is said to experience ER stress. This triggers three UPR signal transducers: PERK, IRE1, and ATF6. Activated PERK dimerizes, autophosphorylates, and then phosphorylates the translation initiation factor subunit eIF2α, causing general attenuation of translation that lessens ER protein client load while also increasing the translation of a subset of genes, including the transcription factor ATF4. Activated IRE1 oligomerizes, triggering its endonuclease activity to initiate splicing of *XBP1* mRNA and synthesis of the UPR transcription factor XBP1s. Activated IRE1 can also degrade a variety of mRNAs in proximity to the ER to reduced ER client protein load; a process termed regulated IRE1-dependent decay (RIDD). ATF6 traffics from the ER to the Golgi apparatus during stress and is cleaved to release a soluble UPR transcription factor, ATF6c.

tumor xenografts demonstrate increased expression of autophagy factors, such as LC3, and increased autophagy. In multiple cell lines, PERK mediates the upregulation of LC3 and autophagy-related gene 5 via ATF4 and CHOP, respectively, promoting phagophore formation (Rouschop et al., 2010). The inability of PERK-deficient cells to replenish LC3 correlates with impaired survival when subjected to hypoxia. Although PERK clearly plays an important role in the survival of hypoxic tumor cells, the IRE1 arm of the UPR is also important. During hypoxia-induced ER stress, IRE1-driven XBP1 splicing (to generate XBP1s) increases tumor cell tolerance to hypoxia, whereas loss of XBP1 impairs hypoxic tumor growth (Romero-Ramirez et al., 2004). Indeed, in breast cancer, increased splicing of *XBP1* is associated with a worse prognosis, perhaps reflecting an increased tolerance of tumor cells to hypoxia (Davies et al., 2008).

Angiogenesis and Invasion

It is well established that tumor hypoxia and glucose deprivation induce angiogenesis. Hypoxia achieves this via HIF, but the mechanism of nutrient limitation has remained obscure until recently. Evidence suggests that the PERK-ATF4 arm of the UPR directly upregulates vascular endothelial growth factor (VEGF) while downregulating inhibitors of angiogenesis (Blais et al., 2006; Wang et al., 2012). Depleting cells of PERK prevents upregulation of VEGF by glucose deprivation, whereas antagonism of HIF1α does not (Wang et al., 2012). Similarly, inhibition of PERK, which reduces the growth of xenograft tumors in mice, decreases tumor vascularity and perfusion (Wang et al., 2012). In addition, hypoxia-induced vascularization is modulated by IRE1α (Drogat et al., 2007). *Ire1α*$^{-/-}$ mouse embryonic fibroblasts and glioblastoma cells expressing dominant-negative IRE1α induce less VEGFA in ischemic conditions, limiting growth and angiogenesis of xenografts. In a human glioma model, it has been demonstrated that IRE1α is involved in the expression of angiogenic factors, including VEGFA and interleukin-6 (IL6), while suppressing the expression of antiangiogenic factors (Auf et al., 2010). Consequently, loss of IRE1α impairs glioma growth with increased overall survival of glioma-implanted animals. However, the relationship between IRE1α signaling and angiogenesis appears to be complex since, in nonmalignant models of ischemia, IRE1 has been shown to impair vascular regeneration by degrading mRNA encoding the neurovascular guidance cue netrin-1 via the process of regulated IRE1 dependent decay (RIDD) (Binet et al., 2013).

It is surprising that, although antagonism of IRE1 can impair tumor vascularity and improve survival, it may promote tumor invasion (Auf et al., 2010). Hypovascularity may contribute to invasiveness, but a more complex picture is likely, as the induction of angiogensis does not fully suppress the infiltrative properties of IRE1-deficient glioma cells. Recent analysis has revealed that the increased migratory phenotype likely reflects changes in the secretome via a reduction in RIDD (Dejeans et al., 2012b). For example, antagonism of IRE1 increases levels of the RIDD target BM-40, promoting cell adhesion and migration (Dejeans et al., 2012b). This suggests that, while suppression of IRE1 signaling may offer a novel approach to target tumor vascularization, it also risks promoting tumor invasion and so deserves further study.

Tumor invasion is also influenced by epithelial-to-mesenchymal transition (EMT), a known characteristic of ER-stressed

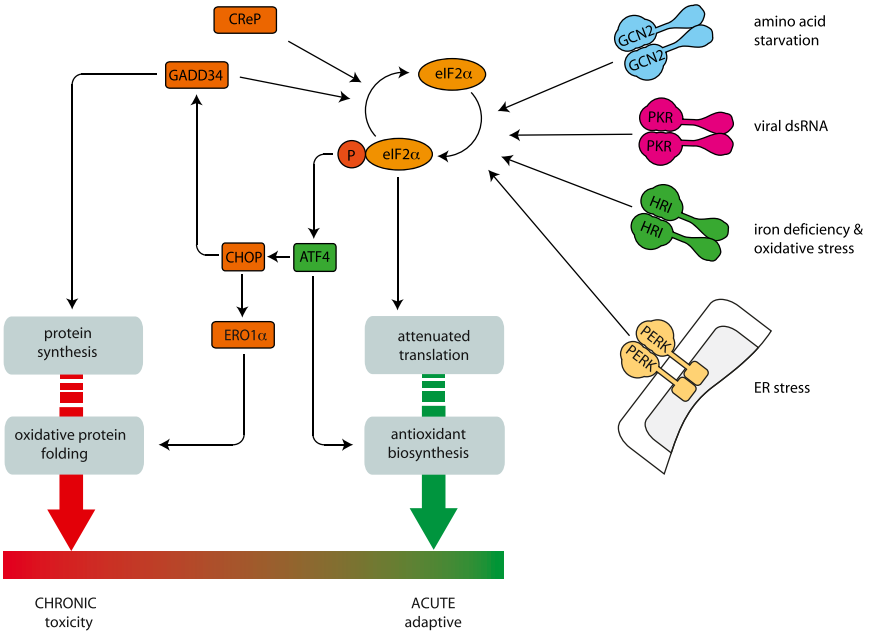

Figure 2. The ISR

Phosphorylation of eIF2α serves as a hub for integration of signals mediated by a family of kinases: PERK responds to ER stress, HRI responds to iron deficiency and to oxidative stress, PKR is activated by dsRNA during some viral infections, and GCN2 is activated during amino acid starvation (Harding et al., 2003). In unstressed conditions, eIF2α supports new protein synthesis as a component of the eIF2 complex that recruits initiator methionyl-tRNA to the ribosome. During its catalytic cycle, the eIF2 complex hydrolyzes bound GTP and must interact with the guanine nucleotide exchange factor eIF2B to be recharged with GTP. Once eIF2α is phosphorylated, it becomes a potent antagonist of eIF2B and thus attenuates the rate of protein translation. Low basal levels of eIF2α phosphorylation are antagonized by the constitutively expressed eIF2α phosphatase CReP, but during stress, this is overwhelmed and phospho-eIF2α accumulates. While translation of most mRNAs is reduced by phosphorylation of eIF2α, a subset is translated more efficiently, most notably, the transcript factor ATF4. This transactivates most genes of the ISR, including amino acid transporters and synthetases, which help counter amino acid limitation while providing the thiol moieties necessary for synthesis of the antioxidant glutathione. Subsequently, ATF4 induces another transcription factor CHOP, which induces the eIF2α phosphatase GADD34 leading to dephosphorylation of eIF2α and the resumption of normal rates of cap-dependent translation. CHOP also induces the ER oxidase ERO1α, thus promoting oxidative protein folding. While the induction of GADD34 and ERO1α can be seen as adaptive during the response to transient ER stress, their induction during chronic stressful circumstances can contributes to worsening stress and result in exaggerated toxicity.

cells. During embryonic development and in malignancy, HIF1α and Notch signaling link hypoxia with EMT, causing loss of epithelial integrity through downregulation of adhesion molecules such as E-cadherin (Lester et al., 2007; Sahlgren et al., 2008). Simultaneously, increased chemotaxis accompanies the induction of the chemokine receptor CXCR4 (Azab et al., 2012; Barriga et al., 2013). Thus, EMT promotes metastasis by removing impediments to the egress of cells from their original tumor while also honing them to new niches (reviewed in Hanahan and Weinberg, 2011). ER stress has been shown to drive EMT in vitro and in animal models of fibrosis through src-mediated signaling (Tanjore et al., 2011; Ulianich et al., 2008). It is therefore plausible that ER stress may contribute to EMT in cancer invasion, although more formal examinations of this are needed. A further consideration is that phenotypic change from epithelium to mesenchyme will affect the secretory capacity of a cell, thus altering its vulnerability to ER stress. Consistent with this, evidence suggests that expression of the ER stress markers CHOP and GADD34 change during dedifferentiation of mesothelioma cells (Dalton et al., 2013).

ER-Mitochondrial Communication

Through a proteostatic network, impaired protein folding in one cellular location leads to the propagation of cell-wide responses. The interplay between the mitochondrial HSP90 chaperone networks and the protein-folding environment of the ER exemplifies such a mechanism. HSP90 and its related chaperone, TRAP-1, are abundant in the mitochondria of tumor cells but not in those of most normal tissues, and they appear to antagonize mitochondrial death pathways (Chae et al., 2012). It is not surprising that impaired function of mitochondrial HSP90 leads to a mitochondrial UPR and the induction of autophagy (Siegelin et al., 2011). More recently, it has been shown that inhibition of mitochondrial HSP90 using the small molecule gamitrinib disrupts tumor bioenergetics to such an extent that ER stress pathways are activated (Chae et al., 2012). Notably, activation of the classical UPR of the ER was necessary for survival of mitochondrial proteotoxicity.

Direct communication between the mitochondrion and ER during stress serves to modulate the function of both organelles. PERK is enriched at mitochondrial-ER contact sites and appears to tether mitochondria to the ER membrane (Figure 3) (Verfaillie et al., 2012). Mitofusin 2 (Mfn2), a GTPase of the mitochondrial outer membrane that mediates mitochondrial fusion, has recently been shown to bind directly to PERK (Muñoz et al., 2013). Because cells lacking Mfn2 experience basal activation of PERK, it has been suggested that Mfn2 may normally inhibit PERK signaling. However, enhanced signaling in all three branches of the UPR in *Mfn2*$^{-/-}$ cells is difficult to explain by this dysinhibition of PERK alone, since exaggerated phosphorylation of eIF2α would reduce ER stress by attenuating protein translation. It therefore seems likely that ER-mitochondrial signaling is affected more extensively. Indeed, PERK modulates mitochondrial morphology and function, with overexpression of PERK causing mitochondrial fragmentation and reduced respiration, while depletion of PERK reduces mitochondrial calcium overload and ROS production in Mfn2-deficient cells (Muñoz et al., 2013). It is interesting that the tethering function of PERK appears independent of its kinase activity and facilitates ROS-mediated proapoptotic signaling between the ER and mitochondrion (Verfaillie et al., 2012). These organelles therefore function in tandem and can both contribute to oxidative stress through production of ROS.

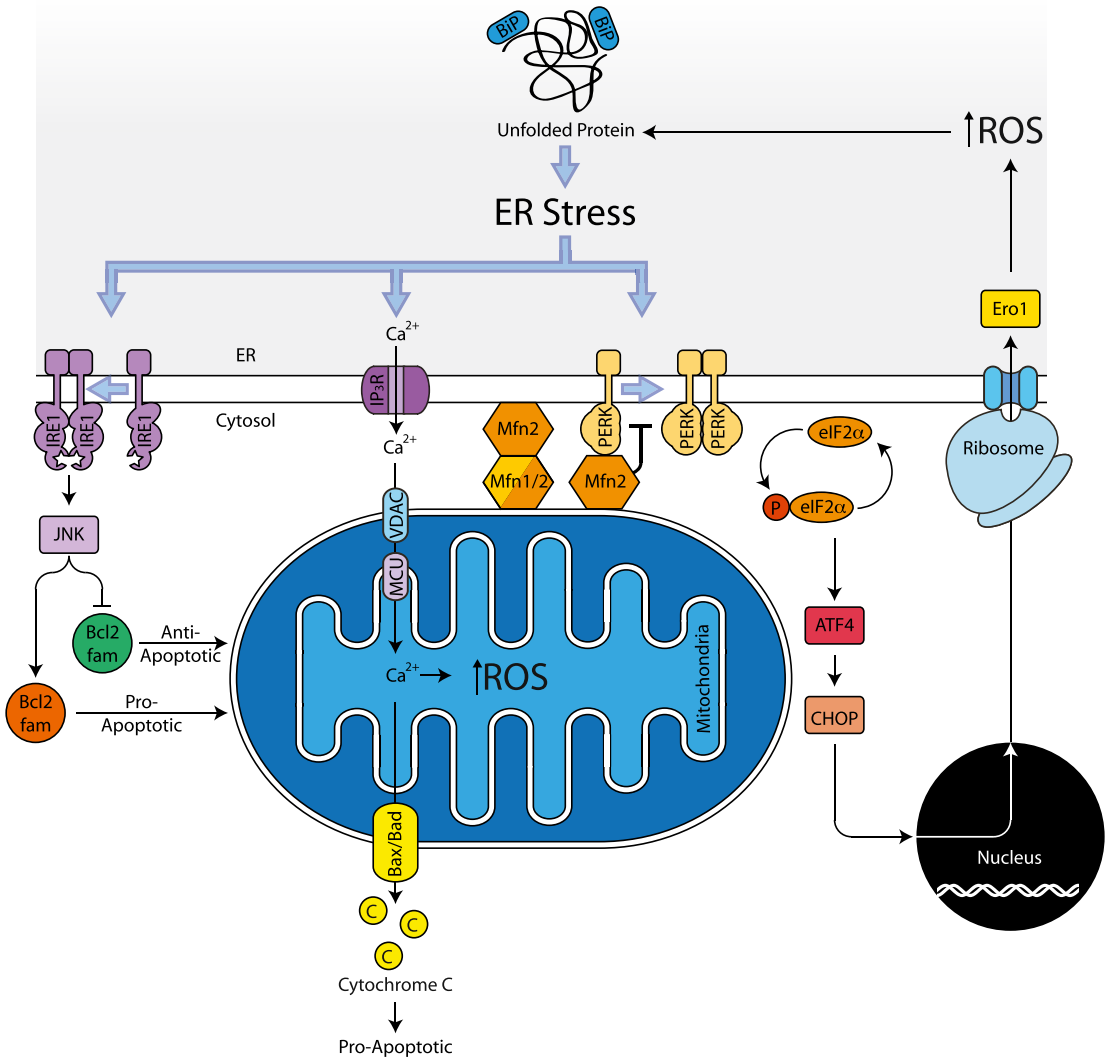

Figure 3. ER-Mitochondrial Communication during ER Stress

During ER stress, IRE1 mediates activation of c-Jun N-terminal kinase (JNK) and apoptosis via activation of pro-apoptotic BH3 only family Bcl-2 proteins, such as Bid, and inhibition of anti-apoptotic Bcl-2 (reviewed in Tabas and Ron, 2011). Calcium released from the ER is taken up by the mitochondrion to stimulate the production of ROS and the release of cytochrome c, both of which promote apoptosis. Prolonged PERK activation induces ERO1α leading to increased production of ROS within the ER. Mfn2, which along with mitofusin 1 (Mfn1) tethers mitochondria to the ER membrane (de Brito and Scorrano, 2008), is thought to inhibit PERK by direct interaction. Ablation of Mfn2 leads to gross mitochondrial dysfunction in a PERK dependent manner, suggesting the existence of further, yet to be discovered, interactions.

Intercellular Signaling

In addition to communication between organelles during failure of proteostasis, it has been suggested that, in cancer, ER stress signals may be transmissible from cell to cell. One proposed mechanism involves the tumor suppressor PAWR, which is known to promote prostate cancer cell apoptosis via WT1. Brief exposure to ER stress causes the secretion of PAWR into the extracellular space that can trigger the apoptosis of nearby cancer cells (Burikhanov et al., 2009). Controversially, the "receptor" on nearby cells has been reported to be the chaperone BiP (Burikhanov et al., 2009), and it has been suggested that ER stress induces surface presentation of ER chaperones that can then modulate the activity of other plasma membrane proteins, such as cripto (Kelber et al., 2009). The apparent surface translocation of ER chaperones in cancer cells must be subjected to further rigorous validation and would be greatly strengthened by further mechanistic insight. However, the observation that cripto-dependent SMAD signaling can be blocked by an antibody recognizing surface-exposed BiP raises the possibility that this putative cancer-cell-specific mechanism may offer novel therapeutic targets (Kelber et al., 2009).

Cancer Cell Death

Depending on the context of ER stress, including intensity and duration, the UPR can promote survival or trigger cell death. In B cell chronic lymphocytic lymphoma, ER stress has been implicated in causing spontaneous tumor cell apoptosis (Rosati et al.,

2010). The initiation of cell death by ER stress involves several partially redundant parallel pathways (reviewed in Tabas and Ron, 2011), but evidence implicates PERK as a major effector (Marciniak et al., 2004). Because loss of CHOP, a downstream target of PERK, renders cells and mice more resistant to ER stress, for some time it was believed that CHOP regulates a cell death program, a theory that was not supported by transcriptional analysis (Han et al., 2013; Marciniak et al., 2004). Instead, CHOP regulates a complex array of more than 200 genes, which promote ongoing protein secretion and autophagy (Marciniak et al., 2004; Rouschop et al., 2010). For example, by inducing the ISR targets GADD34 and ERO1α, PERK and CHOP trigger the upregulation of protein translation and protein oxidation that, in the face of ongoing protein misfolding, represent a worsening to the original toxic insult (Han et al., 2013; Marciniak et al., 2004). Although recovery of protein translation is a necessary part of the adaptive response to acute ER stress, in situations of chronic stress, increased protein synthesis can contribute to toxicity (Figure 2). Recovery of protein translation is brought about by dephosphorylation of eIF2α by GADD34 (Marciniak et al., 2004), and so antagonism of GADD34 protects cells from chronic ER-stress-induced cell death (Tsaytler et al., 2011). In a murine model of medulloblastoma, loss of GADD34 increased eIF2α phosphorylation and promoted tumor growth, invasiveness, and angiogenesis (Lin et al., 2011). This may reflect the increased induction of VEGFA observed in mice lacking functional GADD34.

Although mutation of the *GADD34* gene is a rare event in human carcinogenesis, there is increasing evidence that suppression of the CHOP-GADD34 axis may be a tumor survival mechanism. For example, in malignant mesothelioma, loss of GADD34 expression correlates well with the degree of tumor dedifferentiation and a worse prognosis (Dalton et al., 2013). Reduced expression of CHOP in mammary carcinoma, mediated by the PERK-induced microRNA mir211, promotes tumor cell survival (Chitnis et al., 2012), while attenuation of PERK-CHOP signaling by overexpression of the DNAJ cochaperone p58IPK enables malignant progression under conditions of nutrient limitation (Huber et al., 2013). It therefore appears likely that, while PERK-ATF4 signaling may promote tumor survival, the induction of GADD34 may have broadly tumor-suppressive effects (Figure 2). This hypothesis is testable, since *Gadd34* null mice are viable (Marciniak et al., 2004). It is noteworthy that, in addition to GADD34, which is normally induced by ER stress, cells also possess a constitutively expressed eIF2α phosphatase called CReP. Recently, this has been shown to be downregulated in cells deficient in the tumor suppressor PTEN (Zeng et al., 2011). The resulting increase in phosphorylated eIF2α in these cells leads to increased resistance to oxidative stress.

Tumor-Specific Mechanisms for the Induction of Endoplasmic Stress

A number of mechanisms contribute to cancer-specific induction of the UPR. For example, deficiencies of the tumor suppressors tuberous sclerosis complex (Tsc)-1 or Tsc2, which negatively regulate mTORC1, cause ER stress through uncontrolled protein synthesis (Ozcan et al., 2008). Constitutive activation of the serine/threonine kinase mTORC1 is common to many cancers and stimulates protein synthesis and cell growth. However, mTORC1 activity is often also repressed by homeostatic responses to features of the tumor microenvironment, such as hypoxia and nutrient deprivation, to promote cancer cell survival (Figure 4) (Brugarolas et al., 2004; Inoki et al., 2003). Recently, it has been recognized that an important function of Tsc2 is to enable the cell to regulate its rate of protein synthesis to match the availability of lipids (Düvel et al., 2010). In the secretory pathway, these two processes are intimately linked, since an increased demand for ER protein flux triggers an IRE1-XBP1-dependent expansion of ER membrane to accommodate more folding proteins. Defects in mTORC1 signaling can render hypoxic tumors dependent on exogenous desaturated lipids, an essential nutrient for hypoxic $Tsc2^{-/-}$ tumors (Young et al., 2013). Lipid-deprived $Tsc2^{-/-}$ cells experience an exaggerated ER stress response because IRE-1 activation fails to trigger adequate expansion of the ER (Young et al., 2013). When lipids are limiting, this leads to ER stress-induced cell death that can be blocked by inhibition of mTORC1 with rapamycin. This phenomenon was also seen in kidney tumors arising in $Tsc2^{+/-}$ mice, as well as in multiple cancer cell lines, suggesting that therapies targeting lipid desaturation machinery might enhance ER stress-induced cell death in a tumor-specific manner (Young et al., 2013).

Frequently, transformed cells depend on the activation of prosurvival pathways such as those mediated by the oncogene Myc (reviewed in Dang, 2010), which enhance proliferation through cell cycle deregulation and increased protein synthesis. Increased protein load leads to activation of the UPR, which promotes malignant transformation through PERK-mediated cytoprotective autophagy. Limiting protein synthesis by genetic manipulation abrogates UPR activation by Myc and attenuates lymphomagenesis in a murine model (Hart et al., 2012). Accordingly, PERK inhibition diminished the level of autophagy accompanying Myc activation, leading to reduced colony formation in vitro and decreasing tumor formation in vivo (Hart et al., 2012).

In some situations, ER stress responses appear to be used as antioncogenic mechanisms. In naevi of the skin, components of the MAPK pathways are commonly dysregulated, but although many naevi have oncogenic mutations, few develop into malignant melanomas. Instead, they senesce through incompletely understood mechanisms (Michaloglou et al., 2005). Oncogenic mutations of HRAS have been shown to trigger the UPR via activation of the AKT pathway (Denoyelle et al., 2006), and while the link between AKT and ER stress is unclear, the consequence of UPR activation is to induce senescence and prevent transformation.

The Role of Endoplasmic Reticulum Stress in Tumor Immunogenicity

In cancer, ER stress has the capacity to activate cells of the adaptive immune system (Wheeler et al., 2008) and is sufficient to trigger systemic inflammation by proteolytic activation of the transcription factor CREBH at the ER membrane (Zhang et al., 2006). ER stress in prostate cancer causes the release of proinflammatory cytokines such as IL6 and tumor necrosis factor α (Mahadevan et al., 2010), the promoters of which contain functional XBP1s binding sites (Martinon et al., 2010). These cytokines not only stimulate inflammation but also have been

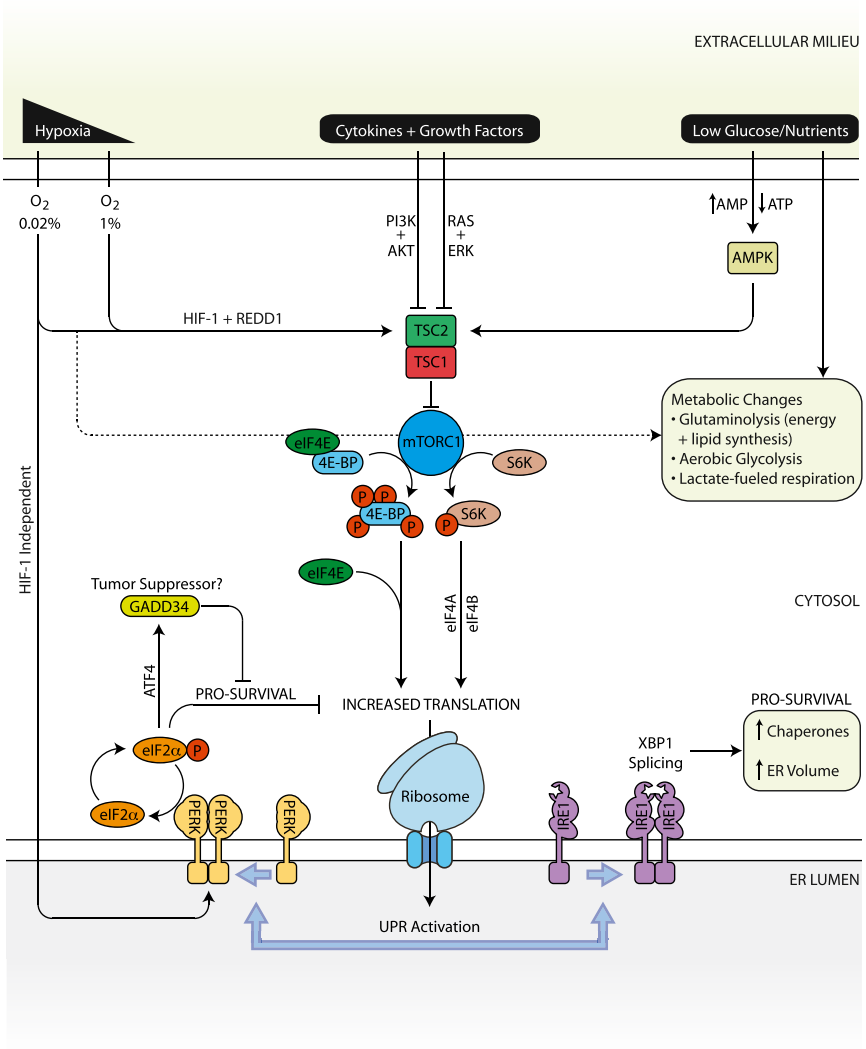

Figure 4. Regulation of Protein Synthesis in Cancer

mTORC1 stimulates protein translation at multiple levels. Release of eukaryotic initiation factor 4E (eIF4E) from its inhibitory binding partner 4E-BP is promoted by 4E-BP phosphorylation by mTORC1, while phosphorylation of S6K leads to activation of eIF4A and eIF4B. Tsc1/2 complex inhibits mTORC1 in response to cues from the tumor microenvironments, while mitogenic factors inhibit Tsc1/2 via PI3-kinase/Akt and Ras/Erk pathways to promoting protein synthesis and growth (reviewed in Mendoza et al., 2011). PERK and IRE1 limit protein synthesis and expand the ER capacity to match ER protein folding to the rate to protein synthesis. GADD34-induced recovery of protein synthesis can have toxic consequences during chronic stress and so may mediate tumor-suppressive effects. When glucose is limiting, reduced cytosolic ATP:AMP ratios trigger Tsc1/2 via AMPK, while hypoxia activates Tsc1/2 via HIF-1 and REDD1. Anoxia ($O_2 \leq 0.02\%$) additionally activates PERK in a HIF-1 independent manner, further limiting translation via eIF2α phosphorylation. Both hypoxia and nutrient deprivation alter cell metabolism, promoting aerobic glycolysis, lactate utilization, and glutaminolysis (reviewed in Dang, 2012).

implicated in promoting tumor survival (Kim et al., 2009; Pikarsky et al., 2004). Remarkably, ER stress appears to be transmissible from cancer cells to cells of the immune system. When cultured in media conditioned by murine cancer cells experiencing ER stress, macrophages show activation of the UPR in a TLR-dependent manner (Mahadevan et al., 2011). It is plausible that this acquired UPR of macrophages might cause the release of proinflammatory mediators and thus contribute to tumor inflammation.

Cell surface expression of damage-associated molecular patterns (DAMPs) offers novel targeting strategies for immunogenic killing of cancer cells. ER-stress-mediated cell surface presentation of calreticulin has emerged as a DAMP of potential importance in cancer (Garg et al., 2012; Obeid et al., 2007). For example, it has been proposed as a mechanism for immune surveillance of hyperploid cancer cells, which display constitutive ER stress (Senovilla et al., 2012). It is interesting that anthracycline chemotherapeutics efficiently induce calreticulin translocation to the cell surface, causing tumor immunogenicity, while knockdown of calreticulin prevents phagocytosis and antigen presentation by dendritic cells (Obeid et al., 2007). In contrast, agents such as cisplatin that do not cause relocalization of calreticulin fail to elicit an anticancer immune response. This therapeutic limitation can be rectified by the coadministration of ER-stress-inducing agents (Martins et al., 2011). The combined insult of ER stress and excess reactive oxygen species appears to be required for translocation of calreticulin in a PERK-dependent process (Garg et al., 2012; Martins et al., 2011; Panaretakis et al., 2009). Phosphorylation of eIF2α is likely to mediate this effect, although one report suggested this to be nonessential (Garg et al., 2012). Although calreticulin appears to be a key molecule in this response, the combination of reactive oxygen species and ER stress also causes surface exposure of additional DAMPs, the relative importance of which remains unexplored (Fucikova et al., 2011; Garg et al., 2012).

Endoplasmic Reticulum Stress as a Therapeutic Target

The homeostatic mechanisms that maintain proteostasis are now sufficiently well understood to be legitimate targets of novel anticancer strategies. For example, the HSP90 family of chaperones are required for the proper folding and stability of many kinases and transcription factors involved in tumor survival, and HSP90 inhibitors have already entered clinical trials. Many of the agents developed to target cytosolic HSP90s appear also to inhibit the ER localized homolog GRP94, but it is currently unclear which of these two targets is the most important for cancer cell toxicity. Elevated levels of GRP94 have been observed in many cancers and have been associated with

advanced clinical stage (Wang et al., 2005; Zheng et al., 2008). Reduction of GRP94 levels has been shown to augment the toxicity of etoposide (Reddy et al., 1999) and actinomycin D (Pan et al., 2009), while overexpression of GRP94 in breast cancer promoted cell proliferation and migration (Dejeans et al., 2012a). Inhibition of GRP94 within the ER lumen was first achieved with the prototype HSP90 inhibitor geldanamycin. This agent and many of its derivatives have been shown to induce ER stress and cell death (Marcu et al., 2002). Recently, agents displaying selectivity for GRP94 were developed but appeared to be less toxic than nonspecific inhibitors of HSP90 (Duerfeldt et al., 2012); however, drug combinations that maximize ER stress and proteotoxicity may prove to be more effective. When combined with rapamycin, the HSP90 inhibitor retaspimycin, or IPI-504, caused catastrophic ER stress and the regression of aggressive ras-driven tumors (De Raedt et al., 2011). Since inhibition of HSP90 induces a robust heat shock response through effects on cytosolic protein folding, cytoprotective induction of cytosolic HSP70-class chaperones is characteristically seen. Consequently, cells depleted of both HSC70 and HSP72 show enhanced toxicity during inhibition of HSP90 (Powers et al., 2008), offering further opportunities for synergistic drug combinations.

Prolonged inhibition of HSP90 may also disable the cell's ability to mount a cytoprotective UPR, since PERK and IRE1 are stabilized by this chaperone (Marcu et al., 2002). These ER stress sensors have been targets of a number of successful small molecule inhibitor screening programs. The specific PERK inhibitor GSK2656157 reduces cancer growth in vivo, most likely via impaired angiogenesis and amino acid metabolism (Atkins et al., 2013), while the inhibitors of IRE1, MKC-3946 and STF-083010, both show antimyeloma activity in animal models (Mimura et al., 2012; Papandreou et al., 2011). While it has been noted that oncolytic viral therapy induces a prosurvival UPR, the cytoprotective effects of this are diminished by inhibition of IRE1, leading to increased oncolytic efficacy (Mahoney et al., 2011). This represents one of many potential utilities of such compounds in combination therapies. It should be noted, however, that agents that antagonize the UPR, such as PERK inhibitors, are likely to cause some systemic toxicity, since conditional ablation of PERK in the adult murine pancreas causes diabetes through ER stress-induced β-cell death (Gao et al., 2012). In addition, administration of the PERK inhibitor GSK2656157 to mice for 2 weeks caused combined degeneration of the endocrine and exocrine components of the pancreas (Atkins et al., 2013). Following this brief treatment, the effects appeared to be reversible on withdrawal of the drug, but longer term effects have yet to be determined.

The proteasome inhibitor bortezomib can induce cell death in Burkitt lymphoma (Shirley et al., 2011) and multiple myeloma (Meister et al., 2007), at least in part via ER stress. It is likely the highly secretory nature of myeloma cells that renders them especially vulnerable to agents that promote ER stress, and in vitro studies have shown that inhibition of the proteasome synergizes with other therapies that increase the load of ER misfolded proteins, such as photodynamic therapy (Szokalska et al., 2009). In addition, bortezomib has been shown to kill hypoxic tumor cells, preferentially through overactivation of the UPR, since hypoxia is a cause of protein misfolding (Fels et al., 2008). The relative selectivity of bortezomib for a subset of cancer cells reflects the dependence of professional secretory cells on their ability to degrade proteins that have terminally misfolded within the ER; the process of ER-associated degradation (ERAD). In addition to direct inhibition of the proteasome, ERAD can be blocked by other means. Eeyarestatin I inhibits the p97-Ufd1-Npl4 complex that mediates dislocation of polyubiquitinated substrates out of the ER into the cytosol and also impairs their deubiquitination (Wang et al., 2008). This agent synergizes with bortezomib in tumor cell killing by inducing the proapoptotic protein NOXA in an ISR-dependent manner (Wang et al., 2009). Similar synergy has been observed with the combination of bortezomib and another p97 inhibitor, DBeQ (Auner et al., 2013).

While the introduction of bortezomib has led to an increased rate of remission in myeloma patients, acquired drug resistance has limited its use in the clinic. Cells cultured in the presence of proteasome inhibitors have been shown to acquire mutations of the bortezomib binding site on proteasome subunit PSMB5 or to overexpress this subunit, although such mutations are rarely observed in the clinical setting (Balsas et al., 2012; Oerlemans et al., 2008). An alternative mechanism involves increased basal expression levels of BiP that allows cells to withstand the accumulation of more misfolded proteins without activation of a cytotoxic UPR (Schewe and Aguirre-Ghiso, 2009). More recently, a subpopulation of bortezomib-refractory B-cell progenitors has been identified in which IRE1-XBP1s signaling is suppressed (Leung-Hagesteijn et al., 2013). It has been known for some time that XBP1s is necessary for the differentiation of B lymphocytes into plasma cells by enabling a dramatic expansion of the ER (Carrasco et al., 2007). Continued signaling by the IRE1-XBP1s axis was thought necessary to maintain healthy plasma cells, but a recent screen identified IRE1 loss of function as promoting bortezomib resistance in myeloma (Leung-Hagesteijn et al., 2013). Despite previous evidence that inhibition of IRE1 is toxic to myeloma (Mimura et al., 2012; Papandreou et al., 2011), knockdown of either IRE1 or XBP1 was surprisingly well tolerated by myeloma-derived cell lines and induced resistance to inhibition of the proteasome, while overexpression of XBP1s restored sensitivity (Leung-Hagesteijn et al., 2013). Moreover, XBP1 target genes were depressed in clinical samples from progressive myeloma. The protective effect of depleting myeloma cells of XBP1s correlated with a partial reversal of plasma cell differentiation, both morphologically and functionally, with a reduced capacity to secrete immunoglobin light chains. Moreover, XBP1s-deficient myeloma progenitors could be isolated from the bone marrow of patients with progressive disease, and it was suggested that these represent a pool of cells intrinsically resistant to proteasome inhibition owing to their arrested development. This may explain the inability of proteasome inhibition to induce an outright cure in myeloma and the appearance of resistance following initial remission. It is encouraging, however, that bortezomib resistance can be overcome in models of disease by potentiating the degree of ER stress with coadministration of an HSP90 inhibitor (Roué et al., 2011). The suppression of the UPR in some bortezomib-resistant cells is thought to be facilitated by reduced phosphorylation of eIF2α. Accordingly, promotion of phosph-eIF2α levels with the agent salubrinal was shown to enhance bortezomib-induced cell killing (Schewe

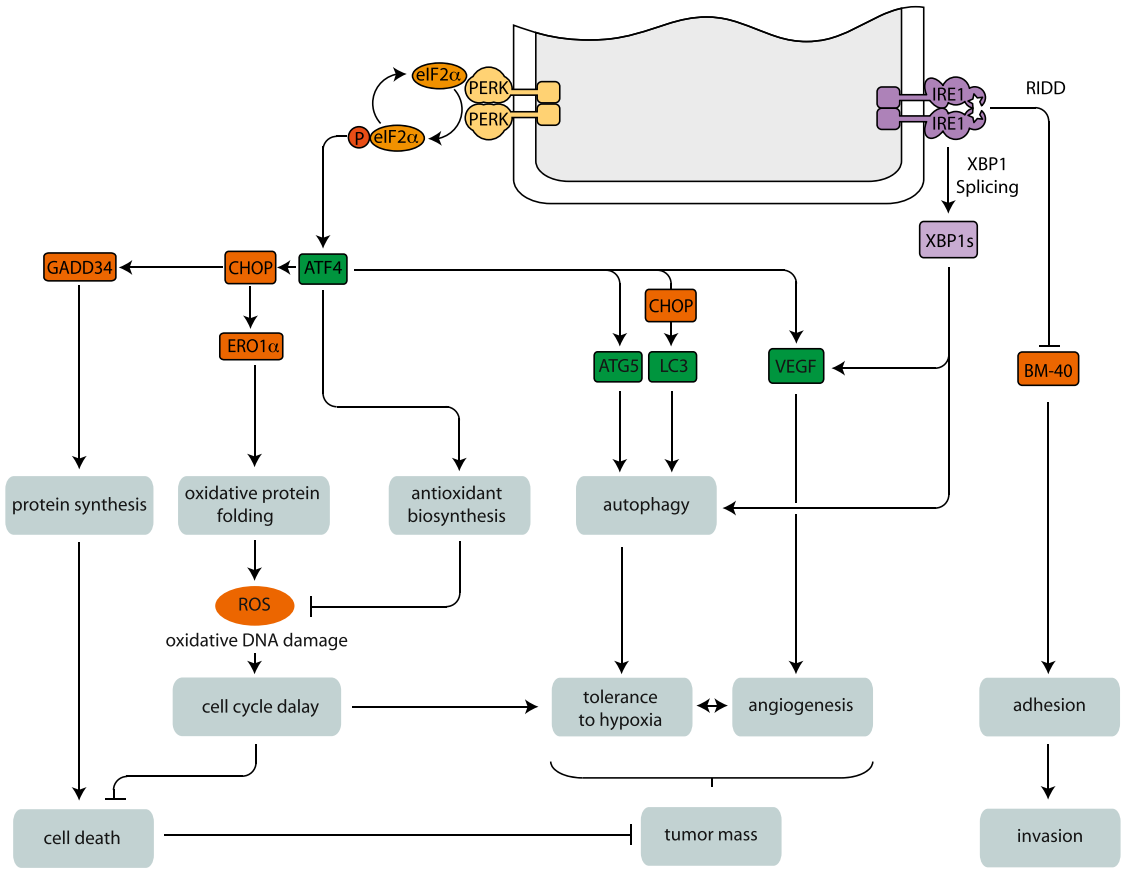

Figure 5. The UPR in Cancer

Activation of the UPR in malignancy results in complex signaling that is neither fully oncogenic nor tumor suppressive. The relative importance of each downstream pathway varies between cells depending on the chronicity of ER stress and on the relative expression of key factors; for example, GADD34. High rates of protein synthesis during ER stress lead to cell death through the accumulation of misfolded protein, which would reduce tumor mass. Loss of GADD34 may serve to antagonize this, leaving PERK unhindered in the attenuation of protein synthesis. Generation of ROS during ER stress, while potentially toxic, may help limit tumor growth to match nutrient supply by initiating DNA damage checkpoints. Excess toxicity from ROS is limited by ATF4-mediated induction of antioxidant pathways. Nutrient limitation within tumors is antagonized by autophagy and angiogenesis, which are induced by the PERK and IRE1 arms of the UPR. Reduced expression of extracellular matrix molecules, such as BM-40, may limit migration and invasion.

and Aguirre-Ghiso, 2009). Conversely, it has also been suggested that enhanced induction of ATF4 may have undesirable effects in the treatment of myeloma. Inhibition of the proteasome is associated with accumulation of the antiapoptotic protein Mcl-1, which can confer resistance to bortezomib (Hu et al., 2012). This accumulation does not reflect stabilization of the protein; instead, Mcl-1 appears to be induced by ATF4. Newer, more selective inhibitors of eIF2α phosphatases are under development and should help to clarify which effect, cytotoxicity or antiapoptotic, is dominant.

Concluding Remarks

Current understanding of the cellular responses to ER stress has made this a valid target for the development of rational therapies, but the role of the UPR in modulating many aspects of tumor behavior from cell proliferation and death to angiogenesis and invasion is becoming clearer (Figure 5). It is perhaps not surprising that the cellular response to ER stress is neither fully oncogenic nor completely tumor suppressive. For example, antioxidant pathways induced downstream of ATF4 appear to promote tumor survival and may largely account for the requirement for PERK in models of solid tumors; while induction of GADD34 may be toxic to cancers experiencing chronic ER stress. Such insights will contribute to better targeting of therapies while also informing our appreciation of the cell biology of physiological ER stress. Remarkably, the precise mechanisms of cell death during ER stress remain unclear, and so better understanding will be required if death pathways are to be invoked with selectivity to ER-stressed cancer cells. It is likely that the relative importance of these death pathways will depend both on the cancer type and on the tumor microenvironment, which will offer further possibilities for personalization of treatment.

Finally, it is important to bear in mind the heterogeneity of cancer as a disease, or rather as a group of diseases. The evolution of an individual tumor over time frequently leads to loss of differentiation and increased propensities for invasion and metastasis, which will affect secretory capacity and reliance on the UPR. Also, there is heterogeneity between subtypes of cancer originating from a single tissue type such the breast, which include basal-like, luminal-like, normal breast-like, and HER2 positive. Given that subtype-dependent differences will lead to differences in metabolic characteristics, it is to be

expected that the importance of ER protein homeostasis for each subtype will vary accordingly. This has already been demonstrated in the context of cytosolic protein folding. HER2 is a client protein of Hsp90, and so HER2-positive tumors are especially sensitive to inhibition of HSP90 (Rodrigues et al., 2012). The highly secretory natures of the rare mucinous and secretory breast cancers make them likely candidates for UPR dependence.

A number of areas require further study. Clearly, there is a need for a more sophisticated appreciation of the oncogenic and tumor-suppressive features of the UPR. This is well illustrated by the antagonism of IRE1 that, while attenuating tumor growth through impaired angiogenesis, might promote tumor invasion. It is unclear what role the mutational burden of a tumor cell plays in the induction of ER stress. Are specific mutations more likely to cause ER stress? A better understanding of how the UPR influences chemo- and radiosensitivity may also direct more effective interventions.

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