Subject Review

The Unfolded Protein Response: A Novel Component of the Hypoxic Stress Response in Tumors

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Abstract

Hypoxia is a physiologically important endoplasmic reticulum (ER) stress that is present in all solid tumors. Numerous clinical studies have shown that tumor hypoxia predicts for decreased local control, increased distant metastases, and decreased overall survival in a variety of human tumors. Hypoxia selects for tumors with an increased malignant phenotype and increases the metastatic potential of tumor cells. Tumor cells respond to hypoxia and ER stress through the activation of the unfolded protein response (UPR). The UPR is an adaptive response to increase cell survival during ER stress. XBP-1 is a critical transcriptional regulator of this process and is required for tumor growth. Pancreatic ER kinase (PKR-like ER kinase) regulates the translational branch of the UPR and is also important in the growth of tumors. Although the exact mechanism has yet to be elucidated, recent data suggest that the UPR affects tumor growth through protection from apoptosis and may influence angiogenic signaling pathways. Targeting various components of the UPR is a promising therapeutic strategy. Understanding the relationship between hypoxia, the UPR, and tumor growth is crucial to improving current cancer therapies.
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Introduction

A defining characteristic of solid tumors is the capacity to divide aggressively and disseminate metastases under conditions of nutrient deprivation and limited oxygen availability. These microenvironmental stresses arise from inadequate perfusion as the primary tumor rapidly outgrows its initial blood supply and from dramatic structural abnormalities of tumor vessels that lead to aberrant microcirculation (1, 2). Therefore, regions of low O_2 tension are heterogeneously distributed within the tumor mass. Although tumor hypoxia stands as a physiologic barrier to cell survival, it paradoxically drives malignant progression by imposing powerful selective pressure on cells that can best adapt to this stress and resume cell division. The demonstration of hypoxia-mediated clonal expansion of cells with diminished apoptotic potential supports this concept (3).

Tumor hypoxia correlates with a more aggressive disease course and limits the effectiveness of anticancer therapy. Hypoxic cells are relatively resistant to killing by radiation. When cells are irradiated under anoxic conditions, a ~3-fold increase in radiation dose is required to achieve the same level of cell killing as cells irradiated under fully oxic conditions. In addition, because hypoxic cells are nonproliferating and relatively isolated from the blood supply, chemotherapies that target rapidly dividing cells may be less effective on this population of cells and the delivery of chemotherapy to these areas may be compromised (4). In 1955, Thomlinson and Gray were the first to show that hypoxia existed in solid tumors (5). Intratumoral measurements using polarographic needle electrodes (Eppendorf, Hamburg, Germany) have conclusively identified areas of hypoxia in a wide variety of human tumors, including cervix, brain, pancreas, head and neck, and prostate cancers (6). Many of these tumors contain regions of hypoxia that could adversely affect therapy (<5 mm Hg oxygen). In a variety of tumor sites, different investigators have correlated the presence of tumor hypoxia with increased tumor recurrence rates (7). For example, in head and neck cancers, Gatenby et al. correlated poor response to radiation with increased hypoxia in these tumors (8). In prostate cancer, hypoxic tumors predicted for increased recurrences (9). Interestingly, even in cancer patients treated with surgery alone, an examination of cervical tumor specimens following radical tumor resection shows that hypoxic tumors were more likely to metastasize compared with well-oxygenated tumors of similar clinical stage and size (10). Fyles et al. reported that patients with hypoxic cervical carcinomas also had a higher rate of disease recurrence outside of the pelvis consistent with the hypothesis that hypoxia drives the metastatic spread of tumors (11). Similarly, in soft tissue sarcomas, investigators have also reported that hypoxic soft tissue sarcomas were more likely to metastasize (12, 13). These clinical studies are supported by laboratory studies indicating that hypoxia is a strong stimulus for the metastatic spread of tumors (7, 14, 15). Collectively, these findings provide strong evidence that hypoxia has a profound effect on tumor growth and clinical outcomes. Understanding the molecular basis for survival under hypoxic conditions therefore holds the promise of revealing fundamental mechanisms that determine malignant progression.

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Hypoxia dramatically reshapes cellular physiology by causing cell cycle arrest, a shift in energy production to glycolysis, elevated secretion of survival and proangiogenic factors, expression of genes involved in drug resistance, and increased cell motility and invasion. An important discovery linking these profound changes to the control of gene expression occurred with the identification of hypoxia-inducible factor (HIF-1), a heterodimeric transcription factor that exerts control over a broad range of cellular pathways, including glycolysis, angiogenesis, and erythropoiesis (16, 17). HIF-1 is composed of a constitutively expressed subunit (HIF-1β) and an oxygen-labile subunit (HIF-1α). HIF-1α protein stability is directly regulated by a post-translational oxygen-dependent hydroxylation mechanism that targets it for ubiquitin-mediated degradation (18-20).

Although HIF controls the expression of many hypoxia-regulated genes, expression profiling studies conducted under hypoxia (21-28) reveal that HIF activation alone cannot account for the full repertoire of changes that occur intracellularly as oxygen becomes limiting. Hypoxic cells elicit additional HIF-1-independent adaptive responses that contribute to increased survival under low oxygen conditions. One immediate reaction to hypoxia is a reduction in the rates of global protein synthesis, which reduces energy demands when oxygen and ATP levels are low (29). Furthermore, hypoxia causes a robust increase in the expression of molecular chaperones, which assist in protein refolding and in the degradation of terminally misfolded proteins. The unfolded protein response (UPR) is a coordinated cellular program that orchestrates these changes, playing a fundamental role during cellular stresses, such as hypoxia.

Hypoxia and Protein Misfolding

In eukaryotic cells, the endoplasmic reticulum (ER) serves as the first compartment of the secretory pathway and as a processing station for all secreted and transmembrane proteins. Unlike their counterparts in the cytosol, secreted proteins undergo a series of post-translational modifications, notably glycosylation and disulfide bonding, which are required for progression to the "native" or folded state. These additional steps increase the likelihood of errors that can cause loss of protein function or potentially toxic accumulation of misfolded proteins in the ER. To ensure proper folding of ER proteins, yeast use a dedicated machinery of molecular chaperones and foldases that associate directly with nascent polypeptides, assisting their correct folding. Foldases, such as protein disulfide isomerase and Ero1, catalyze the formation of disulfide linkages by transiently forming mixed disulfides with their client proteins and acting as an electron relay system for oxidative folding (30). Importantly, molecular oxygen is the major electron acceptor at the end of this relay system, providing the driving force for protein folding in the ER (Fig. 1). Currently, studies are under way to determine if a similar mechanism of oxidative protein folding exists in mammalian cells. Preliminary data from our laboratory and the Koumenis laboratory indicate that in mammalian cells this pathway is similarly dependent on molecular oxygen[1] and future studies will further define the complexities of this pathway. Although Ero1 is transcriptionally induced by HIF during hypoxia (31), in the absence of oxygen, it cannot fold a model substrate (30, 32). These findings underscore the critical role of molecular oxygen in sustaining ER folding and strongly suggest that the accumulation of misfolded proteins in the ER is a natural consequence of intratumoral hypoxia.

[1] C. Koumenis, personal communication.

The UPR Maintains Cellular Homeostasis during ER Stress

The UPR is a conserved, adaptive cellular program activated in response to the accumulation of misfolded proteins in the ER. As a homeostatic mechanism, the UPR matches the folding capacity of the ER with elevated demand. The UPR does this by increasing the expression of ER-resident molecular chaperones while lowering the flux of nascent polypeptides imported into the ER and through the elimination of terminally misfolded proteins by retrotranslocation into the cytosol for proteasomal degradation (33, 34). The UPR modulates these processes by asserting control at both transcriptional and translational levels.

The ER resident transmembrane proteins Ire1, pancreatic ER kinase (PKR-like ER kinase; PERK), and activating transcription factor (ATF) 6 constitute the core stress transducers of the UPR, delivering signals from the ER to the cytoplasm and nucleus following ER stress (33, 34). In the absence of ER stress, the ER chaperone, glucose-regulated protein 78 kDa (GRP78; also called BiP), directly associates with these sensors, suppressing their activation by sterically blocking dimerization or, in the case of ATF6, preventing transport to the Golgi complex and subsequent proteolytic liberation of the transcriptionally active NH_2-terminal domain. GRP78 is critical to the regulation of the ER stress response and considerable redundancy exists between different signaling pathways. There are at least two models to explain how GRP78 functions to regulate ATF6. In the first, misfolded proteins competitively titrate away GRP78 as they accumulate in the ER, initiating stress signaling (35, 36). The other model suggests that GRP78 forms a stable complex with ATF6 and actively dissociates from this complex through a signal initiated by misfolded proteins (37).

Activated ATF6 and Ire1 directly modulate transcriptional induction of UPR target genes. Mammalian cells express two closely related ATF6 family members, ATF6α and ATF6β. The proteolytically activated NH_2-terminal portion of each protein encodes a basic leucine zipper transcription factor that heterodimerizes with the general transcription factor NF-Y and binds to the ER stress response element (ERSE), a *cis*-regulatory motif identified in promoter regions of UPR target genes, such as the ER chaperones GRP78 and GRP94 (38). Mammalian Ire1α, the major functional homologue of yeast Ire1, encodes an ER transmembrane kinase and endoribonuclease that, on activation, excises a 26-nucleotide intron from the mRNA encoding the basic leucine zipper transcription factor XBP-1. This results in a translational frame shift downstream of the splice site to generate XBP-1s, a potent transcriptional activator (39-41). XBP-1s binds as a homodimer preferentially to the UPR element, a *cis*-regulatory sequence containing the consensus sequence TGACGTGG/A (39, 42, 43).

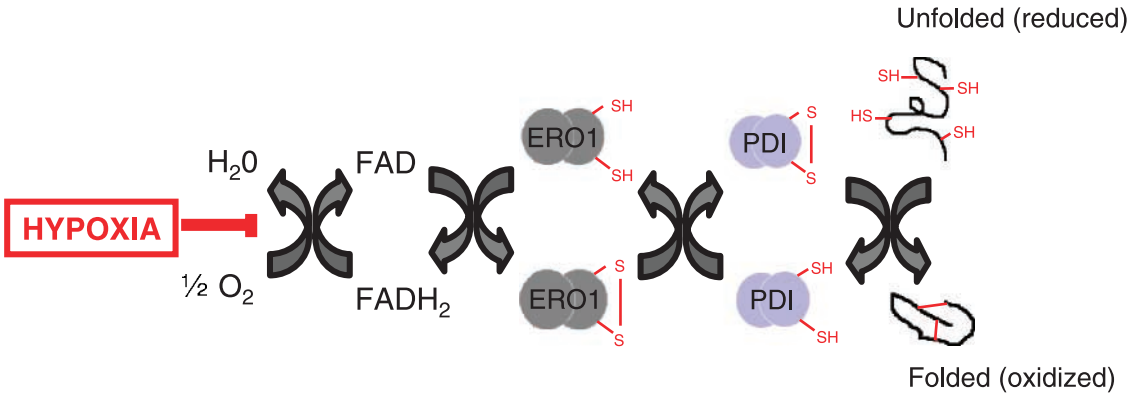

FIGURE 1. Schematic of oxidative folding in yeast (adapted from ref. 32). Protein folding in the ER requires disulfide bond formation catalyzed sequentially by protein disulfide isomerases and Ero1. The final electron acceptor downstream of Ero1 is molecular oxygen.

The ERSE consists of a consensus sequence of CCAAT-N_9-CCACG that is necessary for stress induction of UPR target genes, including GRP78 and GRP94 (38, 44). The general transcription factor NF-Y/CBF binds to the CCAAT motif, and under stress conditions, ATF6 binds to the CCACG portion of this element (45-47). During ER stress, ATF6 is activated rapidly by proteolysis, which generally occurs before the activation of XBP-1s. This observation suggests that ATF6-dependent gene transcription precedes XBP-1-dependent gene transcription and may provide insight into the cellular response during prolonged ER stress (48). However, because XBP-1 can also bind to the ERSE element (39), considerable redundancy exists in ERSE-dependent gene expression. Moreover, XBP-1 and ATF6 can both bind (albeit with different affinity) to the more recently described ERSE II (ATTGG-N-CCACG), an element found in the Herp promoter (42). Interestingly, Herp is strongly induced by ER stress and is regulated by both transcriptional and translational branches of the UPR (49).

A hallmark of UPR activation is global reduction in the rate of protein synthesis, which is controlled by reversible phosphorylation of the α-subunit of eukaryotic initiation factor 2 (eIF2α). Phosphorylation of eIF2α prevents formation of the eIF2-tRNAmet-40s complex, thereby impeding the initiation of protein synthesis. The transmembrane kinase PERK is required for eIF2α phosphorylation in ER stress (50, 51). In addition to reducing the flux of nascent proteins entering the ER, decreased translation results in the selective synthesis of ATF4, a transcription factor that controls the expression of downstream transcriptional regulators, such as ATF3 and CCAAT/enhancer-binding protein homologous protein (CHOP). Under hypoxia, ATF4 is regulated at the translational level and activation occurs independently of HIF-1 (52, 53). Independent of the ERSE, ATF4 also contributes to the ER stress induction of GRP78 (54). Finally, activated PERK directly phosphorylates and activates Nrf2, a transcription factor that controls the expression of antioxidant genes (39, 55, 56).

In tandem with the increased expression of molecular chaperones, the UPR also controls ER-associated degradation, a quality-control pathway dedicated to the elimination from the ER of irretrievably misfolded polypeptides. ER-associated degradation involves the dislocation of selected substrates from the ER to the cytosol for proteolysis via the ubiquitin-proteasome system. This process is mediated by ER lumenal proteins Yos9p and EDEM, which interface directly with sugar moieties on target proteins (57-59) and by a ubiquitin ligase complex composed of the integral ER membrane proteins Hrd1p and Hrd3p (60, 61). Hrd1p contains a NH_2-terminal, multispanning membrane anchor and a COOH-terminal cytosolic domain, which contains a RING-H2 motif homologous to several characterized ubiquitin ligases. The cytosolic chaperone-like AAA ATPase Cdc48 (also known as p97), in conjunction with the integral membrane protein Ubx2 (62), may present ubiquitinylated substrates to the proteasome once they have been removed from the ER. Dissecting out the role of ER-associated degradation in the context of hypoxia and ER stress is important to our overall understanding of the UPR in tumor growth.

Although activation of the UPR serves a protective function to cells under ER stress, prolonged activation of the UPR by excessive or unalleviated ER stress can activate multiple apoptotic pathways in mammalian cells (63). Persistent treatment of neurons with tunicamycin induced expression of the proapoptotic BH3-only protein PUMA, resulting in mitochondrial release of cytochrome *c* and activation of caspase-9 and caspase-3 (64). Furthermore, CHOP transcriptionally activates the expression of GADD34, which directs the protein phosphatase PP1 to eIF2α. Thus, CHOP, acting through GADD34, serves as a feedback inhibitor of PERK-mediated translational attenuation (65). In this manner, CHOP elevates the load of nascent proteins entering the ER, increasing ER stress, and promoting cell death (66). These findings are consistent with the identification and characterization of salubrinal, a selective chemical inhibitor of eIF2α phosphatase complexes that can protect cells from death associated with prolonged ER stress (67). CHOP also inhibits expression of Bcl-2 (68) and directly activates the proapoptotic death receptor 5 protein (69), which recruits procaspase-8 to form a death-inducing signaling complex.

Several reports implicate Ire1 in promoting proapoptotic signaling via its kinase domain, which can activate the adaptor protein TRAF2 and downstream effector kinases

apoptosis signal-regulating kinase 1 and c-Jun NH_2-terminal kinase. Apoptosis signal-regulating kinase 1 (−/−) fibroblasts fail to activate c-Jun NH_2-terminal kinase following ER stress and remain resistant to cell death induced by ER stress (70). TRAF2 (−/−) fibroblasts are also resistant to oxidative stress-induced cell death (71). The Ire1-TRAF2 module directly associates with procaspase-12 and promotes the clustering and activation of procaspase-12 by cleavage in response to ER stress (72). However, the role of caspase-12 and downstream effector caspase-4 in ER stress-induced cell death is controversial, as human and murine cells deficient in these caspases can still undergo apoptosis following treatment with chemical inducers of ER stress (73). The ER stress-induced death observed in these cell lines may be driven by other caspases. Overall, these findings highlight the dual nature of the UPR both as a critical protector of cells during ER stress and as a potent inducer of a multitude of proapoptotic pathways. Cell death may ensue from an inadequate response to ER stress, as evidenced by the stress-sensitive phenotype of UPR mutants, or from prolonged activation of the UPR. Thus, regulation of the UPR must be precisely calibrated to the level of ER stress in the cell and multiple pathways function simultaneously to maintain this balance (Fig. 2).

A Role for the UPR in Tumorigenesis

Diminished oxygen and glucose availability are hallmarks of the tumor microenvironment. Because the delivery of nutrients to the tumor is a dynamic process determined in part by fluctuating blood flow, different regions of the tumor must constantly respond to fluctuating levels of ER stress to survive. During prolonged or severe ER stress, the UPR cannot match the ER stress demands required for survival. However, within a heterogeneous tumor, a subpopulation of cells may exist that is relatively resistant to ER stress and these cells may contribute to tumor recurrences.

The functional link between ER stress response and hypoxia was shown by antisense-mediated inhibition of GRP78 expression during hypoxia. In these studies, cells that were not able to induce GRP78 under hypoxia were sensitized to hypoxia *in vitro* (74). In addition, inhibition of GRP78 inhibited the growth of implanted fibrosarcoma cells *in vivo* (75). Additional studies have shown that expression of ER chaperones in tumors increases resistance to DNA damage and chemotherapeutic agents, such as etoposide and cisplatin (76, 77). Up-regulation of ER chaperones and classic UPR targets GRP78 and GRP94 has been shown convincingly in a variety of primary tumors, such as breast, prostate, gastric, and colon carcinomas (78, 79).

The first demonstration that the UPR was necessary for tumor growth came from studies examining the role of the Ire1/XBP-1 branch during hypoxia. During hypoxia, Ire1 processes XBP-1 into its activated form, XBP-1s, in a HIF-1-independent manner. Mouse embryonic fibroblasts genetically deleted for XBP-1, or cells expressing a small interfering RNA that diminishes expression of XBP-1, are severely impaired for survival following exposure to hypoxia. Strikingly, the XBP-1 (−/−) cells do not grow into tumors when implanted into mice, although they secrete normal levels of angiogenic and survival factors, such as vascular endothelial growth factor and basic fibroblast growth factor (28). Expression of truncated, dominant-negative Ire1α also significantly reduces survival of HT1080 fibrosarcoma and MiaPACA-2 pancreatic carcinoma cells following exposure to hypoxia due to a defect in splicing XBP-1 and in part to proapoptotic signaling through the Ire1 kinase

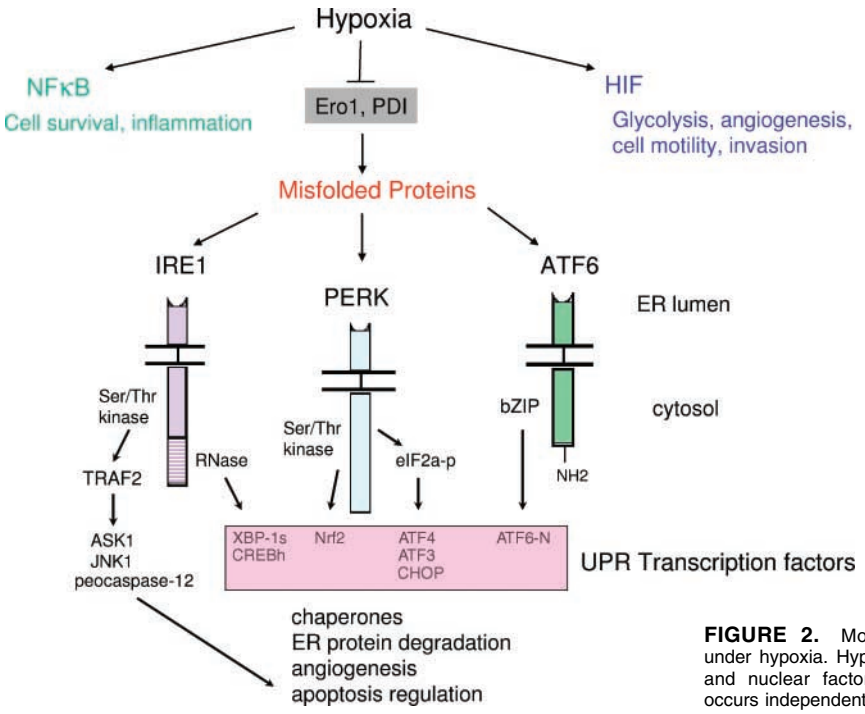

FIGURE 2. Model of UPR signaling under hypoxia. Hypoxia regulation of HIF-1 and nuclear factor-κB (*NF-κB*) signaling occurs independently of the UPR.

domain.[2] The importance of Ire1/XBP-1 in tumor growth is supported by examination of human tumor xenografts expressing a XBP-luciferase fusion protein in which luciferase is fused in frame with XBP-1s. In these cells, luciferase activity is detected only when XBP-1 is spliced into its active form. As shown in Fig. 3, we were able to detect luciferase activity in tumors of varying sizes using a bioluminescence imaging system (IVIS, Xenogen, Alameda, CA). These findings show that even relatively small tumors have detectable XBP-1 splicing, suggesting that ER stress occurs throughout tumor growth. The importance of Ire1/XBP-1 is further supported by the finding that the chemotherapeutic agent PS-341, a proteasome inhibitor, blocks Ire1-mediated splicing of XBP-1 and stabilizes the unspliced form, which can function as a dominant negative (80). Taken together, these results show the key contribution of the Ire1/XBP-1 branch of the UPR in hypoxia survival and tumor progression.

In a separate report, Koumenis et al. showed activation of PERK and phosphorylation of eIF2α during hypoxia (81). Mouse embryonic fibroblasts stably expressing a dominant-negative PERK allele, or mouse embryonic fibroblasts with a homozygous deletion of PERK, exhibited attenuated phosphorylation of eIF2α and reduced inhibition of protein synthesis in response to hypoxia. Under hypoxia, there is a biphasic response to eIF2α phosphorylation, suggesting that following the initial attenuation in protein translation there may be a transient period in which additional protein synthesis is allowed before a more permanent decrease in protein synthesis occurs (Fig. 4). PERK (−/−) mouse embryonic fibroblasts fail to phosphorylate eIF2α and exhibit lower survival after prolonged exposure to hypoxia compared with wild-type fibroblasts. This was the first demonstration that adaptation of cells to hypoxic stress requires activation of PERK, directly implicating the UPR in hypoxia survival. Subsequent studies have shown that PERK activation in hypoxia confers resistance to apoptosis and that hypoxic regions of PERK (−/−) mouse tumors closely overlap with apoptotic regions. A critical role for PERK-mediated translational control in hypoxia survival is further substantiated by results from mouse embryonic fibroblasts expressing a "knock-in" mutant allele of eIF2α (S51A) that cannot be phosphorylated by PERK. These cells exhibit an increased susceptibility to hypoxia, with virtually no survival after a 48-hour exposure compared with a 40% survival rate for cells expressing wild-type eIF2α (82). Importantly, impairment of PERK signaling in HT29 colorectal carcinoma cells causes increased apoptosis following hypoxia and significantly attenuates the growth of subcutaneously implanted tumor xenografts. These findings further support the significance of the PERK-eIF2α-ATF4 branch of the UPR in regulating tumor growth.

Immunohistochemical studies from human tumors have also substantiated the significance of various branches of the UPR in cancer. Molecular analysis of hepatocellular carcinoma tissue revealed extensive activation of ATF6 and Ire1-mediated processing of XBP-1 in tumor cells but not in adjacent normal tissues (83). Moreover, elevated mRNA expression of GRP78, ATF6, and XBP-1s occurs in carcinoma tissues with increased histologic grading.

Overcoming Proteotoxicity and Beyond

Although it is expected, a positive role in hypoxia survival and tumorigenesis for ATF6 family members ATF6α and ATF6β has not been validated yet by either biochemical or genetic studies. Furthermore, because of redundancy in UPR signaling for some target genes, blocking multiple branches of the UPR may be necessary to prevent compensatory changes in survival signaling pathways. Inhibiting multiple branches of the UPR may have a greater effect on tumor growth and therapeutic resistance to this type of cancer therapy. Nonetheless, the widespread occurrence of hypoxia in tumors and the severe impairment of growth in XBP-1-deficient and PERK-deficient tumors show that ER stress is inextricably linked to tumorigenesis (84).

Indirectly, the UPR may affect tumor growth by mechanisms that extend beyond its traditional role in the alleviation of protein misfolding and proteotoxicity. Expression of oxygen-regulated protein 150, a hypoxia-inducible ER chaperone, suppresses hypoxia-induced cell death and seems necessary for the correct processing and secretion of vascular endothelial growth factor, a potent inducer of angiogenesis (85-88). Furthermore, inhibitors of glycosylation, such as tunicamycin, known to activate ER stress response pathways, also induce the expression of vascular endothelial growth factor (88, 89). The vascular endothelial growth factor mRNA transcript is enriched in translationally active polysome fractions following exposure to hypoxia (53). These findings reveal the involvement of UPR effectors in tumor growth via increased expression and processing of vascular endothelial growth factor.

Oxidative stress in the form of excess peroxides and reactive oxygen species is an additional consequence of ER stress that can influence cellular survival pathways. ER proteins incapable of productively folding undergo futile cycles of disulfide formation, reduction, and rearrangement, generating reactive oxygen species as a byproduct (30, 90). This is supported by the observation of chronically elevated reactive oxygen species levels in PERK-deficient and ATF4-deficient cells (91). Reactive oxygen species has been linked to the accumulation of HIF (92) and may be an important factor in tumor cells undergoing successive cycles of hypoxia/reoxygenation. Reactive oxygen species–mediated signaling have also been reported to affect apoptosis and the c-Jun NH_2-terminal kinase pathway through inhibition of mitogen-activated protein kinase phosphatases (93, 94).

Separately, ER stress antagonizes insulin pathway signaling via Ire1-mediated serine phosphorylation of IRS-1 downstream of the insulin receptor (95). This mutual antagonism between Ire1 and insulin signaling is supported by the finding that protein tyrosine phosphatase 1B promotes ER stress-induced Ire1 signaling (96) but inhibits signaling downstream of the insulin receptor (97). Finally, an intriguing possibility is that ER stress may inhibit the function of the p53 tumor suppressor protein by redistributing p53 out of the nucleus and stimulating its

[2] D.E. Feldman and A.C. Koong, in preparation.

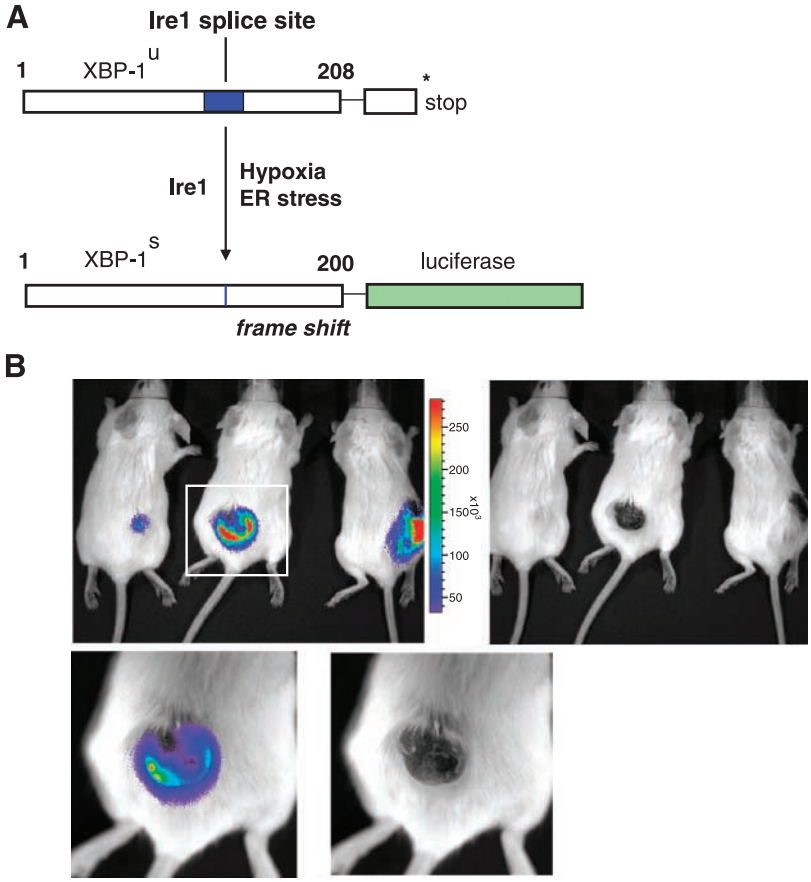

FIGURE 3. Activation of Ire1/XBP-1 pathway in implanted tumor xenografts. **A.** XBP-luciferase reporter construct. Firefly luciferase was inserted downstream of the Ire1 splice site in human XBP-1 to permit translation of luciferase on ER stress in an Ire1-dependent manner. **B.** HT1080 human fibrosarcoma cells stably expressing the XBP-luciferase reporter were implanted subcutaneously into severe combined immunodeficient mice. Mice were injected with luciferase substrate D-luciferin immediately before anesthesia and were imaged with a Xenogen CCD camera.

degradation *in vivo* (98). These findings need to be further substantiated by additional studies but suggest that ER stress may influence tumor progression through multiple pathways. Overall, these findings show that ER stress and the UPR can exert control over cellular processes relevant to cancer, such as cell survival, tumor progression, angiogenesis, and apoptosis, through direct and indirect effects.

Therapeutic Targeting of the UPR in Cancer

The requirement for the UPR in tumor growth raises the possibility of targeting this pathway as a novel chemotherapeutic strategy. One promising therapeutic avenue entails the expression of a virally delivered cytotoxin under control of the ER stress-activated GRP78 promoter, which robustly eradicates human tumors in mouse xenograft experiments (99). Another approach is the development of small molecule-based therapies that directly inactivate key upstream activators of the UPR, such as PERK and Ire1, which are transmembrane kinases amenable to high-throughput screening for chemical inhibitors. In principle, these drug-like molecules should be relatively specific to tumors where activation of the UPR is greatest. However, secretory organs, such as the pancreas, should be closely monitored for normal tissue toxicity. UPR inhibitor compounds would also be ideally suited for the treatment of secretory cell malignancies, such as multiple myeloma, an aggressive plasma cell neoplasm characterized by the secretion of monoclonal immunoglobulins (80, 100). Because the secretory capacity of these malignant B cells is sustained by a dramatically expanded ER and is maintained by an active UPR, chemical inhibitors of the UPR may decrease immunoglobulin secretion while causing B-cell apoptosis.

The clinical potential for chemotherapeutic agents that target the UPR is illustrated by the recent demonstration that versipelostatin, an inhibitor of glucose deprivation-induced transcription from the GRP78 promoter, functions as a cytotoxin during glucose starvation and enhances the cisplatin sensitivity of tumors *in vivo* (101). The mechanism by which versipelostatin blocks glucose deprivation-induced transcription remains unclear, but it is unlikely that the drug directly inhibits upstream UPR activators because there is no effect on the activation of Ire1 or ATF6 by tunicamycin or calcium iono-phores, well-characterized inducers of ER stress. Nevertheless, these results show that disruption of the UPR can elicit an antitumor effect, particularly when given in combination with other cytotoxic therapies.

These findings provide important support for the pursuit of selective inhibitors of PERK and Ire1 that function without regard to the origin of ER stress. A screen for inhibitors of Ire1-mediated splicing of a XBP-1-luciferase reporter substrate identified several compounds targeting the Ire1 pathway that exhibit potent, hypoxia-specific cytotoxicity.[3] The antitumor

[3] D.E. Feldman and A.C. Koong, unpublished data.

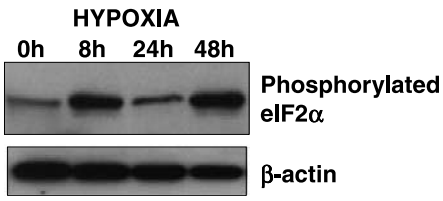

FIGURE 4. eIF2α phosphorylation in Panc1 cells exposed to hypoxia (<0.5% oxygen) shows a biphasic response. These results suggest that following the initial block in protein synthesis (8-hour hypoxia) there is a transient period in which translation resumes under hypoxia before a more permanent decrease in translation occurs (48-hour hypoxia).

properties of these compounds are currently under investigation. Simultaneous inhibition of Ire1 and PERK may prove most effective in killing hypoxic cells, because the downstream effectors of PERK and Ire1 are largely nonoverlapping, and deletion of either Ire1 or XBP-1 is synthetically lethal with the deletion of PERK in the nematode *Caenorhabditis elegans* (102). Identification of downstream transcriptional effectors of the UPR by gene expression profiling strategies in tumor cells may also uncover promising new targets for chemotherapy (103).

Concluding Remarks

The Ire1/XBP-1 and PERK pathways have been directly implicated in the regulation of tumor growth. These results suggest a broader role for the UPR in cancer, particularly with regard to tumor microenvironmental effects of hypoxia, low glucose, and pH changes. These pathophysiologic stresses occur in tumors and lead to ER stress. The adaptation to ER stress is an important survival mechanism for tumor cells. Prolonged exposure to ER stress eventually saturates the ability of the UPR to protect cells and results in cell death. Cell death by apoptosis or necrosis, however, is not the only consequence of impairment of the UPR. Elucidating the mechanism(s) of how the UPR regulates tumor growth will require the identification of downstream effectors that participate in the UPR and a detailed examination of cells carrying mutations that disable individual components of the UPR. The use of small interfering RNA techniques and the identification of selective inhibitors of PERK and Ire1 that enable a "chemical genetic" approach will be indispensable in determining the function of these and other UPR activators and their effect on tumor growth. Nevertheless, the data to date show that the UPR is activated in tumors and modulation of UPR signaling represents a promising strategy for anticancer therapy.

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