



ASIP OUTSTANDING INVESTIGATOR AWARD LECTURE

Endoplasmic Reticulum Stress Signaling in Cancer Cells



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To survive, cancer cells must resist numerous internal and environmental insults associated with neoplasia that jeopardize proteostasis within the endoplasmic reticulum (ER). Solid and hematopoietic tumors often experience genomic instability, oncogene activation, increased protein secretion demands, and somatic mutations in proteins handled by the secretory pathway that impede their folding. Invasion or metastasis into foreign environments can expose tumor cells to hypoxia, oxidative stress, lack of growth signals, inadequate amino acid supplies, glucose deprivation, and lactic acidosis, all of which pose challenges for protein processing in the ER. Together, these conditions can promote the buildup of misfolded proteins in the ER to cause ER stress, which then activates the unfolded protein response (UPR). An intracellular signaling network largely initiated by three ER transmembrane proteins, the UPR constantly surveils protein folding conditions within the ER lumen and when necessary initiates counteractive measures to maintain ER homeostasis. Under mild or moderate levels of ER stress, the homeostatic UPR sets in motion transcriptional and translational changes that promote cell adaptation and survival. However, if these processes are unsuccessful at resolving ER stress, a terminal UPR program dominates and actively signals cell suicide. This article summarizes the mounting evidence that cancer cells are predisposed to ER stress and vulnerable to targeted interventions against ongoing UPR signaling. (*Am J Pathol* 2020, 190: 934–946; <https://doi.org/10.1016/j.ajpath.2020.01.010>)

The ER Is the Gateway to the Secretory Pathway

The endoplasmic reticulum (ER) is the foremost intracellular compartment of the secretory pathway in eukaryotic cells. In addition to its essential roles in calcium handling and lipid biosynthesis, the ER is a specialized organelle where the synthesis, folding, and maturation begin for approximately a third of the proteome, including nearly all proteins destined for secretion or residence in the plasma membrane. After cotranslational translocation into the lumen of the ER, these polypeptides are actively folded and modified by an assembly line of ER-resident enzymes, including chaperones, foldases, glycosylating enzymes, and oxidoreductases. Despite the efficiency of these protein-folding machines, a third of all polypeptides translocated into the ER fail to fold correctly.¹ Misfolded proteins are targeted for elimination by quality

control systems, including the ER-associated degradation pathway, which directs them to the cytoplasm for ubiquitination and 26S proteasomal degradation, as well as autophagy. Numerous physiological and pathologic insults can perturb protein folding and cause accumulation of misfolded

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forms in the ER, including nutrient fluctuations, increased demands on protein secretion, hypoxia, mutations in client proteins of the secretory pathway that stabilize or promote aggregation of intermediate folding forms, and reduction in calcium levels with inhibitory effects on calcium-dependent chaperones. If its capacity to either properly fold proteins or dispose of those that fail quality control is overwhelmed, the ER lumen will begin to accumulate misfolded proteins, a harmful situation called ER stress that threatens the function and survival of the cell.

The Unfolded Protein Response

Homeostatic versus Terminal Responses

Proteostasis, a portmanteau constructed by combining the words proteome and homeostasis, is a state of overall protein health maintained through the coordinated control of the biogenesis, folding, transport, and degradation of polypeptides in the cell. When misfolded proteins pile up in the ER above an acceptable level, this loss in ER proteostasis triggers an intracellular signal transduction pathway known as the unfolded protein response (UPR).² In mammalian cells, the UPR is initiated by three single-pass ER transmembrane proteins that possess an ER luminal domain capable of detecting misfolded proteins: inositol-requiring enzyme 1 α (IRE1 α ; officially known as endoplasmic reticulum to nucleus signaling), protein kinase R (PRK)-like ER kinase (PERK; also officially known as eukaryotic translation initiation factor 2 α kinase 3), and activating transcription factor 6 (ATF6).³ When the ER is operating under homeostatic conditions, the luminal domains of IRE1 α , PERK, and ATF6 are held in a monomeric and inactive state through interaction with an abundant ER chaperone called binding Ig protein [BiP; alias glucose-regulated protein, 78kD (GRP78) and heat shock 70 kDa protein 5 (HSPA5)]. However, given its higher affinity for the exposed hydrophobic polypeptide domains contained within misfolded proteins, BiP/GRP78 is titrated off the ER stress sensors and onto misfolded proteins as they begin to accumulate within the ER lumen, thereby priming the UPR stress sensors to be ready to signal. Misfolded proteins are then capable of serving as direct ligands for the ER luminal domains of the unrestrained UPR sensors, which leads to their oligomerization and activation.⁴ The initial outputs of the UPR work to restore ER homeostasis by decreasing demand and increasing capacity on the protein-folding machinery in an effort to preserve cell function, a process termed homeostatic UPR.⁵ However, if these corrective measures prove inadequate to restore homeostasis, then the ER sensors initiate an alternative program called the terminal UPR, which actively signals cell destruction.^{6,7}

The Three Branches of the UPR

IRE1 α is a bifunctional kinase/endoribonuclease (RNase) that employs autophosphorylation as a readout of ER stress

severity to govern cell outcome (Figure 1).^{8–10} Rectifiable ER stress causes low-level, transient kinase autophosphorylation and dimerization/tetramerization that confines IRE1 α 's RNase activity to excising a 26-nucleotide nonconventional intron from the mRNA encoding X-box binding protein 1 (XBP1). Religation of spliced *XBP1* by a cytoplasmic ligase called RNA 2',3'-cyclic phosphate and 5'-OH ligase changes the open reading frame such that its translation produces the homeostatic transcription factor spliced XBP1 (XBP1s),^{11,12} which up-regulates dozens of genes that code for ER biogenesis, protein folding, and quality control constituents.¹³ In contrast, persistent and unresolved ER stress leads to sustained, high-level IRE1 α autophosphorylation, which drives higher-order oligomerization of IRE1 α . When oligomerized, IRE1 α 's RNase further increases its *XBP1* splicing activity, but, more important, it lowers its specificity to endonucleolytically degrade hundreds of mRNA species as they attempt translation at the ER membrane^{8,9,14}—a process known as regulated IRE1 α -dependent decay (RIDD). Although depletion of secretory pathway transcripts through RIDD may initially limit ER stress by reducing the protein-folding burden on the ER, the unselective destruction of mRNAs at the ER membrane eventually results in the depletion of critical enzymatic and structural components of the ER. The net consequence of RIDD is that it actively destroys ER function. Moreover, RIDD reduces select miRNAs, such as miR-17, that are repressing proapoptotic targets, like the pro-oxidant protein thioredoxin-interacting protein, which leads to their up-regulation and consequent prodeath effects.^{15,16} Thus, hyperactivated in response to severe ER stress, IRE1 α 's RNase promotes a terminal over homeostatic UPR in which RIDD-mediated ER destruction and proapoptotic signals overwhelm the adaptive signaling through *XBP1* mRNA splicing.

On activation by ER stress, PERK has a cytosolic kinase that trans-autophosphorylates and then phosphorylates eukaryotic translation initiation factor 2 α (eIF2 α), which prevents association of the eIF2-GTP-Met-tRNA ternary complex to reduce protein translation, thereby lessening protein-folding burden in the ER.¹⁷ A second target phosphorylated by PERK is nuclear factor erythroid 2-related factor 2,¹⁸ which then transcriptionally up-regulates antioxidants and other factors that protect against oxidative stress. Moreover, PERK's inhibitory efforts on Cap-dependent protein translation promote ER homeostasis because translation machinery becomes biased for mRNAs containing an internal ribosome entry site, such as activating transcription factor 4 [ATF4; alias cAMP-response element binding protein 2 (CREB2)], a major adaptive factor that induces genes involved in protein folding, resistance to oxidative stress, autophagy, and amino acid metabolism.¹⁹ Although a hiatus in global protein translation can be advantageous for ER stressed cells by giving them extra time to process a stockpile of proteins, a prolonged block in translation from persistent PERK signaling is incompatible with cell viability. Therefore, ATF4 transcriptionally up-regulates growth arrest and DNA damage-inducible

protein, a key regulatory subunit of protein phosphatase 1, which then dephosphorylates eIF2 α to restore mRNA translation.²⁰ However, if ER stress remains unresolved, sustained PERK activation will result in up-regulation of the C/EBP homologous protein [alias growth arrest and DNA damage 153 (GADD153)] transcription factor, which rapidly reduces expression of short-lived antiapoptotic proteins B-cell lymphoma—extra large (normal half-life 4 to 18 hours) and myeloid cell leukemia 1 (normal half-life 0.5 hours) and increases expression of proapoptotic BCL2 family proteins to promote cell death.^{21,22} Hence, like IRE1 α , prolonged PERK activation is detrimental toward cell survival (Figure 1).

ATF6 is a type II transmembrane protein that contains a basic leucine zipper transcription factor within its cytoplasmic domain. On detection of ER misfolded proteins, ATF6 shuttles to the Golgi apparatus, where it undergoes intramembrane cleavage by the site-1 and site-2 proteases to release the p50 ATF6(N) transcription factor, which then translocates to the nucleus.²³ In cooperation with XBP1s, ATF6(N) up-regulates many genes (eg, BiP and DnaJ homolog subfamily C member 3, alias P58IPK) that enlarge ER size and protein-folding capacity as well as up-regulate ER-associated degradation pathway components.²⁴ Under high ER stress, ATF6 has been reported to reduce antiapoptotic proteins, such as Mcl-1,²⁵ but, in general, the contributions of ATF6 to ER stress-induced cell death are not yet well understood (Figure 1).

ER Stress in Cancer

Does the UPR Prevent or Promote Cancer?

One of the most common features of cancer cells is their ability to locally spread or metastasize into other tissues, where hostile environmental conditions, such as hypoxia, low glucose, growth factor deficiency, lactic acidosis, oxidative stress, and amino acid starvation, jeopardize the fidelity of protein folding in the ER.²⁶ Moreover, intrinsic stresses shared by many tumor cells, including oncogene activation, aneuploidy, and increased glycolysis, often lead to a higher steady-state level in protein translation and protein secretion, adding pressure on the secretory pathway.²⁷ Furthermore, genomic instability and somatic mutations in secretory pathway proteins can disrupt their folding and cause ER stress. As such, numerous studies have documented high-level activation of all three branches of the UPR (IRE1 α , PERK, and ATF6) in a wide range of human hematopoietic (leukemia, lymphoma, and myeloma) and solid tumors, including glioblastoma and carcinomas of the breast, stomach, colon, esophagus, lung, prostate, pancreas, and liver.²⁶ Somatic mutations in or near one of the enzymatic domains (kinase or RNase) of IRE1 α have been found rarely (<1%) in glioblastoma, lung carcinoma, and ovarian cancer.²⁸ Previous studies have found that most of these cancer-associated mutations in IRE1 α disable its

apoptotic outputs,^{9,29} suggesting that some cancer cells may shut down the terminal UPR to survive. Likewise, a loss-of-function mutation (c.499C>A) in XBP1 that prevents its proper mRNA splicing has been reported in rare myeloma cases and may be associated with resistance to proteasome inhibitors.³⁰ Furthermore, the ER chaperones BiP/GRP78 and GRP94, which promote adaptation to ER stress, are overexpressed in a variety of cancer types.³¹ Both GRP78 and GRP94 are also up-regulated by a variety of oncogenic viruses, including hepatitis C virus and human papillomavirus.^{32,33} Taken together, these studies demonstrate UPR activation in many neoplasms.

Despite the vast evidence of ongoing ER stress in multiple cancer types, whether the UPR restrains or promotes tumor growth in patients has been hotly debated. For example, pharmacologically inhibiting IRE1 α in >300 cancer cell lines in culture failed to impair tumor cell viability.³⁴ Moreover, PERK haploinsufficiency has been reported to promote melanoma development.³⁵ Conceptionally, targeting the UPR might block ER stress-induced apoptosis and inadvertently promote cancer progression, raising concerns about the safety of therapeutics against this pathway. However, over the past few years, multiple preclinical studies have provided strong evidence that *in vivo* the UPR supports various aspects of tumor growth, including angiogenesis, metabolism, metastasis, and chemoresistance. For example, studies have found that IRE1 α 's homeostatic target XBP1 promotes tumor progression in multiple preclinical models of triple-negative breast cancer.³⁶ Genetic deletion of IRE1 α in a human glioma cell line inhibited angiogenesis and decreased tumor growth when these cells were subsequently injected into mice.³⁷ The IRE1 α -XBP1s pathway has also been shown to support prostate cancer growth through activating c-MYC signaling,³⁸ raising the possibility that other MYC-driven tumors will have a similar dependency on the UPR. PERK has been documented to support tumor growth in murine models of mammary cancer by limiting oxidative damage,³⁹ and PERK inhibition reduces breast cancer metastasis to the lung after tail vein injection into immunocompromised mice.⁴⁰ Moreover, PERK signaling in HT29 colon cancer cell lines promotes chemoresistance.⁴¹ Fewer studies have examined the role of ATF6 in cancer. However, a recent report suggests that high ATF6 expression is a marker of precancerous lesions in the setting of colorectal carcinoma.⁴² Together, these data strongly suggest that the UPR has tumor-promoting functions *in vivo*.

The Role of the UPR in Neoplasms of Professional Secretory Cells

Recently, the UPR has emerged as an attractive target in two neoplasms that derive from professional secretory cells and retain or even exceed their normally high protein secretion burden into malignancy. The first such neoplasm is myeloma, a tumor of malignant plasma cells that can often

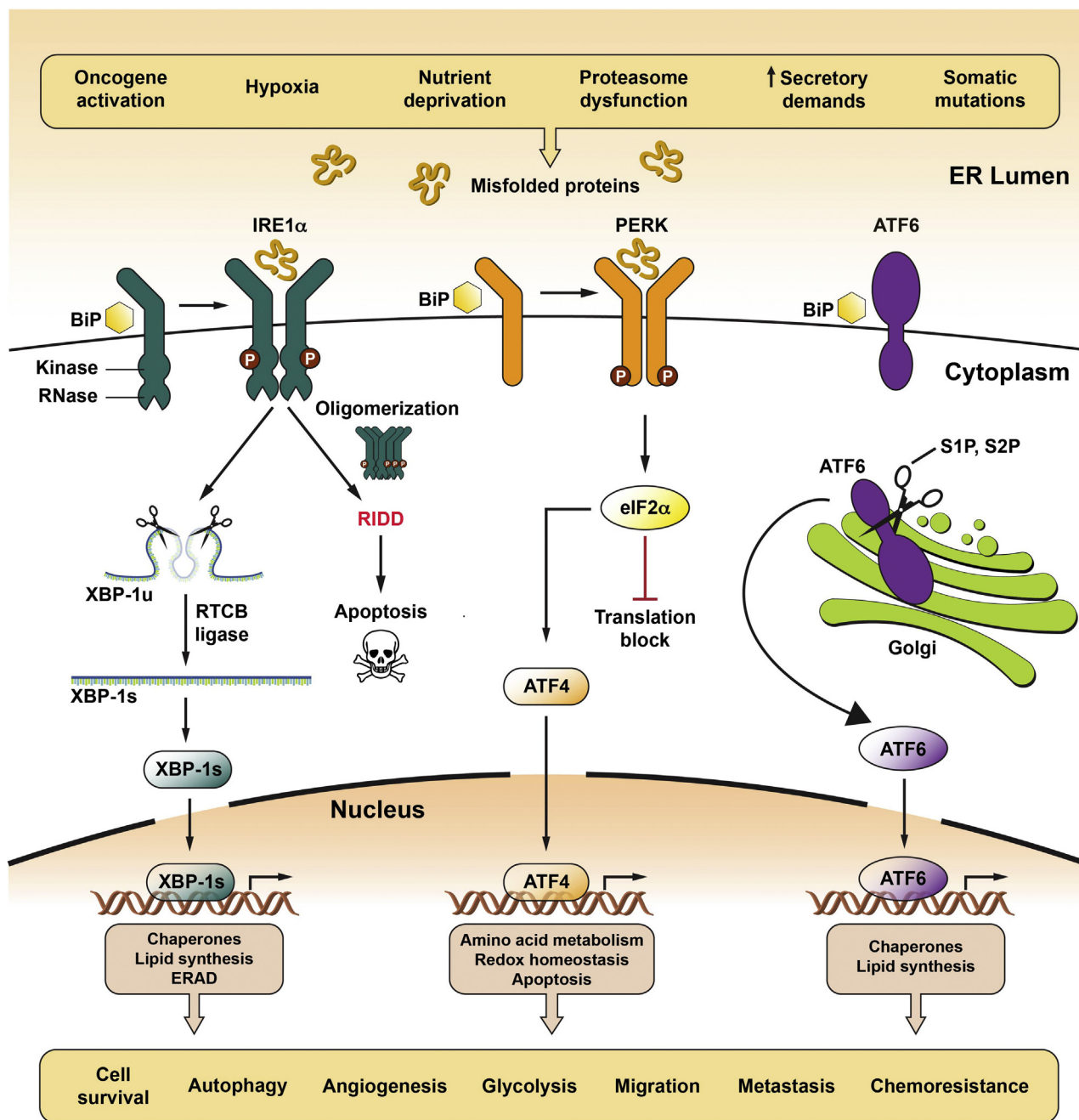


Figure 1 Role of the unfolded protein response (UPR) in cancer. Cancer cells frequently encounter extrinsic stresses that challenge protein folding in the endoplasmic reticulum (ER), including hypoxia, nutrient deprivation, and growth factor withdrawal. Intrinsic stresses, such as oncogene activation, proteasome dysfunction, increased glycolysis, and increased protein secretion, can lead to additional demands on the secretory pathway. Furthermore, genomic instability and somatic mutations that cripple protein folding can lead to ER stress. On detecting an accumulation of ER misfolded proteins, the UPR is initiated by three transmembrane ER proteins: inositol-requiring enzyme 1 α (IRE1 α ; alias endoplasmic reticulum to nucleus signaling), PRK-like ER kinase (PERK; alias eukaryotic translation initiation factor 2 α kinase 3), and activating transcription factor 6 α (ATF6 α ; see text for signaling details). The combined outputs of the UPR can regulate tumor growth at many levels, including cell survival, autophagy, angiogenesis, glycolysis, migration, metastases, and chemoresistance. Modified from Oakes¹⁰ with permission from APS Publications. BiP, binding Ig protein; eIF2 α , eukaryotic translation initiation factor 2 α ; ERAD, ER-associated degradation; Redox, oxidation-reduction; RIDD, regulated IRE1 α -dependent decay; RTCB, RNA 2',3'-cyclic phosphate and 5'-OH ligase; S1P, site-1 protease; S2P, site-2 protease; XBP1, X-box binding protein 1-unspliced; XBP-1s, spliced X-box binding protein 1.

secrete their own weight in Ig each day. The earliest hint that the UPR might be particularly important to plasma cells came from developmental studies. In mice, deletion of *Irel1 α* or *Xbp1* prevents the differentiation of B lymphocytes into plasma cells,⁴³ a process that requires a massive expansion

of the ER to accommodate the enormous Ig production levels of this cell type. It was later reported that at least 50% of primary human myelomas have elevated levels of XBP1s.⁴⁴ This set up the prediction that if IRE1 α /XBP1 signaling is required for normal plasma cell differentiation,

then perhaps constitutive signaling through XBPs could lead to extended plasma cell survival and a permissive environment for the accumulation of additional oncogenic events. Further supporting this idea, transgenic expression of *Xbp1s* in B lymphocytes of mice leads to a plasma cell malignancy with many features of myeloma.⁴⁴ There is also evidence to suggest that proteasome inhibitors that the Food and Drug Administration approved as first-line therapy for myeloma work in part by halting the disposal of misfolded proteins through the ER-associated degradation pathway and thus triggering ER stress—induced apoptosis,⁴⁵ again implicating the UPR as a potential target in this disease. The strongest evidence comes from a recent report showing that genetic disruption of IRE1 α or XBPs, or pharmacologic inhibition of IRE1 α 's kinase activity, significantly reduced s.c. or orthometastatic myeloma tumor growth in mice.⁴⁶ Mechanistically, blockade of IRE1 α /XBP1 signaling leads to a reduction in cytokines and chemokines known to promote myeloma growth.

Pancreatic neuroendocrine tumors (PanNETs) are the second secretory neoplasm for which the UPR has been shown to play a critical role on the basis of recent work from our laboratory. PanNETs originate from professional secretory cells, which are critically dependent on a functional UPR for proper development and maintenance. Homozygous deletion of *Perk* in mice causes rapid pancreatic β -cell apoptosis, leading to infantile diabetes, pancreatic exocrine insufficiency, and early growth defects.^{47,48} This phenocopies Wolcott-Rallison syndrome, a rare human diabetic syndrome caused by homozygous loss-of-function mutations in *PERK*.⁴⁸ Similarly, the genetic removal of *Irel1 α* in adult murine β cells results in their severe dysfunction and defective insulin secretion,⁴⁹ whereas deleting *Xbp1* specifically in the β -cell compartment leads to upstream IRE1 α hyperactivation, degeneration of β cells, and hyperglycemia.⁵⁰ Although healthy endocrine cells can secrete large amounts of protein in response to appropriate signals, the vast majority of PanNETs constitutively hypersecrete one or more hormones. For example, although each normal pancreatic β cell is capable of releasing an estimated 1 million molecules of insulin per minute,⁵¹ some insulinoma PanNET cells secrete >10-fold higher amounts of this hormone, even under normoglycemic conditions.⁵² On the basis of these observations, we predicted that PanNETs would be particularly dependent on the UPR to manage ER stress.

Consistent with this notion, human PanNETs were found to have elevated ER stress markers (eg, BiP) and up-regulation of homeostatic outputs of the IRE1 α and PERK arms of the UPR.⁵³ More important, rat insulinoma INS-1 cells (a well-established PanNET model) grown in culture have low levels of ER stress and UPR activation; however, the same cells grown in mice as xenografts show marked up-regulation of the UPR, demonstrating that these tumor cells face unique challenges to ER proteostasis *in vivo* (eg, hypoxia and nutrient deprivation). As such, although loss of

IRE1 α or PERK had little effect on the growth of INS-1 cells in culture, it markedly halted their ability to form tumors *in vivo*. Pharmacologic modulation of IRE1 α and PERK in INS-1 xenografts or in the spontaneous genetic (RIP-Tag2) mouse PanNET model showed that UPR signaling was optimized for adaptation *in vivo*, and that inhibiting either IRE1 α or PERK led to hyperactivation and apoptotic signaling through the reciprocal arm, thereby halting the growth and survival of the tumor cells.

Taken together, these preclinical studies argue that the homeostatic UPR is frequently activated in cancer, especially highly secretory neoplasms, and necessary for the survival and/or growth of the tumor cells under conditions that stress the ER (Figure 2). However, if the UPR is to be targeted effectively to control cancer, the mechanisms by which UPR activation is promoting tumor growth must be understood. Although much work remains, recent studies have started to uncover how a deregulated UPR contributes to cancer.

Protumorigenic Outputs of the UPR

The UPR-Autophagy Connection

Autophagy is an evolutionarily conserved pathway that regulates lysosomal degradation of long-lived proteins and damaged organelles, including the ER. In mammalian cells, >30 autophagy-related genes (ATGs) orchestrate each step in the autophagy process, including induction, vesicle initiation, autophagosome maturation, fusion with the lysosome, and lysosomal digestion. Through recycling damaged or surplus cell components, autophagy can provide energy or macromolecular building blocks to maintain cellular homeostasis and promote survival. The role of autophagy in cancer is complex and context dependent.⁵⁴ The cellular homeostasis functions of autophagy, especially those of limiting the accumulation of reactive oxygen species and preventing DNA damage, seem to play an important role in preventing tumorigenesis. For example, monoallelic deletion of Beclin-1 (mammalian ortholog of yeast Atg6 encoded by the *BECN1* gene) occurs in approximately 50% of breast, ovarian, and prostate cancers⁵⁵; and mice heterozygous for one copy of *Becn1* show a high incidence of lymphoma, hepatocellular carcinoma, and lung cancer.^{56,57} However, autophagy induction in tumors exposed to hypoxia and/or low nutrient conditions can provide energy sources that promote cancer cell survival and resistance to chemotherapy.⁵⁸ Furthermore, autophagy activity in nearby tumor stromal fibroblasts can lead to the delivery of metabolites, such as lactate, ketone bodies, and amino acids, to serve as fuel to promote tumor growth.⁵⁹ As such, blocking autophagy has antitumor activity in many murine cancer models, with the evidence strongest for tumors driven by oncogenic RAS.⁶⁰

Over the past several years, a great deal of cross talk has been found between the UPR and autophagy pathways.

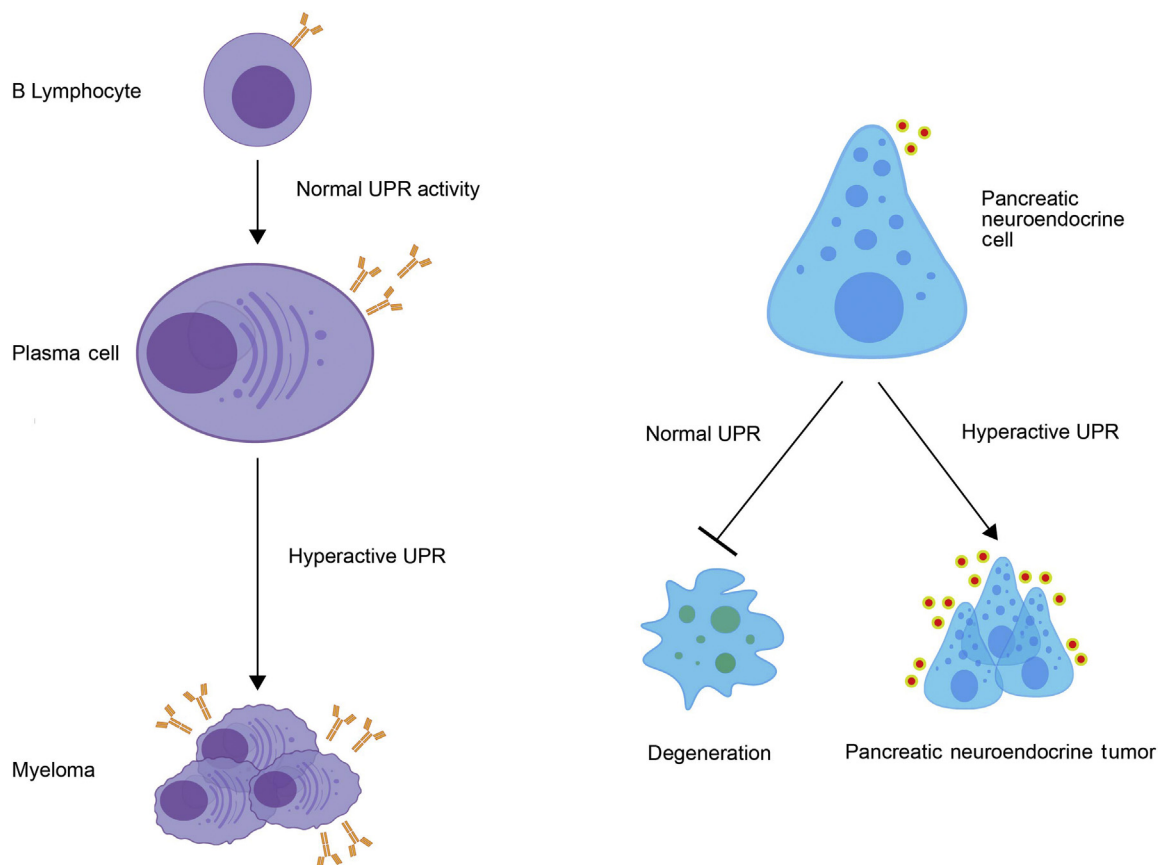


Figure 2 Normal and malignant professional secretory cells are critically dependent on the unfolded protein response (UPR). Plasma cells and pancreatic neuroendocrine cells, two cell types with a high secretory burden, require the UPR for their development and/or function. However, elevated UPR signaling is commonly seen in neoplasms derived from these cells (eg, myeloma and pancreatic neuroendocrine tumors) and appears critical for their growth and survival.

Although the molecular mechanisms linking these two ancient survival pathways are still being worked out, it has been reported that each arm of the UPR is capable of regulating autophagy in distinct ways.⁵⁴ For example, the IRE1 α /XBP1 pathway has been shown to induce autophagy in multiple cell types. This is in part because of the ability of XBP1s to bind directly to the *BECN1* promoter and enhance expression of Beclin-1.⁶¹ However, despite its proautophagy effects when activated, XBP1 deficiency has been reported to result in enhanced autophagy in cells from amyotrophic lateral sclerosis patients⁶² and in neurons through a mechanism that involves elevated expression of the transcription factor forkhead box protein O1.⁶³ Hence, it appears that either insufficient or excess IRE1 α /XBP1 signaling can promote autophagy. The PERK pathway has been shown to be required for autophagy triggered by polyglutamine aggregates.⁶⁴ The PERK output ATF4 is critical for the up-regulation of ATG12,⁶⁵ a key component of the complex responsible for elongation of autophagosomes. In addition, C/EBP homologous protein can increase the expression of ATG5.⁶⁶ Finally, there have been multiple reports that ATF6 is required for autophagy induction through a mechanism that involves transcriptionally up-regulating death-associated kinase 1, which promotes autophagosome

formation by phosphorylating Beclin-1.⁶⁷ Finally, the UPR and autophagy pathways have both been shown to coordinately regulate several histone deacetylases implicated in cancer, such as the cytoplasmic deacetylase histone deacetylase 6, which acts on α -tubulin, heat shock protein 90, and stress granules to promote tumor cell survival and migration.⁶⁸ Hence, although there is much to learn about the molecular interplay between these pathways, the UPR-autophagy connection is likely to be important in the context of neoplasia.

UPR Signals Tumor Angiogenesis in Response to Hypoxia

Solid tumor growth is limited by the ability to provide sufficient oxygen, glucose, and other nutrients to the expanding cell mass, especially those cells in the center of the tumor. When challenged with hypoxia and glucose deprivation, tumor cells up-regulate expression of vascular endothelial growth factor (VEGF), fibroblast growth factor 2, and other proangiogenic signals to promote neoangiogenesis. These same conditions cause ER stress and activate the UPR, which regulates the transcriptional and post-translational control of several proangiogenic factors. All

three arms of the UPR have been shown to strongly up-regulate VEGF-A,⁶⁹ which stimulates angiogenesis to protect rapidly growing tumor cells from hypoxia. The UPR transcription factor ATF4 directly binds the VEGF-A promoter,⁷⁰ and XBP1 transcriptionally up-regulates VEGF-A and IL-6 expression, possibly through a hypoxia-inducible factor-1 α (HIF1 α)—dependent mechanism.^{36,37} In several human cancer lines, hypoxia increases the transcription of two essential autophagy genes, microtubule-associated protein 1 light chain 3 β and *ATG5*, through the PERK outputs ATF4 and C/EBP homologous protein, respectively.⁶⁶ By up-regulating autophagy in this way, PERK can promote tumor cell survival under hypoxic conditions. The ER chaperone BiP/GRP78 also promotes cancer growth and chemoresistance, in part through up-regulating tumor angiogenesis.⁷¹ Another UPR-regulated ER chaperone, called oxygen-regulated protein 150, promotes the VEGF secretion.⁷² Interestingly, VEGF has also been reported to activate the UPR in endothelial cells in the absence of ER stress through a noncanonical mechanism involving phospholipase C and mammalian target of rapamycin signaling.⁷³ In such a way, VEGF seems capable of maintaining its own expression through triggering the UPR in endothelial cells, a feed-forward loop with the potential to strongly promote angiogenesis.⁷⁴

The UPR, Cell Migration, and Metastasis

Evidence has emerged that the UPR can regulate cytoskeleton remodeling and cell migration. Independent of its canonical role as a UPR signal transducer, IRE1 α was reported to control cytoskeleton dynamics and cell motility through directly binding filamin A.⁷⁵ Consistent with this notion, there have been studies suggesting that the UPR may govern cancer cell metastasis, at least in preclinical models. Tumor cells often undergo epithelial-to-mesenchymal transition before metastasis, which involves acquisition of a highly secretory phenotype and increased UPR signaling. In breast tumor models, PERK inhibition attenuates metastasis to the lung after tail-vein injection into an immunocompromised mouse.⁴⁰ ATF4 has been reported as critical for metastatic potential of breast cancer cells subjected to hypoxia through a mechanism involving activation of lysosome-associated membrane protein 3.^{76,77} On the other hand, expressing a dominant negative mutant of IRE1 α in glioma tumor cells led to reduced growth and angiogenesis *in vivo*, but increased invasiveness.^{37,78} Hence, although blocking IRE1 α signaling may inhibit primary tumor progression, it has the potential to promote a more invasive phenotype. In this model, IRE1 α regulates glioma cell migration through RIDD-mediated decay of the mRNA for secreted protein acidic and rich in cysteine (alias osteonectin), a matrix-associated protein important for the interaction between glioma cells and the extracellular matrix. The inhibition of IRE1 α 's RNase leads to increased secreted protein acidic and rich in cysteine and promotes tumor cell

invasion.⁷⁹ The proinvasive phenotype these cells acquire in response to IRE1 α inhibition is akin to that of some cancer types treated with direct angiogenesis inhibitors (eg, those targeting VEGF).⁸⁰

The UPR Regulates Metabolism

When faced with low nutrient conditions, cancer cells are capable of modifying their metabolism through UPR activation.⁸¹ In response to IRE1 α -mediated splicing, XBP1s transcriptionally up-regulates components of the hexosamine biosynthetic pathway that catalyze the conversion of glucose to UDP-acetylglucosamine, which serves as substrates for O- and N-linked glycosylation to promote proteostasis.^{82,83} Moreover, another known target of XBP1 is HIF1 α , which up-regulates glucose transporters to facilitate glycolysis.⁸⁴

The UPR and Resistance to Treatment

Despite major advances in the effectiveness of cytotoxic chemotherapy and targeted therapies, most ultimately fail because of the emergence of resistant clones. The mechanisms involved in chemotherapy resistance vary widely, depending on the therapy employed and cancer type. However, common mechanisms include drug inactivation through detoxification pathways, down-regulation of drug transporters, up-regulation of efflux pumps, up-regulation of prosurvival proteins, and enhanced DNA repair.⁸⁵

Mounting evidence over the past decade suggests that the UPR plays a key role in resistance to chemotherapy, hormone therapy, and targeted therapies for cancer.⁸¹ The initial link was noted when UPR activation was found to correlate with drug resistance in solid tumors, in particular breast cancer and colorectal carcinoma.^{86–89} Increased expression of the UPR sensors and their downstream outputs (including XBP1s) is correlated with tamoxifen resistance, decreased time to recurrence, and shortened survival.⁹⁰ Whether and how the UPR is directly contributing to chemoresistance *in vivo* remain poorly understood. However, *in vitro* studies on cultured cancer cell lines have demonstrated that UPR activation can promote survival against chemotherapy agents. Many studies have shown that GRP78 overexpression can prevent drug-induced apoptosis, in part through inhibition of proapoptotic Bcl-2—associated protein expression and caspase-7 activation.⁹¹ In HT29 colon cancer cells, PERK was reported to promote chemoresistance through the regulation of nuclear factor erythroid 2—related factor 2,⁴¹ and genetic or pharmacologic inhibition of PERK sensitized human colon cancer cells to 5-fluorouracil chemotherapy.⁹² Genetic deletion of IRE1 α sensitized KRAS-mutant colon cancer cells to mitogen-activated protein kinase kinase (MEK) inhibitors.⁹³ However, more studies are needed to determine the mechanisms through which each arm of the UPR can contribute to

chemoresistance and whether inhibiting the UPR is a beneficial strategy for increasing response to chemotherapy.

Cross Talk between UPR and Other Oncogenic Signaling Pathways

In addition to its distinctive signaling outputs, the UPR communicates in complex ways with other major signaling pathways that impact tumor development. For example, the UPR can have a major impact on the NF- κ B and HIF1 α pathways, two signaling pathways widely known to promote tumorigenesis in some settings. PERK-mediated inhibition of Cap-dependent protein translation results in a relative reduction in I κ B, leading to nuclear translocation of NF- κ B and transcription of its target genes.⁹⁴ Through its association with tumor necrosis factor receptor-associated factor 2, IRE1 α activation can lead to I κ B phosphorylation and degradation, again resulting in NF- κ B signaling.⁹⁵ In triple-negative breast cancer cell lines, IRE1 α 's adaptive output XBP1 was found to form a transcriptional complex with HIF1 α and regulate expression of HIF1 α targets through the recruitment of RNA polymerase II.³⁶ These examples exemplify the elaborate wiring between the UPR and other signaling pathways that together will determine tumor cell behavior and the challenges in trying to isolate the UPR-specific roles in cancer.

The Impact of the UPR on Tumor Immunity

As a point of reference, this review is focused on how tumor cell intrinsic ER stress and UPR signaling impact cancer growth. However, it is now well established that the UPR plays an essential role in the differentiation and function of many immune cell types, and therefore has consequences on antigen presentation, antibody production, inflammation, and tumor immunity. In particular, signaling through the IRE1 α /XBP1 pathway in tumor-associated dendritic cells blunts antitumor immunity. As such, genetic or pharmacologic inhibition of this pathway has been shown to enhance T-cell antitumor immunity and increase the survival of cancer-bearing mice.⁹⁶ Moreover, several approved chemotherapeutics work in part through inducing immunogenic cell death and antitumor immunity. The process of immunogenic cell death involves the translocation of calreticulin from the ER lumen to the plasma membrane, where it enhances the transfer of tumor antigens to dendritic cells.^{97,98} Recently, PERK activation and eIF2 α phosphorylation have been found to be a marker for immunogenic cell death and tumors susceptible to therapies that induce this process.^{99,100} Hence, it appears that targeting the UPR can have anticancer benefits at both the level of the tumor cells themselves and the antitumor capacity of T cells. Readers interested in learning more about the UPR and tumor immunosurveillance can find several recent reviews on the topic.^{101,102}

Targeting the UPR in Cancer

Considering the evidence of aberrant UPR signaling in cancer, it should come as no surprise that there is strong interest in pharmacologically controlling its outputs as a strategy to limit tumor growth. As the UPR first responders, IRE1 α , PERK, and ATF6 are obvious targets through which to control this pathway. In particular, there has been much effort in developing small-molecule modulators of the enzyme active sites of IRE1 α and PERK. Various small molecules have been identified that directly bind and inhibit IRE1 α 's RNase.^{103–106} Most direct inhibitors identified contain a reactive electrophile that covalently binds the RNase active site of IRE1 α , with the most common pharmacophore being a salicylaldehyde, including SFT-083010, MKC-3946, and 4 μ 8c. These salicylaldehyde-based inhibitors form a Schiff base with K907 in the active site of the RNase.^{106,107} These compounds inhibit IRE1 α 's RNase activity in cell culture, and several have shown antitumor activity in xenograft models.¹⁰⁸ For example, IRE1 α RNase inhibitors were found to decrease the tumor cell secretome and enhance the response to chemotherapy in xenograft models of triple-negative breast cancer.¹⁰⁹ However, the selectivity of these compounds is not well understood given their likely reactivity against many intracellular proteins.

As a strategy to generate more specific IRE1 α inhibitors, the first-in-class ATP-competitive IRE1 α kinase-inhibiting RNase attenuators that bind into the kinase domain and allosterically inhibit IRE1 α 's RNase were developed.¹¹⁰ Subsequently, second-generation, more advanced kinase-inhibiting RNase attenuators capable of dose dependently inhibiting endogenous IRE1 α 's kinase activity, oligomerization, ER-localized mRNA decay, and Xbp1 mRNA cleavage *in vivo* were developed.⁹ A group at Amgen (Thousand Oaks, CA) subsequently published a series of potent and selective IRE1 α kinase inhibitors, which did not impair the growth of >300 tumor cell lines when administered for 24 hours *in vitro*.³⁴ The *i.p.* delivery of the best compound from this series (compound 18 or kinase-inhibiting RNase attenuator 8) showed efficacy in preclinical models of myeloma and pancreatic neuroendocrine tumors.^{46,53}

A team at GlaxoSmithKline (Brentford, UK) developed highly potent and selective inhibitors of PERK's kinase domain, including GSK2606414 and GSK2656157.¹¹¹ Oral administration of GSK2606414 leads to therapeutic doses in the central nervous system and protects against preclinical models of neurodegeneration.¹¹² Moreover, GSK2656157 showed antitumor effects in xenograft models of multiple myeloma and pancreatic adenocarcinoma in immunocompromised mice.¹¹¹ However, most of the PERK inhibitors tested to date quickly cause pancreatic β -cell loss and diabetes, which represent a major barrier for clinical development. The β -cell toxicity seen with PERK inhibition phenocopies Wolcott-Rallison syndrome, the previously mentioned rare diabetic syndrome in humans caused by *PERK* mutations.

Small molecules have also been identified that attenuate eIF2 α phosphorylation without inhibiting PERK per se. Recently, a symmetric bis-glycolamide, named integrated stress response inhibitor, was discovered that binds to and activates elongation initiation factor 2 B, a guanine nucleotide exchange factor that then releases eIF2 α phosphorylation-mediated inhibition of protein translation.¹¹³ Integrated stress response inhibitor administration is generally well tolerated by rodents, and it has been described to enhance their memory formation through mechanisms that are still not completely understood.¹¹⁴ Recently, integrated stress response inhibitor was shown to cause tumor regression and prolong survival in patient-derived models of advanced prostate cancer.¹¹⁵

Although by no means exhaustive, these examples of small-molecule inhibitors against IRE1 α and PERK highlight the therapeutic potential and risks of targeting the UPR in cancer. For a more complete discussion of therapeutics against the UPR, readers should refer to several recent reviews on the topic.^{116,117}

Conclusions

The UPR is a signal transduction pathway that becomes activated when the cell is unable to maintain proteostasis within the ER. In response to a buildup of misfolded proteins in the ER lumen, a condition called ER stress, the UPR sets in motion adaptive outputs that decrease protein-folding load and increase the capacity of the ER secretory pathway to restore homeostasis. However, if these corrective actions are unsuccessful at restoring proteostasis within the ER, the UPR triggers prodeath signals to cause cell demise. Cancer cells face many threats to ER proteostasis, including hypoxia, nutrient deprivation, proteasome dysfunction, increased demands on the secretory pathway, and somatic mutations in its client proteins. UPR hyperactivation is well documented in many types of solid and hematopoietic malignancies. Xenograft studies in mice suggest that the UPR supports tumor growth. Depending on the tumor model, UPR activation has been shown to regulate cell survival, angiogenesis, inflammation, invasion, metastasis, and chemoresistance. As such, the UPR is emerging as an attractive therapeutic target in cancer, but many more studies are needed to understand the benefits and risks of modulating the UPR in any particular tumor type. Fortunately, the recent development of selective small-molecule inhibitors of upstream UPR components has finally made these experiments feasible.

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