



Published in final edited form as:

Oncogene. 2013 February 14; 32(7): . doi:10.1038/onc.2012.130.

The critical roles of endoplasmic reticulum chaperones and unfolded protein response in tumorigenesis and anti-cancer therapies

Biquan Luo, B.S.¹ and Amy S. Lee, Ph.D.^{1,2}

¹Department of Biochemistry and Molecular Biology, University of Southern California (USC) Norris Comprehensive Cancer Center, Keck School of Medicine of USC, Los Angeles, CA, USA

Abstract

Cancer progression is characterized by rapidly proliferating cancer cells that are in need of increased protein synthesis. Therefore, enhanced endoplasmic reticulum (ER) activity is required to facilitate the folding, assembly and transportation of membrane and secretory proteins. These functions are carried out by ER chaperones. It is now becoming clear that the ER chaperones have critical functions outside of simply facilitating protein folding. For example, cancer progression requires GRP78 for cancer cell survival and proliferation, as well as angiogenesis in the microenvironment. GRP78 can translocate to the cell surface acting as a receptor regulating oncogenic signaling and cell viability. Calreticulin, another ER chaperone, can translocate to the cell surface of apoptotic cancer cells and induce immunogenic cancer cell death and antitumor responses *in vivo*. Tumor-secreted GRP94 has been shown to elicit antitumor immune responses when used as antitumor vaccines. Protein disulfide isomerase is another ER chaperone that demonstrates pro-oncogenic and pro-survival functions. Due to intrinsic alterations of cellular metabolism and extrinsic factors in the tumor microenvironment, cancer cells are under ER stress, and they respond to this stress by activating the unfolded protein response (UPR). Depending on the severity and duration of ER stress, the signaling branches of the UPR can activate adaptive and pro-survival signals, or induce apoptotic cell death. The PERK signaling branch of the UPR has a dual role in cancer proliferation and survival, and is also required for ER stress-induced autophagy. The activation of the IRE1 branch promotes tumorigenesis, cancer cell survival, and regulates tumor invasion. In summary, perturbation of ER homeostasis plays critical roles in tumorigenesis, and therapeutic modulation of ER chaperones and/or UPR components presents potential antitumor treatments.

Introduction

The endoplasmic reticulum (ER) is a perinuclear, cytosolic compartment essential for the synthesis, folding and modification of secretory and membrane proteins. It is also the site for lipid synthesis and a major intracellular site for Ca²⁺ storage. Physiological and pathologic conditions that perturb the ER, such as nutrient deprivation, hypoxia, ER Ca²⁺ depletion, impaired glycosylation or disulfide bond formation, oxidative stress, and viral or bacterial infection, may lead to ER stress. ER stress occurs when the protein load exceeds the ER capacity to fold or degrade them, and is manifested by the accumulation of misfolded

²Correspondence: Dr. Amy S. Lee, Department of Biochemistry and Molecular Biology, USC Norris Comprehensive Cancer Center, 1441 Eastlake Ave., Room 5308, Los Angeles, CA 90089-9176, USA. Telephone: (323) 865-0507, Fax: (323) 865-0094, amylee@ccnt.usc.edu.

Conflict of Interest

None

proteins in the ER. ER stress triggers an evolutionarily conserved quality control mechanism, the unfolded protein response (UPR), which aims at restoring ER homeostasis by activating a cascade of signaling molecules to transiently arrest protein translation, to induce ER molecular chaperones and enzymes that enhance the protein folding capacity, and to initiate a process to export and degrade the misfolded ER proteins^{1,2}. The most abundant and well-characterized ER chaperone proteins include GRP78, GRP94, CRT, and PDI. Due to increased protein synthesis in proliferating cancer cells, tumor cells require increased ER capacity and function. ER chaperones serve a host of important roles in maintaining ER homeostasis contributing to cancer cell survival and progression. For example, GRP78 is critical for tumorigenesis and therapeutic resistance. Recently, much progress has been made in identifying diverse roles for ER chaperones in cancer. Due to the interconnectivity of the ER with other cellular compartments, it is now becoming clear that chaperones traditionally thought to remain in the ER, can function beyond this compartment and are involved in processes beyond protein folding and posttranslational modification. In particular, the cell surface form of GRP78, GRP94, CRT and PDI assume novel signaling functions that regulate proliferation, apoptosis and immunity. In view of the many excellent general reviews on the UPR, this review focuses on the diverse functions of major ER chaperones inside and outside of the ER and updates the contribution of the UPR signaling pathways within the context of cancer progression and anti-cancer therapy.

Endoplasmic reticulum homeostasis and the unfolded protein response

There are three canonical branches in the UPR signaling pathway, which are mediated by three ER stress sensors: protein kinase RNA (PKR)-like ER kinase (PERK), inositol-requiring kinase 1 (IRE1), and the activating transcription factor 6 (ATF6). A major ER chaperone, the 78 kDa glucose regulated protein (GRP78), also referred to as BiP/HSPA5, acts as a master regulator of the UPR through direct interaction with all three sensors and maintains them in an inactive form in non-stressed situations (Figure 1)³⁻⁵. Upon ER stress, GRP78 is titrated away by the accumulated malformed proteins, releasing the UPR sensors, which allows the activation and transduction of UPR signals across the ER membrane to the cytosol and the nucleus. PERK is an ER transmembrane protein with an ER luminal stress-sensing domain and a cytosolic kinase domain. An important function of PERK in the UPR is to facilitate the attenuation of global protein synthesis via the phosphorylation of eIF2 γ , which suppresses 80S ribosome assembly. This results in the inhibition of cyclin D1 translation and cell cycle arrest⁶. While global translation is suppressed under conditions of eIF2 γ phosphorylation, select mRNAs containing regulatory sequences in the open reading frame in 5'-untranslated regions require the phosphorylation of eIF2 γ for translation. The transcription factor ATF4 is one example, and the translational upregulation of ATF4 can induce the expression of UPR target genes that promote ER folding capacity and adaptation to stress.

IRE1 is a transmembrane Ser/Thr protein kinase that also has site-specific endoribonuclease (RNase) activity. Upon ER stress, IRE1 dimerizes and autophosphorylates, and thereby activates its RNase activity to cleave a 26 base intron from the mRNA encoding X-box-binding protein 1 (XBP1), resulting in a translational frameshift and a translation of a spliced form of XBP1 (XBP1-s) which is a more stable and potent transcription factor of target genes including *DnaJ*, *p58*, *ERdj4*, *EDEM* and *PDI*, all involved in protein folding and ER-associated degradation (ERAD)^{7,8}. ATF6, a basic leucine zipper (bZIP) transcription factor, when released from GRP78 upon ER stress, translocates from the ER to the Golgi apparatus, where it is cleaved by S1P and S2P proteases to generate the active nuclear form of ATF6 (p50). Cleaved ATF6 and spliced XBP1 act in parallel to mainly induce the transcription of genes encoding ER chaperones and enzymes that facilitate protein folding and maturation. Interestingly, while the PERK/

peIF2 /ATF4 pathway is canonically regarded as the translational controlling arm of the UPR, a recent report suggests that it is also required for the activation of ATF6 and its target genes, thereby fully integrating the regulatory networks of the UPR⁹.

In addition to these ER stress pathways, autophagy is activated upon ER stress and has been implicated as a defensive mechanism for maintaining ER homeostasis that promotes survival^{10,11}. Autophagy is an intracellular protein degradation system required for the normal turnover of cellular components and for the starvation response. When autophagy is induced, a double-membrane structure called autophagosome is formed *de novo* or from existing membranes, possibly from the ER, to enclose the subcellular components. Loss of the integrity of the ER, for example through depletion of GRP78, suppresses autophagosome formation induced by ER stress as well as nutrient starvation¹². How might autophagy relieve ER stress? As shown in the yeast system, upon ER stress when the ERAD system is saturated, autophagy removes both soluble and aggregated forms of unfolded proteins¹³. It has also been suggested that autophagy counterbalances ER expansion in the face of continuously accumulating unfolded proteins¹⁴. On the other hand, the onset of autophagy upon ER stress requires the UPR. Studies using MEFs with deficiency in various UPR stress sensors showed that ER stress-induced autophagy was inhibited¹⁵⁻¹⁷. While the precise requirement of specific UPR pathways to induce autophagy under different stress conditions awaits further resolution, the UPR and autophagy are integrated processes playing important roles in restoring homeostasis upon stress, counteracting apoptotic mechanisms (Figure 1).

While UPR activation leads to adaptations that may sustain cell survival, under severe and prolonged ER stress conditions where the cells fail to restore ER homeostasis, the UPR activates pathways that lead to apoptotic cell death^{2,3}. Such measures protect the organism by eliminating damaged cells beyond repair. ER stress-induced apoptosis can be carried out in a number of ways. Examples include the translocation of the death effectors BAX and BAK from the ER to the mitochondria triggered by ER Ca²⁺ flux, caspase activation (C-12 and C-4 in mice and human, respectively) by tumor necrosis factor receptor (TNFR)-associated factor-2, and the activation of c-Jun-N-terminal kinase (JNK) by IRE1 via phosphorylation and the inactivation of the anti-apoptotic protein BCL-2. Additionally, the synthesis of BCL-2 can be suppressed by CHOP, downstream of the PERK-eIF2 -ATF4 pathway. However, it has been proposed that in stressed cells the pro-apoptotic effect of CHOP may be attributed more to its activation of GADD34 which promotes eIF2 dephosphorylation, thereby acting in a negative feedback loop which leads to excessive recovery of protein synthesis that exceeds the ER folding capacity².

ER stress in tumorigenesis

Neoplastic progression is a multistep process resulting from genetic alterations that drive the progressive transformation of normal cells into malignant states by overriding growth arrest or senescence controls, and at the same time, suppressing the pro-apoptotic signals. Rapidly proliferating cancer cells require increased ER activity to facilitate the folding, assembly and transport of membrane and secretory proteins, and are thereby subjected to ER stress. Due to inadequate vascularization and rapid growth, tumor cells encounter growth-limiting conditions such as hypoxia and nutrient deprivation. An inadequate supply of glucose affects protein glycosylation and the production of ATP, both of which could lead to the accumulation of unfolded proteins in the ER, resulting in ER stress. Thus, in response to ER stress, activation of the UPR has been observed in various tumors¹⁸. In the case of leukemic cells, retroviral infection, the pathological expression of fusion protein PML-RAR and the accumulation of reactive oxygen species (ROS) can all induce ER stress^{19,20}. The hypoxic bone marrow (BM) environment and the high glucose demands resulting from rapid proliferation also contribute to ER stress in leukemia cells. Indeed, ER stress signaling has

been implicated in the spontaneous apoptosis of leukemia cells^{21,22} and inducible heterozygous knockout of GRP78 in the BM suppresses PTEN-mediated leukemogenesis²³.

During the early stages of tumor development, ER stress and the activation of the UPR could affect tumorigenesis in multiple ways (Figure 2). For example, ER stress promotes angiogenesis through stimulating VEGF expression and secretion^{24,25}. It also induces cancer cell dormancy through G1 arrest in response to decreased cyclin D1 downstream of PERK activation^{26,27}. During tumorigenesis, the robust upregulation of GRP78 and other ER chaperones by the UPR can enhance the ER protein folding capacity and maintain ER homeostasis. Additionally, the anti-apoptotic property of GRP78 can also counterbalance the cell death pathways that are still functional in the cancer cells. This, coupled with the induced dormancy, dually protects the cancer cells from apoptosis and allows for recurrence once favorable growth conditions return. A recent report further suggests a novel role of the tumor ER stress response in promoting macrophage activation and inflammation in the tumor microenvironment²⁸. Macrophages cultured in conditioned medium from ER stressed tumor cells became activated, and they undergo ER stress with upregulation of GRP78, GADD34, CHOP and XBP-1 splicing, and were able to recapitulate, amplify and expand the proinflammatory response of tumor cells, favoring tumor progression (Figure 2).

Despite the protective effects of the UPR, severe, persistent ER stress leads to cell death. Thus, overactivation of the ER stress pathways in hypoxic tumor cells has been shown to render them more sensitive to proteasome inhibitors such as bortezomib, resulting in increased cytotoxicity²⁹. Interestingly, HSP90-inhibitor IPI504, another agent known to enhance proteotoxic stress, induces tumor regression only when combined with rapamycin, by promoting irresolvable ER and oxidative stress that result in catastrophic ER and mitochondria damage³⁰. Nonetheless, in cancer cells where mutations often inactivate their apoptotic potential, mild, chronic ER stress could be beneficial for cancer cell survival. In a reconstituted cell culture system, it was demonstrated that chronic ER stress can be a predominantly adaptive pathway, primarily by maintenance of expression of UPR targets that facilitate survival, in particular ER chaperones such as GRP78 by ATF6 activation³¹. At the same time, mild, chronic ER stress leads to intrinsic instabilities of mRNAs and proteins that promote apoptosis such as CHOP and GADD34 (Figure 2). Thus, the UPR can be structured to allow cells to avert death as they adapt and both posttranscriptional and posttranslational mechanisms influence this outcome. Nonetheless, considering the complexity and heterogeneity within the tumor as well as the microenvironment around the tumor, the interplay between UPR and the various signaling pathways will likely cooperate to dictate whether apoptosis, growth arrest or proliferation will occur.

The pleiotropic role of the ER master chaperone GRP78 in cancer

GRP78 is a multifunctional protein that can impact a wide range of human diseases via diverse mechanisms^{4,5,32-34}. While traditionally GRP78 is regarded as a lumen ER chaperone whose major function is to fold and process ER proteins, bind ER Ca²⁺ and maintain ER homeostasis, recent studies have established that in specific cell types or when subjected to stress, GRP78 can be located in compartments outside the ER, including the cell surface, the cytosol, the mitochondria and the nucleus, and it can even be secreted, where it binds interacting partners and exerts new effects on cell growth and signaling³⁵⁻³⁷. These discoveries change the paradigm on GRP78 function and offers novel therapeutic approaches to target GRP78 (Figure 3).

GRP78 regulates cell survival and proliferation

GRP78 is widely observed to be upregulated in cancer cells^{4,5,33,38}. One major function of GRP78 is protection against stress-induced apoptosis. The ability of GRP78 to block

apoptosis includes binding and inactivating pro-apoptotic components such as BIK and caspase-7 that localize to the ER, as well as suppressing the induction of CHOP, which mediates the apoptotic arm of the UPR^{39,40}. Recently, a novel mechanism for the anti-apoptotic function of GRP78 has been uncovered involving functional interactions between GRP78, BIK, NOXA and BCL-2 (ref. 41). This new study showed that GRP78 is able to negate apoptosis resulting from BIK induction, even if it is assisted by another BH-3 protein such as NOXA. GRP78 binds to BIK in a BH-3 domain independent manner through its amino portion, consistent with previous findings that a subfraction of GRP78 may exist in a transmembrane configuration with the amino portion exposed to the cytosol^{39,42}. Interestingly, GRP78 and BCL-2 form separate complexes with different domains of BIK, and increasing amounts of GRP78 expressed in cells leads to a reduction of BCL-2 binding to BIK and vice versa⁴¹. These results suggest that when the expression of BIK is elevated under circumstances such as estrogen starvation or anti-estrogen treatment of breast cancer cells, it promotes the formation of BIK/BCL-2 heterodimers at the outer surface of the ER. The high ratio of BIK:BCL-2 changes the set of proteins BCL-2 interacts with the ER, leading to Ca²⁺ release and initiation of the apoptosis including the translocation of BAX to the mitochondria and the release of cytochrome c to the cytosol. However, when GRP78 is expressed at high levels in cancer cells due to ER/metabolic stress associated with long-term estrogen deprivation, GRP78 binds to and sequesters BIK through complex formation. With reduced binding to BIK, BCL-2 is able to suppress ER Ca²⁺ release, thereby suppressing apoptosis. This mechanistic explanation is consistent with another report implicating BCL-2 in the survival pathway activated by GRP78 overexpression⁴³. Considering that the apoptotic function of BIK has also been implicated in response to chemotherapy and proteasome inhibitors^{44,45}, this mechanism may also apply in these systems. In addition to being a regulator of apoptosis, GRP78 is also identified as a regulator of autophagy due to its role in maintaining ER integrity and homeostasis¹².

Evidence is accumulating that GRP78 is critical for cell proliferation. Genetic knockout of *Grp78* leads to a much reduced embryonic cell proliferation as well as massive apoptotic death of the inner cell mass, leading to embryonic lethality at day E3.5 (ref. 46). This provides the first hint that GRP78 may be important for pluripotent cell homeostasis and survival. In adult cells, GRP78 is expressed at low levels in the normal adult brain but is highly elevated in malignant glioma specimens and human malignant cell lines, correlating with their rate of proliferation, and knockdown of GRP78 by small interfering RNA leads to a slowdown in glioma cell growth³⁸. This is further supported by the observation that in an endogenous breast cancer model, GRP78 haploinsufficiency inhibits tumor cell proliferation⁴⁷. How might GRP78 facilitate cell proliferation? This could be due to the ER chaperone function of GRP78 in growth factor secretion and/or the maturation of growth factor receptors. Another major discovery is that ER stress actively promotes the localization of GRP78 to the cell surface⁴⁸, and selected cell types, notably cancer cells, express cell surface GRP78 which acts as a multifunctional receptor^{36,49}. One function of cell surface GRP78 is to enhance PI3K/AKT signaling, leading to both increased proliferation and survival. It is recently reported that ligation of prostate cancer cell surface GRP78 by activated κ -2-macroglobulin (κ -2-M) upregulates the prostate-specific antigen (PSA), which is then secreted into the medium and binds κ -2-M. The resultant PSA/ κ -2-M complex then binds to cell surface GRP78, causing activation of multiple kinase signaling pathways, coupling with an increase in proliferation and protein synthesis⁵⁰. Additionally, GRP78 and Cripto can form a complex at the cell surface and collaborate to inhibit transforming growth factor β -signaling and enhance cell growth⁵¹. Cell surface GRP78 is also reported to complex with Cripto in adult hematopoietic stem cells (HSCs) and maintains them in the hypoxic endosteal niche in the BM⁵².

GRP78 is required for solid tumor progression

Fast proliferating solid tumors are characterized with increasing hypoxia, nutrient starvation and acidosis, factors that activate the UPR. Correspondingly, GRP78 has been reported to be upregulated in solid tumors in various organs including breast, liver, gastric, esophagus, brain, prostate, head and neck and melanoma, correlating with aggressive tumor behavior and recurrence^{5,18,33,38,53–57}. While xenograft studies with knockdown of GRP78 supported its requirement for tumor formation and progression⁵⁸, the pathophysiologic role of GRP78 in tumorigenesis has been directly validated using the heterozygous *Grp78* mice and mouse models of conditional knockout of the *Grp78* in specific tissues. These studies revealed that without affecting mouse growth rate, organ development, and antibody production, GRP78 haploinsufficiency prolongs the latency period and retards the progression of the oncogene-induced mammary tumors in the MMTV-PyVT breast adenocarcinoma mouse model⁴⁷. The underlying mechanisms for this suppression include lowered tumor proliferation, increased apoptosis and dramatically reduced tumor angiogenesis. Likewise, heterozygous or homozygous deletion of *Grp78* specifically in the mouse prostate epithelium suppresses prostate tumorigenesis mediated by a loss of the tumor suppressor gene PTEN without affecting postnatal prostate development and growth⁵⁹. Strikingly, AKT activation in the PTEN-null prostate epithelium was potently suppressed by the loss of GRP78, and a similar suppression of stress-mediated AKT activation was observed in human prostate cancer cells where GRP78 was knocked down by siRNA. Since PTEN mutation and AKT activation are key drivers of human cancer, inactivation of GRP78 may represent a novel approach to block tumorigenesis resulting from loss of PTEN tumor suppression or activation of oncogenic AKT, or both.

Cancer initiating cells (CICs) with self-renewal capacity have recently been implicated as the root cause of cancer metastasis, therapeutic resistance, and recurrence. GRP78 has been reported to be highly elevated in breast disseminated tumor cells, which shared similar biological properties of CICs⁶⁰. In agreement, differential systemic analysis revealed elevated GRP78 expression in head and neck cancer initiating cells (HN-CICs)⁵⁶. This same study also reported that such cells with cell surface expression of GRP78 contain cancer stemness properties of self-renewal, differentiation and radioresistance; whereas knockdown of GRP78 by shRNA promoted HN-CIC cell differentiation and apoptosis and impaired their tumorigenic properties both *in vitro* and *in vivo*. These observations, though still at an early stage, suggest that GRP78 could play a critical role in regulating CIC proliferation and survival, as well as in stem cell biology in general, as evidenced by the previous observation that homozygous knockout of *Grp78* led to massive apoptosis of cells within the inner cell mass which are precursors of embryonic stem cells⁴⁶.

GRP78 is an effector for leukemogenesis and AKT oncogenic signaling

While GRP78 is established to protect cancer cells against the adverse hypoxic and nutrient-deprived microenvironment of solid tumors, its role in the initiation and progression of hematologic cancers is just emerging. Proteomic analysis reveals that GRP78 is differentially expressed in the HSC-like fractions from the BM of leukemic patients⁶¹. In addition to being a tumor suppressor, PTEN also contributes to the maintenance of the HSCs. Induced deletion of PTEN in the hematopoietic system of postnatal mice exhausted normal HSCs and promoted exhaustive proliferation of leukomogenic stem cells, resulting in the development of myeloproliferative disorders and eventually leukemia^{62,63}. These studies further showed that the mTOR inhibitor rapamycin effectively suppressed growth of the leukemia initiating cells and prevented the exhaustion of normal HSCs. Through creation of a biallelic conditional knockout mouse model of GRP78 and PTEN in the hematopoietic system, it was demonstrated that GRP78 haploinsufficiency potently suppresses

leukemogenesis and AKT/mTOR signaling in PTEN null BM cells²³. Importantly, *Grp78* heterozygosity by itself has no apparent effect on development or survival, nor does it alter total BM cell number or HSC population^{23,46,47}. Thus, partial GRP78 expression is sufficient to maintain normal organ homeostasis whereas tumor progression requires an optimal level of GRP78, both in solid and blood tumors.

How might GRP78 contribute to the PTEN-null mediated leukemogenesis? While GRP78 is able to confer multiple anti-apoptotic effects on cancer cells, a key mechanistic explanation may be that the activation of AKT, a prominent effector that is activated by the loss of PTEN, is compromised by the reduction of GRP78 in the hematopoietic system. Thus, PTEN-null mediated AKT/mTOR signaling is potently suppressed in the BM of the *Pten* null *Grp78* heterozygous mice. It has been reported that ligation of cell surface GRP78 in human cancer cells with antibodies directed against its carboxyl domain suppresses PI3K/AKT/mTOR signaling⁶⁴. The observation that knockdown of GRP78 in leukemia cells suppresses serum-stimulated phosphorylation of the p85 regulatory subunit of PI3K and inhibits PI(3,4,5)P3 production further suggests that GRP78 regulates AKT activation through functional regulation of PI3K²³. Upregulation of Wnt signaling is also suggested to be associated with leukemogenesis⁶⁵. Hypoxia-induced ER stress was reported to inhibit normal Wnt protein processing and secretion as ER stress causes dissociation between GRP78 and Wnt which is essential for its correct posttranslational processing⁶⁵. Hence, the delayed onset of leukemogenesis observed in the *Pten* null, *Grp78* heterozygous mice may be partially attributed to the attenuation of Wnt protein processing due to GRP78 knockdown, which awaits further investigation. The expression of anti-apoptotic protein BCL-2 has been implicated in hematologic malignancies⁶⁶. Considering that GRP78 level may influence the binding of pro-apoptotic BH-3 protein BIK to BCL-2 (ref. 41), one speculation is that the suppressed leukemic phenotype in the *Pten* null, *Grp78* heterozygous mice may in part be due to modulation of the BCL-2 activity. While the role of GRP78 in the development of human leukemia remains to be validated, emerging evidence shows elevated *Grp78* mRNA and protein expression in patient leukemic blasts compared to normal controls, notably in AML and in high grade B-lineage lymphoid malignancies^{21-23,67}. With the *Grp78* knockout leukemic mouse model providing proof-of-principle that a partial reduction of GRP78 can arrest leukemogenesis while having no harmful effect on the hematopoietic system, targeting GRP78 may represent a novel therapeutic target against leukemia and other stem cell related diseases.

GRP78 is required for angiogenesis in the tumor microenvironment

The tumor microenvironment contains a plethora of cells that support tumor growth and progression. Tumor vasculature is essential for tumor growth and metastasis, as it supplies the nutrients and oxygen that are critical for the growth and maintenance of the tumor. Endothelial cells are therefore the requisite members of the tumor microenvironment. The first hint that GRP78 may play a critical role in tumor angiogenesis is the constitutive high-level expression of GRP78 within the tumor vasculature of glioblastoma, suggestive of the constant state of stress of these activated tumor associated endothelial cells⁶⁸. Multiple lines of evidence support the critical requirement of GRP78 for tumor neoangiogenesis. In the MMTV-PyVT transgene induced mammary tumor model, *Grp78* heterozygosity showed a dramatic reduction in the microvessel density (MVD) of endogenous mammary tumors, while having no effect on the MVD of normal organs⁴⁷. Wild-type syngeneic mammary tumor cells injected into *Grp78* heterozygous host mice showed suppressed tumor growth and the early phase of angiogenesis. As neoangiogenesis is critical for supporting metastatic growth, injection of WT, syngeneic melanoma cells in the *Grp78* heterozygous host mice also resulted in potent suppression of pulmonary metastatic lesions⁶⁹. Furthermore, creation of a conditional heterozygous deletion of GRP78 in the host endothelial cells showed a

severe reduction of tumor angiogenesis and metastatic growth, again with minimal effect on normal tissue MVD⁶⁹. Knockdown of GRP78 expression by siRNA in immortalized human endothelial cells revealed that GRP78 regulates endothelial cell proliferation, survival and migration⁶⁹. While intracellular GRP78 can contribute to tumor angiogenesis, other forms of GRP78 may also be important. Cell surface GRP78 is induced by VEGF and is required for its angiogenic signaling⁷⁰. Interestingly, some tumor cell lines are capable of secreting a high amount of GRP78 into the tumor microenvironment. By binding to the cell surface receptors of endothelial cells, extracellular GRP78 activates the ERK and AKT pathways, and protects endothelial cells from anti-angiogenic effects of bortezomib³⁵. Angiogenesis inhibitors targeting VEGF and other pro-angiogenic growth factor pathways, while showing antitumor effects, concomitantly elicit tumor adaptations leading to accelerated metastasis^{71,72}. Considering that GRP78 regulates a much larger repertoire of cellular function than a single pro-angiogenic pathway and is a key modulator of cellular adaptation to stress, targeting GRP78 may offer a new approach to dually suppress tumor growth and angiogenesis. The role of GRP78 in the microenvironment may well extend beyond the endothelial cells. The observation that GRP78 expression is upregulated in tumor-associated macrophages but not in normal organs⁷³ suggests that stromal cells supporting tumor growth are likely to experience stress associated with the tumor microenvironment and require GRP78 for proliferation, survival and migration. Thus, suppression of the stress induction of GRP78 may then have the added benefit of also impeding the ability of tumor-associated macrophages and other stromal cells to support tumor growth and this warrants future investigation.

Targeting GRP78 sensitizes cancer cells to therapy

Therapeutic resistance remains a major challenge in the treatment of cancer. While this issue is complex and involves multiple mechanisms, the induction of the protective elements of the UPR, such as GRP78, in both the tumor and the tumor microenvironment, particularly at the necrotic borders that are highly chemoresistant, can be a major contributing factor^{18,32,73}. In agreement, an elevated expression of GRP78 correlates with resistance to a wide range of therapies including chemotoxic, anti-hormonal, DNA damaging and anti-angiogenesis agents in a variety of cancers, in both proliferating and dormant cancer cells as well as tumor associated endothelial cells and inversely, knockdown of GRP78 sensitizes the cells to these treatments^{27,38,39,68,74}. As the role of intracellular and cell surface GRP78 in cancer therapy and drug resistance has been summarized in previous reviews^{5,33}, here we focus on some new, unanticipated findings.

Histone deacetylase (HDAC) inhibitors represent a new class of anti-cancer compounds with great therapeutic potential through their ability to modulate the expression of both histone and non-histone genes resulting in growth arrest, increased differentiation and apoptosis⁷⁵. However, this treatment could also lead to resistance that is not well understood. It was recently discovered that HDAC inhibitors specifically induce GRP78 without concomitantly inducing an ER or heat shock stress response⁷⁶. This is due to HDAC1 binding to and acting as a repressor for the basal transcription of *Grp78* and HDAC inhibitors such as trichostatin A and MS-275 relieve this suppression. Overexpression of GRP78 confers resistance to HDAC inhibitor induced apoptosis, whereas knockdown of GRP78 sensitizes cancer cells to the treatment. This provides an example that drugs that do not induce ER stress can still upregulate GRP78 through mechanisms distinct from the UPR. Another interesting observation is that in the case of estrogen-receptor positive breast cancer cells, while estrogen starvation initially lowers GRP78 expression, prolonged exposure to this adverse condition, which mimics the action of aromatase inhibitors, leads to the upregulation of GRP78, associating with hormonal resistance, which can be suppressed by the knockdown

of GRP78 (ref. 41). In prostate cancer, GRP78 upregulation associates with androgen receptor status and the development of castration resistance and recurrence^{53,57}.

With regard to drug resistance in leukemia, PI3K/AKT is constitutively active in primary AML cells from patients and blocking PI3K with inhibitor (LY294002) potentiates the response to AraC⁷⁷. GRP78 is required for PI3K/AKT signaling in leukemia cells and modulation of GRP78 expression alters sensitivity to AraC²³. Since knockdown of GRP78 by siRNA downregulates both intracellular and cell surface GRP78 (ref. 69), both forms of GRP78 could contribute to AraC resistance, with mechanisms involving cell surface GRP78 promoting AKT survival signaling and intracellular GRP78 suppressing caspase-7 activation by AraC²³. Resistance of B-ALL cells to the anti-leukemic drug vincristine was suppressed by (–)-epigallocatechin gallate (EGCG), which inhibits the anti-apoptotic function of GRP78 by targeting its ATP-binding domain^{67,78}. Chemosensitive B-ALL cells underwent apoptosis when exposed to a doxorubicin-conjugated penetrating cyclic anti-GRP78 peptide that targets cell surface GRP78 (ref. 67). There is also new evidence linking GRP78 overexpression to early relapse in childhood ALL²³, as well as GRP78 overexpression in relapsed ALL compared to initial diagnosis⁶⁷. Furthermore, ER stress induces alternative splicing of the *Grp78* transcript, leading to the production of a cytosolic isoform of GRP78 (GRP78va) that also protects leukemic cells from ER stress-induced cell death⁷⁹.

Collectively, these discoveries identify GRP78 as a novel therapeutic target against chemoresistance in cancer cells. In agreement, recent studies showed that therapies capable of reducing the level of GRP78 in cancer cells are effective in enhancing cancer cell death, sensitizing them to chemotherapy treatment and reducing xenograft roles^{5,33,67,80–85}.

Cell surface GRP78 is a novel anti-cancer target

Evidence is emerging that a subfraction of GRP78 can be localized on the cell surface of cancer cells as well as cells undergoing ER stress^{36,48}. The preferential expression of GRP78 on the surface of tumor cells *in vivo* enables specific tumor targeting by peptidic GRP78 ligands linked to cytotoxic agents for anti-cancer therapy without harmful effects on normal organs^{86–89}. This is consistent with a report that a phage display-derived human monoclonal antibody that recognizes GRP78 binds strongly to multiple types of cancer cells, but shows weak or no binding to the corresponding normal tissues⁹⁰. However, it should be cautioned that studies on cell surface GRP78 expression performed in tissue culture show much variability among different cell lines³⁶. This is probably in part due to the inherent differences among cell lines that have been maintained long term in tissue culture. In addition, the stressful conditions of tissue culture may artificially induce cell surface localization of GRP78 in non-transformed cells. Another possibility is rapid endocytosis of cell surface GRP78 making its detection by acute methods not being reliable. Thus far, *in vivo* studies consistently showed preferential expression of surface GRP78 in tumors compared to normal tissues. A human monoclonal IgM antibody (SAM6) that was isolated from a gastric cancer patient bound to an *O*-linked glycosylated form of cell surface GRP78 in malignant cells and induced intracellular lipids accumulation prior to apoptosis induction in cancer cells⁹¹. Currently, a Phase I melanoma study is being conducted to test the efficacy of this antibody. Furthermore, hypoxic/stressed endothelial cells express cell surface GRP78, which is induced by VEGF⁷⁰. Taken together, these findings raise the exciting idea that agents against cell surface GRP78 can dually target tumor cells and tumor vasculature while sparing normal organs.

Cell surface calreticulin and immunogenic chemotherapy

Calreticulin (CRT) is an evolutionarily conserved 46 kDa ER luminal protein traditionally regarded as a Ca²⁺ homeostasis regulator and an ER chaperone^{92–95}. Additionally, CRT

regulates other cellular processes, including cell adhesion, MHC class I molecules assembly and hormone-sensitive gene expression. While the majority of CRT resides in the ER, evidence from various studies indicates that CRT is also found on the cell surface and can be secreted from cells under specific conditions^{92,96}.

Cell surface CRT induces immunogenic tumor cell death

CRT was demonstrated to be expressed on the surface of tumor cells treated with apoptotic inducing agents such as anthracyclines, oxaliplatin, and ionizing irradiation^{97,98}. Additionally, cell surface CRT (ecto-CRT) functions as an “eat me” signal that is recognized by dendritic cells (DC), and elicits DC-mediated phagocytosis of tumor cells⁹⁹. Phagocytosis leads to increased tumor antigen cross-presentation, which activates cytotoxic T cells and triggers an antitumor immune response and immunogenic tumor cell death. In human cancer samples, ecto-CRT expression is enhanced in both solid tumors and hematologic malignancies when compared to the corresponding normal tissues, which could possibly explain the increased susceptibility of tumors in immune-based cancer therapy compared to normal cells¹⁰⁰. In acute myeloid leukemia patients, the presence of ecto-CRT on leukemia blast cells undergoing anthracyclin treatment positively correlates with the cellular anti-cancer immune response¹⁰¹.

The recent discovery that CRT is presented on the cell surface offers a new anti-cancer therapeutic strategy based on ecto-CRT mediated immunogenic chemotherapy¹⁰². For example, human colon cancer cells that were treated with anthracycline in culture, and then injected into mice, prevented the tumor formation induced by the administration of non-anthracycline treated colon cancer cells. Furthermore, intratumoral injection of recombinant CRT combined with the chemotherapeutic agent Mitomycin C cured already established tumors in the same mouse model¹⁰². These observations were also confirmed in fibrosarcoma and mammary carcinoma cells. Importantly, this tumor immunity was specific against autologous tumors, as opposed to unrelated tumors¹⁰². Interestingly, the immunogenic tumor cell death induced by ecto-CRT appears to require both cell surface CRT and apoptotic signals. CRT exposure driven by ER stress restores the immunogenicity caused by cisplatin, an apoptosis inducer that fails to induce CRT exposure by itself¹⁰³. To induce immunogenicity, ecto-CRT must present on the surface of apoptotic cells, as opposed to the surface of adjacent live cells. Recombinant CRT that was absorbed onto the surface of tumor cells could trigger immunogenic tumor cell death when substituted for endogenous CRT that has translocated to the cell surface^{99,102}, demonstrating the direct requirement of cell surface CRT in triggering immunogenic tumor cell death. Collectively, such tumor specificity raises the possibility of personalized tumor vaccination where primary tumor cells from a patient could be treated with anthracyclines *ex vivo*, and subsequently injected back into the patient in order to both eradicate existing tumor cells and to induce long-term antitumor immunity.

Cell surface CRT exposure requires PERK activation and apoptotic effectors

The cell surface exposure of CRT in immunogenic cell death results from the translocation of endogenous CRT and is unrelated to the enhanced expression of CRT within the cell¹⁰⁴. Upon anthracycline treatment, only CRT and ERp5 are detected on the surface of tumor cells while other ER chaperones, including GRP78, GRP94, PDI or CNX are not. ERp57 and CRT form a complex in the lumen of the ER, and translocate together to the cell surface^{105,106}. The translocation process requires the activation of PERK, but not IRE1 or ATF6. Blocking the function of PERK-eIF2 activation using PERK-specific shRNA or non-phosphorylatable eIF2 -S51A mutant abolished CRT translocation, whereas inhibiting

the function of GADD34 and PP1 complex, which is involved in eIF2 dephosphorylation, enhanced the surface exposure of CRT^{98,103,104,107}. However, ER stress alone does not induce CRT/ERp57 translocation; additional apoptotic effectors are required. Caspase-8 cleavage downstream of PERK-activated p-eIF2 phosphorylation is required for the cleavage of an ER-sessile protein called Bap31. Bap31 is a Bcl-2 binding protein and cleaved Bap31 binds to Bcl-2 (ref. 108). This binding may compete with the binding of Bcl-2 to Bax, and could potentially lead to the activation of Bax and Bak, which serve as the apoptotic effectors required for CRT/ERp57 translocation downstream of PERK¹⁰⁴. The physical translocation of CRT/ERp57 to the cell surface can be blocked by the anterograde protein transport inhibitor brefeldin A, the actin skeleton inhibitor latrunculin B, or the knockdown of SNAREs, suggesting a role for the ER-Golgi secretory pathway¹⁰⁴. Nonetheless, the precise roles of Bap31, Bax and Bak, or how CRT/ERp57 translocates from the ER to the cell surface have not been well elucidated.

The role of GRP94 and other chaperones in antitumor vaccination

GRP94, also known as gp96, is a major glycoprotein in the ER with functions beyond protein folding and processing⁴. GRP94 plays an important role in immune responses by facilitating MHC class I molecule mediated antigen presentation, and by inducing the maturation and activation of various cells involved in innate and adapted immune responses, including macrophages, DCs, T cells and B cells^{109–111}. Therefore, it might be expected that GRP94 could elicit an antitumor response. Indeed, several studies demonstrated that a vaccination of lethally irradiated cancer cells expressing various non-ER-retainable autologous GRP94 fusion proteins (devoid of the ER retention/retrieval signal KDEL) protected mice from primary tumor growth as well as metastasis^{112–114}. One explanation for this effect could be that autologous tumor-derived secretory GRP94 stimulates the maturation of macrophages and DCs, enhances the antigen cross-presentation and amplifies the inflammatory signals^{109,110}. As an alternative to autologous GRP94, a pooled GRP94 vaccine derived from different multiple myeloma cells was shown to be as effective as the autologous GRP94 vaccine when tested in mouse models¹¹⁵. Taken together, these studies demonstrate that the use of GRP94 as an antitumor vaccination can prevent cancer development. However, GRP94 vaccination in mice appears less promising in the treatment of established tumors^{114,116,117}, and clinical trials across various types of tumors suggest that autologous tumor-derived GRP94 vaccination may have limited efficacy^{118–121}.

Tumor-derived GRP78 is also capable of eliciting an antitumor response and inhibiting tumor growth in mouse models with established tumors as well as inducing an antitumor memory response in mice rechallenged with the tumor cells¹²². One explanation for this effect could be that KDEL-deleted secretable GRP78 is recognized by DCs and cross-presented with associated antigens by MHC class I molecules, leading to the activation of cytotoxic T cells. Additionally, heat shock proteins HSP70, HSP110 and GRP170 derived from tumors have been shown to function as antitumor vaccines^{123–126}. Evidence suggests that vaccination with mixed heat shock proteins or heat shock proteins with other antigens may increase the antitumor effect. In a mouse model injected with sarcoma cells, tumor regression and long-term survival is shown in the mice treated with a mixed heat shock protein antitumor vaccine (GRP94, HSP60, HSP70 and HSP110)¹²⁷.

The role of PDI in tumorigenesis

Protein disulfide isomerase (PDI) is a thiol-disulfide oxidoreductase that is recognized for catalyzing the formation of disulfide bonds in newly synthesized proteins in the lumen of the ER^{128–130}. PDI is also found in the cytosol, nucleus, on the cell surface, and can be secreted¹³¹ (Figure 3). Studies on the role of PDI in cancer have demonstrated a pro-

oncogenic, pro-survival function for PDI in cancer and therapeutic resistance. Similar to other ER chaperones, PDI upregulation in response to ER stress helps ameliorate misfolded proteins and stress-induced apoptosis^{132,133}. Thus, inhibiting PDI activity sensitizes cells to stress-induced apoptosis. Treatment of melanoma cells with the PDI inhibitor bacitracin enhanced the stress response and apoptosis in the presence of chemotherapeutic drugs¹³⁴. PDI is highly expressed in invasive glioma cells and the invasive edge of human glioblastomas. Furthermore, suppression of PDI activity with an anti-PDI antibody, or the specific PDI inhibitor bacitracin, reduced tumor cell migration and invasion *in vitro*¹³⁵. Beyond these roles in tumor progression, PDI may also be important for tumor formation. A recent study demonstrated a novel role of PDI in AML tumorigenesis, in which PDI suppressed the expression of CEBPA by binding to the stem loop region of its mRNA, and therefore blocking normal neutrophil differentiation¹³⁶. PDI has also been reported to induce antibody production and evoke a potent humoral immune response in GM-CSF secreting cancer cells¹³⁷. Therefore, because PDI is expressed on the surface of various murine and human cancer cell lines, and plays important roles in tumorigenesis, it presents a potential immune target which may enhance the efficacy of anti-cancer therapy^{135,137}.

The role of PERK in tumorigenesis

PERK is an ER transmembrane serine/threonine protein kinase that is activated in response to ER stress^{1,2}. Upon UPR activation, PERK phosphorylates its two major substrates: eukaryotic translation initiation factor 2 (eIF2) and nuclear factor-like 2 (Nrf2)¹³⁸ (Figure 1). The phosphorylation of eIF2 acts to attenuate global protein translation, while at the same time enhancing the translation of select mRNAs, such as ATF4 (ref. 139). The phosphorylation of Nrf2 activates the expression of enzymes that are required for ROS quenching^{140,141}.

PERK plays a critical role during fetal development since the PERK^{-/-} mice that survive are characterized by severe postnatal growth retardation, skeletal dysplasia and neonatal diabetes, all of which are also symptoms presented in the human Wolcott-Rallison syndrome¹⁴². PERK is required for osteoblast proliferation, differentiation and bone matrix collagen I secretion¹⁴³. PERK is also required for pancreatic β cell development. Despite displaying a morphologically and functionally normal pancreas at birth, mice with global PERK knockout rapidly developed hyperglycemia and died^{142,144,145}. It was demonstrated that β cell development occurs at E13.5–16.5, and impaired β cell proliferation and differentiation was observed in PERK^{-/-} mice from day E13.5 to 18.5 (ref. 145). Interestingly, mice with β cell specific PERK knockout expressed postnatally are normoglycemic and maintain glycemia throughout the lifespan¹⁴⁵. Taken together, these studies suggest that the defect of PERK^{-/-} β cell development at the embryonic stage is permanent such that when β cells begin to play an important role in regulating blood glucose, the PERK deficiency starts to exert its effect and eventually leads to the hyperglycemic and neonatal diabetic phenotypes.

Dual role of PERK in tumor proliferation and survival

In the case of tumorigenesis and cancer progression, PERK exhibits both pro- and antitumor properties. PERK promoted tumor survival and angiogenesis under conditions of ER stress caused by hypoxia, as was demonstrated in a xenograft tumor model derived from K-Ras or Ki-RasV12 transformed Per^{-/-} mouse embryonic fibroblasts (MEFs)^{146,147}. Similar observations were made in a xenograft model using human colorectal carcinoma cells expressing a dominant negative PERK¹⁴⁶. In these models, PERK-deficient tumors showed increased apoptosis in hypoxic regions, were poorly vascularized, and these effects were attributed to the loss of eIF2 and ATF4. The requirement of PERK in tumor angiogenesis

was further confirmed using a mouse PERK^{-/-} insulinoma model, in which PERK^{-/-} tumors had lower vascularity compared to size-matched PERK^{+/+} tumors¹⁴⁸. PERK is not the only kinase that can phosphorylate eIF2 and regulate cell survival. GCN2 is another eIF2 kinase demonstrated to work in parallel to PERK to regulate eIF2-mediated cell survival under conditions of hypoxia and nutrient deprivation in immortalized MEFs, human fibrosarcoma, and colorectal adenocarcinoma cells¹⁴⁹⁻¹⁵¹. Mechanisms by which eIF2 phosphorylation promoted cell survival were attributed to cell cycle G1 arrest, mediated by the downregulation of cyclin D1 and the induction of HIF-1 and p21^{WAF1} (refs. 149,150), and autophagy, mediated by ATF4 and CHOP¹⁵²⁻¹⁵⁴. Moreover, it has been shown that the expression of asparagine synthetase (ASNS), which is a downstream target of ATF4, partially restored growth in tumors with compromised ATF4 function¹⁵¹. Although phosphorylation of eIF2 attenuates protein translation and protects cells from apoptosis under ER stress, under severe stress such as bortezomib treatment, the downstream pro-apoptotic factor CHOP was activated and the cancer cells underwent apoptosis¹⁵⁵. Bortezomib-resistant multiple myeloma cells displayed attenuated eIF2 phosphorylation, and became susceptible when eIF2 dephosphorylation was inhibited.

The role of PERK in cell proliferation has been controversial. In early studies, PERK-eIF2 was indicated to function as a barrier in the transformation of normal cells to cancer cells. Expression of a nonphosphorylatable eIF2 mutant led to the transformation of mouse fibroblasts, and facilitated the transformation of human fibroblasts in conjunction with hTERT and the SV40 large T-antigen^{156,157}. Non-tumorigenic mammary epithelial cells with a dominant negative PERK or a kinase dead PERK mutant showed increased proliferation, displayed hyperplastic growth, and developed into small tumor nodules after orthotopic implantation¹⁵⁸. In human squamous carcinoma cells, pharmacologically induced PERK activation caused growth arrest *in vitro* and suppressed tumor growth *in vivo*¹⁵⁹. This study implies that PERK activation in established tumors is sufficient to inhibit tumor proliferation. This suppression of tumor cell proliferation by PERK may be attributed to the eIF2-mediated inhibition of cyclin D1 synthesis^{26,149,159}. However, other studies indicate that PERK is required for cancer cell proliferation. PERK^{-/-} insulinoma induced by -cell specific expression of SV40 large T-antigen displayed reduced proliferation compared to PERK^{+/+} controls¹⁴⁸. Cell cycle arrest was observed upon PERK knockdown by shRNA in human breast carcinoma and esophageal carcinoma cells via reduced Nrf2 activity. The same study demonstrated that mammary gland specific PERK deletion delayed tumor onset in MMTV-Neu breast cancer mice¹⁶⁰. Phosphorylated Nrf2 activates the expression of enzymes required for ROS elimination. Therefore, reduced Nrf2 activity leads to ROS accumulation which causes cell cycle arrest mediated by increased DNA damage. Collectively, these observations demonstrate the complexity and specificity of PERK in tumorigenesis and cancer progression, and illustrate that the role of PERK is dependent on its expression and activation, as well as cancer cell type, proliferation status, tumor microenvironment, and the model system studied. Therefore, one can imagine the challenges involved in targeting PERK as an effective and generalized anti-cancer therapy. For instance, the suppression of tumor growth by either over-activation or depletion of PERK might also drive proliferative cancer cells into dormancy and thereby protect them from chemotherapy.

The role of PERK in autophagy

Autophagy can be activated upon ER stress as a defensive mechanism for maintaining ER homeostasis^{10,16,161}. Depending on the context, autophagy either enhances cell survival by counterbalancing ER expansion or commits the cell to non-apoptotic death¹⁶². Studies have shown that autophagy alleviated ER stress-induced cell death in cancer cells while promoting cell death in non-transformed cells¹⁰. As two important mechanisms regulating

ER homeostasis, the UPR and autophagy are coupled via the PERK-eIF2 β -ATF4 pathway. The requirement of the PERK-eIF2 β -ATF4 pathway in mediating autophagy is shown in different models, in which autophagy is induced by polyglutamine, radiation, ECM detachment and hypoxia^{17,152–154,163}. In response to ER stress, PERK phosphorylates eIF2 and activates its downstream transcription factor ATF4, which induces the expression of another transcription factor CHOP. Studies have shown that ATF4 and CHOP bind to the promoter of two genes essential for autophagy. ATF4 activates the expression of *MAP1LC3B*, while CHOP activates the transcription of *ATG5* (ref. 152). MAP1LC3B and ATG5 are two essential proteins for autophagy. ATG5-dependent conversion of free LC3-I to membrane-bound LC3-II is an essential step for autophagosome formation¹⁷, whereas MAP1LC3B induction is necessary for replenishing the rapid degradation of MAP1LC3B during the prolonged autophagy¹⁵². Interestingly, ATF4 also induces autophagy in a PERK-independent manner. The induction of autophagy by Bortezomib treatment was dependent on the proteasome stabilization of ATF4 and the upregulation of LC3B by ATF4 (ref. 164). However, it was also suggested that the UPR and autophagy are linked by IRE1, rather than PERK. The accumulation of LC3-positive vesicles triggered by tunicamycin or thapsigargin was shown to depend on IRE1 but not PERK or ATF6, using MEFs or embryonic stem cells deficient for IRE1 α , ATF6 or PERK¹⁶.

The role of IRE1-XBP1 axis in tumorigenesis

XBP1 is a transcription factor downstream of the IRE1 α endonuclease^{165–167}. It has been shown to be essential for the development and function of various organs, especially secretory organs. XBP1 knockout mouse embryos died of liver failure and severe anemia. Mice with liver-specific overexpression of XBP1 were rescued from the embryonic lethality, but died shortly postnatal with impaired secretory apparatus including the pancreas and salivary glands¹⁶⁵. XBP1 and its splicing are also essential for plasma cell differentiation and antibody secretion^{7,168}.

XBP1 splicing is widely observed in both solid and hematological tumors, and enhanced XBP-1 splicing has been associated with more malignant phenotypes and poor survival^{169–171}. XBP1 activation was required for tumor survival in solid tumors that undergo hypoxic stress¹⁷². In breast cancer cells, XBP1 was shown to be a key factor affecting estrogen dependency. Overexpression of XBP1-s in estrogen-dependent breast cancer cell lines conferred an increased expression of estrogen receptors and estrogen-independent growth¹⁷³. In the case of virus-associated cancers such as the Epstein-Barr virus associated nasopharyngeal carcinoma, XBP1 was shown to bind to the promoter of EBV oncoprotein LMP1 and activate its expression¹⁷⁴. Other than promoting cancer cell survival and facilitating tumorigenesis along with other oncogenes, XBP1 splicing itself may lead to tumorigenesis, as the expression of XBP1-s alone in B cells and plasma cells drove the pathogenesis of multiple myeloma with features mimicking the classic hallmarks of human multiple myeloma¹⁷⁵.

Since XBP1 is the only known substrate of IRE1 α ¹⁶⁷, studies on IRE1 α also shed light on the role of the IRE1 α -XBP1 pathway in tumorigenesis. Using prostate cancer cell lines with IRE1 α mutants that are either kinase-defective or with drug-inducible RNase activity, IRE1 α was reported to promote cell proliferation *in vitro* by regulating the expression of cyclin A1 through XBP1 splicing¹⁷⁶. In a xenograft model with glioma cells expressing a dominant-negative IRE1 α (dnIRE1 α), dnIRE1 α expressing cancer cells had a lower proliferation rate *in vitro* and *in vivo*¹⁷⁷. This xenograft model also revealed that IRE1 α was required for angiogenesis and functioned as a key switch between the angiogenesis and the invasion of glioma. Wild-type gliomas showed the angiogenic/massive phenotype whereas tumors expressing dnIRE1 α displayed the avascular/diffuse phenotype. Inhibition of IRE1

function diminished the expression of potent pro-angiogenic factors, leading to a compensatory response in glioma cells to migrate and hijack host blood vessels in response to their poor angiogenesis ability. The requirement of IRE1 in tumor angiogenesis under hypoxia or glucose deprivation could be attributed to its role in regulating VEGF-A expression¹⁷⁸. However, the link between the IRE1-XBP1 axis, angiogenesis, and tumor growth can also occur in a VEGF-independent manner. It was shown that secretion of VEGF was not compromised when XBP1 was inhibited using an shRNA approach^{172,179}. Additionally, IRE1 was recently reported to regulate the survival/apoptosis switch in the UPR¹⁸⁰. Under mild ER stress conditions, IRE1 preferentially splices XBP1 to promote cell survival; while under conditions of overwhelming stress or chronic ER stress, it causes endonucleolytic decay of ER-localized mRNA, including those encoding ER chaperones such as GRP78, and renders apoptosis¹⁸⁰⁻¹⁸².

The important role of the IRE1-XBP1 pathway in cancer progression presents a potential therapeutic target in anti-cancer therapy. Recently a small-molecule inhibitor targeting the endonuclease activity of IRE1 was identified and tested in a multiple myeloma model¹⁸³. This inhibitor blocked XBP1 splicing induced by ER stress both *in vitro* and *in vivo*. Treatment with this small molecule inhibited the growth of human multiple myeloma cells xenografted in host mice, and displayed cytotoxicity specifically on the multiple myeloma cells rather than normal blood cells freshly isolated from patients. Along with this small molecule inhibitor, another IRE1 inhibitor that is salicylaldehyde-based was used in rhabdovirus mediated oncolytic viral therapy¹⁸⁴, in which natural or genetically engineered replicating viruses selectively targeted tumor cells based on their altered genetic properties¹⁸⁵. Blockage of the UPR by this IRE1 inhibitor sensitized cancer cells to apoptosis in response to rhabdovirus infection and dramatically improved the oncolytic efficacy¹⁸⁴.

Conclusion

While extensive cell culture studies have clearly implicated the contribution of ER chaperones and the UPR in cancer cell proliferation, survival and therapeutic resistance, the fruition of mouse models of cancer which allowed for the dissection of the physiological roles of various components of the UPR in relation to their effects on normal organ development and homeostasis validated their unique roles in tumorigenesis as well as in tumor angiogenesis. Evidence is also emerging that ER stress not only arms cancer cells with prosurvival adaptive pathways to combat the host surveillance mechanisms, it also promotes a vicious feedback cycle where cancer cells and stroma cells in the tumor microenvironment cross stimulate each other leading to tumor progression and metastasis. Knowledge of the involvement of ER stress and the UPR in the tumor microenvironment is at an early stage and offers exciting new opportunities for investigation. The discovery that major ER chaperones such as GRP78, GRP94, CRT and PDI all exhibit a cell surface form with activities distinct from those of the ER forms changes the paradigm of their function and expands their role in tumor progression and therapeutic resistance. While ER stress can be exploited to enhance tumor cell death, tumor cells are likely to acquire resistance to such therapy, in part by upregulating the pro-survival arms of the UPR such as the ER chaperones. With therapeutics targeting ER chaperones and UPR pathways currently in development, the next major breakthrough will come from directly testing whether these agents will be effective in reversing the course of cancer, either alone or in combination with existing therapy, and their impact on normal cell physiology.

Acknowledgments

B. L. is a recipient of the University of Southern California Norris Comprehensive Cancer Center Wang Scholarship in Cancer Research. We thank Costas Koumenis, Albert Koong, Kyle Pfaffenbach and Kate Ott for helpful discussions. This work was supported in part by National Institutes of Health grants CA027607 and 1 P01 AG034906 to A.S.L.

References

1. Rutkowski DT, Kaufman RJ. A trip to the ER: coping with stress. *Trends Cell Biol.* 2004; 14:20–28. [PubMed: 14729177]
2. Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol.* 2007; 8:519–529. [PubMed: 17565364]
3. Wu J, Kaufman RJ. From acute ER stress to physiological roles of the unfolded protein response. *Cell Death Differ.* 2006; 13:374–384. [PubMed: 16397578]
4. Ni M, Lee AS. ER chaperones in mammalian development and human diseases. *FEBS Lett.* 2007; 581:3641–3651. [PubMed: 17481612]
5. Wang M, Wey S, Zhang Y, Ye R, Lee AS. Role of the unfolded protein response regulator GRP78/BiP in development, cancer, and neurological disorders. *Antioxid Redox Signal.* 2009; 11:2307–2316. [PubMed: 19309259]
6. Diehl JA, Fuchs SY, Koumenis C. The cell biology of the unfolded protein response. *Gastroenterology.* 2011; 141:38–41. 41 e31–32. [PubMed: 21620842]
7. Calton M, Zeng H, Urano F, Till JH, Hubbard SR, Harding HP, et al. IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature.* 2002; 415:92–96. [PubMed: 11780124]
8. Lee AH, Iwakoshi NN, Glimcher LH. XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. *Mol Cell Biol.* 2003; 23:7448–7459. [PubMed: 14559994]
9. Teske BF, Wek SA, Bunpo P, Cundiff JK, McClintick JN, Anthony TG, et al. The eIF2 kinase PERK and the integrated stress response facilitate activation of ATF6 during endoplasmic reticulum stress. *Mol Biol Cell.* 2011; 22:4390–4405. [PubMed: 21917591]
10. Ding WX, Ni HM, Gao W, Hou YF, Melan MA, Chen X, et al. Differential effects of endoplasmic reticulum stress-induced autophagy on cell survival. *J Biol Chem.* 2007; 282:4702–4710. [PubMed: 17135238]
11. Yorimitsu T, Klionsky DJ. Endoplasmic reticulum stress: a new pathway to induce autophagy. *Autophagy.* 2007; 3:160–162. [PubMed: 17204854]
12. Li J, Ni M, Lee B, Barron E, Hinton DR, Lee AS. The unfolded protein response regulator GRP78/BiP is required for endoplasmic reticulum integrity and stress-induced autophagy in mammalian cells. *Cell Death Differ.* 2008; 15:1460–1471. [PubMed: 18551133]
13. Kruse KB, Brodsky JL, McCracken AA. Characterization of an ERAD gene as VPS30/ATG6 reveals two alternative and functionally distinct protein quality control pathways: one for soluble Z variant of human alpha-1 proteinase inhibitor (A1PiZ) and another for aggregates of A1PiZ. *Mol Biol Cell.* 2006; 17:203–212. [PubMed: 16267277]
14. Bernales S, McDonald KL, Walter P. Autophagy counterbalances endoplasmic reticulum expansion during the unfolded protein response. *PLoS Biol.* 2006; 4:e423. [PubMed: 17132049]
15. Momoi T. Conformational diseases and ER stress-mediated cell death: apoptotic cell death and autophagic cell death. *Curr Mol Med.* 2006; 6:111–118. [PubMed: 16472118]
16. Ogata M, Hino S, Saito A, Morikawa K, Kondo S, Kanemoto S, et al. Autophagy is activated for cell survival after endoplasmic reticulum stress. *Mol Cell Biol.* 2006; 26:9220–9231. [PubMed: 17030611]
17. Kourouk Y, Fujita E, Tanida I, Ueno T, Isoai A, Kumagai H, et al. ER stress (PERK/eIF2alpha phosphorylation) mediates the polyglutamine-induced LC3 conversion, an essential step for autophagy formation. *Cell Death Differ.* 2007; 14:230–239. [PubMed: 16794605]
18. Ma Y, Hendershot LM. The role of the unfolded protein response in tumour development: friend or foe? *Nat Rev Cancer.* 2004; 4:966–977. [PubMed: 15573118]

19. Khan MM, Nomura T, Chiba T, Tanaka K, Yoshida H, Mori K, et al. The fusion oncoprotein PML-RARalpha induces endoplasmic reticulum (ER)-associated degradation of N-CoR and ER stress. *J Biol Chem*. 2004; 279:11814–11824. [PubMed: 14701861]
20. Zhang Y, Soboloff J, Zhu Z, Berger SA. Inhibition of Ca²⁺ influx is required for mitochondrial reactive oxygen species-induced endoplasmic reticulum Ca²⁺ depletion and cell death in leukemia cells. *Mol Pharmacol*. 2006; 70:1424–1434. [PubMed: 16849592]
21. Tanimura A, Yujiri T, Tanaka Y, Hatanaka M, Mitani N, Nakamura Y, et al. The anti-apoptotic role of the unfolded protein response in Bcr-Abl-positive leukemia cells. *Leuk Res*. 2009; 33:924–928. [PubMed: 19237191]
22. Rosati E, Sabatini R, Rampino G, De Falco F, Di Ianni M, Falzetti F, et al. Novel targets for endoplasmic reticulum stress-induced apoptosis in B-CLL. *Blood*. 2010; 116:2713–2723. [PubMed: 20628148]
23. Wey S, Luo B, Tseng CC, Ni M, Zhou H, Fu Y, et al. Inducible knockout of GRP78/BiP in the hematopoietic system suppresses Pten-null leukemogenesis and AKT oncogenic signaling. *Blood*. 2012; 119:817–825. [PubMed: 21937694]
24. Ghosh R, Lipson KL, Sargent KE, Mercurio AM, Hunt JS, Ron D, et al. Transcriptional regulation of VEGF-A by the unfolded protein response pathway. *PLoS One*. 2010; 5:e9575. [PubMed: 20221394]
25. Pereira ER, Liao N, Neale GA, Hendershot LM. Transcriptional and post-transcriptional regulation of proangiogenic factors by the unfolded protein response. *PLoS One*. 2010; 5 pii: e12521.
26. Brewer JW, Diehl JA. PERK mediates cell-cycle exit during the mammalian unfolded protein response. *Proc Natl Acad Sci USA*. 2000; 97:12625–12630. [PubMed: 11035797]
27. Ranganathan AC, Adam AP, Zhang L, Aguirre-Ghiso JA. Tumor cell dormancy induced by p38SAPK and ER-stress signaling: an adaptive advantage for metastatic cells? *Cancer Biol Ther*. 2006; 5:729–735. [PubMed: 16861922]
28. Mahadevan NR, Rodvold J, Sepulveda H, Rossi S, Drew AF, Zanetti M. Transmission of endoplasmic reticulum stress and pro-inflammation from tumor cells to myeloid cells. *Proc Natl Acad Sci USA*. 2011; 108:6561–6566. [PubMed: 21464300]
29. Fels DR, Ye J, Segan AT, Kridel SJ, Spiotto M, Olson M, et al. Preferential cytotoxicity of bortezomib toward hypoxic tumor cells via overactivation of endoplasmic reticulum stress pathways. *Cancer Res*. 2008; 68:9323–9330. [PubMed: 19010906]
30. De Raedt T, Walton Z, Yecies JL, Li D, Chen Y, Malone CF, et al. Exploiting cancer cell vulnerabilities to develop a combination therapy for ras-driven tumors. *Cancer Cell*. 2011; 20:400–413. [PubMed: 21907929]
31. Rutkowski DT, Arnold SM, Miller CN, Wu J, Li J, Gunnison KM, et al. Adaptation to ER stress is mediated by differential stabilities of pro-survival and pro-apoptotic mRNAs and proteins. *PLoS Biol*. 2006; 4:e374. [PubMed: 17090218]
32. Li J, Lee AS. Stress induction of GRP78/BiP and its role in cancer. *Curr Mol Med*. 2006; 6:45–54. [PubMed: 16472112]
33. Lee AS. GRP78 induction in cancer: therapeutic and prognostic implications. *Cancer Res*. 2007; 67:3496–3499. [PubMed: 17440054]
34. Pfaffenbach KT, Lee AS. The critical role of GRP78 in physiologic and pathologic stress. *Curr Opin Cell Biol*. 2011; 23:150–156. [PubMed: 20970977]
35. Kern J, Untergasser G, Zenzmaier C, Sarg B, Gastl G, Gunsilius E, et al. GRP-78 secreted by tumor cells blocks the antiangiogenic activity of bortezomib. *Blood*. 2009; 114:3960–3967. [PubMed: 19713465]
36. Ni M, Zhang Y, Lee AS. Beyond the endoplasmic reticulum: atypical GRP78 in cell viability, signalling and therapeutic targeting. *Biochem J*. 2011; 434:181–188. [PubMed: 21309747]
37. Ouyang YB, Xu LJ, Emery JF, Lee AS, Giffard RG. Overexpressing GRP78 influences Ca²⁺ handling and function of mitochondria in astrocytes after ischemia-like stress. *Mitochondrion*. 2011; 11:279–286. [PubMed: 21047562]
38. Pyrko P, Schonthal AH, Hofman FM, Chen TC, Lee AS. The unfolded protein response regulator GRP78/BiP as a novel target for increasing chemosensitivity in malignant gliomas. *Cancer Res*. 2007; 67:9809–9816. [PubMed: 17942911]

39. Reddy RK, Mao C, Baumeister P, Austin RC, Kaufman RJ, Lee AS. Endoplasmic reticulum chaperone protein GRP78 protects cells from apoptosis induced by topoisomerase inhibitors: role of ATP binding site in suppression of caspase-7 activation. *J Biol Chem.* 2003; 278:20915–20924. [PubMed: 12665508]
40. Fu Y, Li J, Lee AS. GRP78/BiP inhibits endoplasmic reticulum BIK and protects human breast cancer cells against estrogen-starvation induced apoptosis. *Cancer Res.* 2007; 67:3734–3740. [PubMed: 17440086]
41. Zhou H, Zhang Y, Fu Y, Chan L, Lee AS. Novel mechanism of anti-apoptotic function of 78 kDa glucose-regulated protein (GRP78). *J Biol Chem.* 2011; 286:25687–25696. [PubMed: 21622563]
42. Rao RV, Peel A, Logvinova A, del Rio G, Hermel E, Yokota T, et al. Coupling endoplasmic reticulum stress to the cell death program: role of the ER chaperone GRP78. *FEBS Lett.* 2002; 514:122–128. [PubMed: 11943137]
43. Penas C, Font-Nieves M, Fores J, Petegnief V, Planas A, Navarro X, et al. Autophagy, and BiP level decrease are early key events in retrograde degeneration of motoneurons. *Cell Death Differ.* 2011; 18:1617–1627. [PubMed: 21436843]
44. Nikrad M, Johnson T, Puthalalath H, Coultas L, Adams J, Kraft AS. The proteasome inhibitor bortezomib sensitizes cells to killing by death receptor ligand TRAIL via BH3-only proteins Bik and Bim. *Mol Cancer Ther.* 2005; 4:443–449. [PubMed: 15767553]
45. Oppermann M, Geilen CC, Fecker LF, Gillissen B, Daniel PT, Eberle J. Caspase-independent induction of apoptosis in human melanoma cells by the proapoptotic Bcl-2-related protein Nbkl/Bik. *Oncogene.* 2005; 24:7369–7380. [PubMed: 16007125]
46. Luo S, Mao C, Lee B, Lee AS. GRP78/BiP is required for cell proliferation and protecting the inner cell mass from apoptosis during early mouse embryonic development. *Mol Cell Biol.* 2006; 26:5688–5697. [PubMed: 16847323]
47. Dong D, Ni M, Li J, Xiong S, Ye W, Virrey JJ, et al. Critical role of the stress chaperone GRP78/BiP in tumor proliferation, survival, and tumor angiogenesis in transgene-induced mammary tumor development. *Cancer Res.* 2008; 68:498–505. [PubMed: 18199545]
48. Zhang Y, Liu R, Ni M, Gill P, Lee AS. Cell surface relocalization of the endoplasmic reticulum chaperone and unfolded protein response regulator GRP78/BiP. *J Biol Chem.* 2010; 285:15065–15075. [PubMed: 20208072]
49. Gonzalez-Gronow M, Selim MA, Papalas J, Pizzo SV. GRP78: a multifunctional receptor on the cell surface. *Antioxid Redox Signal.* 2009; 11:2299–2306. [PubMed: 19331544]
50. Misra UK, Payne S, Pizzo SV. Ligation of prostate cancer cell surface GRP78 activates a proliferative and antiapoptotic feedback loop: a role for secreted prostate-specific antigen. *J Biol Chem.* 2011; 286:1248–1259. [PubMed: 21056970]
51. Shani G, Fischer WH, Justice NJ, Kelber JA, Vale W, Gray PC. GRP78 and Cripto form a complex at the cell surface and collaborate to inhibit transforming growth factor beta signaling and enhance cell growth. *Mol Cell Biol.* 2008; 28:666–677. [PubMed: 17991893]
52. Miharada K, Karlsson G, Rehn M, Rorby E, Siva K, Cammenga J, et al. Cripto regulates hematopoietic stem cells as a hypoxic-niche-related factor through cell surface receptor GRP78. *Cell Stem Cell.* 2011; 9:330–344. [PubMed: 21982233]
53. Potrakul L, Datar RH, Shi SR, Cai J, Hawes D, Groshen SG, et al. Expression of stress response protein Grp78 is associated with the development of castration-resistant prostate cancer. *Clin Cancer Res.* 2006; 12:5987–5993. [PubMed: 17062670]
54. Daneshmand S, Quek ML, Lin E, Lee C, Cote RJ, Hawes D, et al. Glucose-regulated protein GRP78 is up-regulated in prostate cancer and correlates with recurrence and survival. *Hum Pathol.* 2007; 38:1547–1552. [PubMed: 17640713]
55. Zhuang L, Scolyer RA, Lee CS, McCarthy SW, Cooper WA, Zhang XD, et al. Expression of glucose-regulated stress protein GRP78 is related to progression of melanoma. *Histopathology.* 2009; 54:462–470. [PubMed: 19309398]
56. Wu MJ, Jan CI, Tsay YG, Yu YH, Huang CY, Lin SC, et al. Elimination of head and neck cancer initiating cells through targeting glucose regulated protein78 signaling. *Mol Cancer.* 2010; 9:283. [PubMed: 20979610]

57. Tan SS, Ahmad I, Bennett HL, Singh L, Nixon C, Seywright M, et al. GRP78 up-regulation is associated with androgen receptor status, Hsp70-Hsp90 client proteins and castrate-resistant prostate cancer. *J Pathol.* 2011; 223:81–87. [PubMed: 21125667]
58. Jamora C, Dennert G, Lee AS. Inhibition of tumor progression by suppression of stress protein GRP78/BiP induction in fibrosarcoma B/C10ME. *Proc Natl Acad Sci USA.* 1996; 93:7690–7694. [PubMed: 8755537]
59. Fu Y, Wey S, Wang M, Ye R, Liao CP, Roy-Burman P, et al. Pten null prostate tumorigenesis and AKT activation are blocked by targeted knockout of ER chaperone GRP78/BiP in prostate epithelium. *Proc Natl Acad Sci U S A.* 2008; 105:19444–19449. [PubMed: 19033462]
60. Bartkowiak K, Effenberger KE, Harder S, Andreas A, Buck F, Peter-Katalinic J, et al. Discovery of a novel unfolded protein response phenotype of cancer stem/progenitor cells from the bone marrow of breast cancer patients. *J Proteome Res.* 2010; 9:3158–3168. [PubMed: 20423148]
61. Ota J, Yamashita Y, Okawa K, Kisanuki H, Fujiwara S, Ishikawa M, et al. Proteomic analysis of hematopoietic stem cell-like fractions in leukemic disorders. *Oncogene.* 2003; 22:5720–5728. [PubMed: 12944923]
62. Yilmaz OH, Valdez R, Theisen BK, Guo W, Ferguson DO, Wu H, et al. Pten dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells. *Nature.* 2006; 441:475–482. [PubMed: 16598206]
63. Zhang J, Grindley JC, Yin T, Jayasinghe S, He XC, Ross JT, et al. PTEN maintains haematopoietic stem cells and acts in lineage choice and leukaemia prevention. *Nature.* 2006; 441:518–522. [PubMed: 16633340]
64. Misra UK, Pizzo SV. Ligation of cell surface GRP78 with antibody directed against the COOH-terminal domain of GRP78 suppresses Ras/MAPK and PI 3-kinase/AKT signaling while promoting caspase activation in human prostate cancer cells. *Cancer Biol Ther.* 2010; 9:142–152. [PubMed: 20368692]
65. Verras M, Papandreou I, Lim AL, Denko NC. Tumor hypoxia blocks Wnt processing and secretion through the induction of endoplasmic reticulum stress. *Mol Cell Biol.* 2008; 28:7212–7224. [PubMed: 18824543]
66. Konopleva M, Zhao S, Hu W, Jiang S, Snell V, Weidner D, et al. The anti-apoptotic genes Bcl-X(L) and Bcl-2 are over-expressed and contribute to chemoresistance of non-proliferating leukaemic CD34+ cells. *Br J Haematol.* 2002; 118:521–534. [PubMed: 12139741]
67. Uckun FM, Qazi S, Ozer Z, Garner AL, Pitt J, Ma H, et al. Inducing apoptosis in chemotherapy-resistant B-lineage acute lymphoblastic leukaemia cells by targeting HSPA5, a master regulator of the anti-apoptotic unfolded protein response signalling network. *Br J Haematol.* 2011; 153:741–752. [PubMed: 21517817]
68. Virrey JJ, Dong D, Stiles C, Patterson JB, Pen L, Ni M, et al. Stress chaperone GRP78/BiP confers chemoresistance to tumor-associated endothelial cells. *Mol Cancer Res.* 2008; 6:1268–1275. [PubMed: 18708359]
69. Dong D, Stapleton C, Luo B, Xiong S, Ye W, Zhang Y, et al. A critical role for GRP78/BiP in the tumor microenvironment for neovascularization during tumor growth and metastasis. *Cancer Res.* 2011; 71:2848–2857. [PubMed: 21467168]
70. Katanasaka Y, Ishii T, Asai T, Naitou H, Maeda N, Koizumi F, et al. Cancer antineovascular therapy with liposome drug delivery systems targeted to BiP/GRP78. *Int J Cancer.* 2010; 127:2685–2698. [PubMed: 20178102]
71. Ebos JM, Lee CR, Cruz-Munoz W, Bjarnason GA, Christensen JG, Kerbel RS. Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis. *Cancer Cell.* 2009; 15:232–239. [PubMed: 19249681]
72. Paez-Ribes M, Allen E, Hudock J, Takeda T, Okuyama H, Vinals F, et al. Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. *Cancer Cell.* 2009; 15:220–231. [PubMed: 19249680]
73. Dong D, Dubeau L, Bading J, Nguyen K, Luna M, Yu H, et al. Spontaneous and controllable activation of suicide gene expression driven by the stress-inducible grp78 promoter resulting in eradication of sizable human tumors. *Hum Gene Ther.* 2004; 15:553–561. [PubMed: 15212714]

74. Dong D, Ko B, Baumeister P, Swenson S, Costa F, Markland F, et al. Vascular targeting and antiangiogenesis agents induce drug resistance effector GRP78 within the tumor microenvironment. *Cancer Res.* 2005; 65:5785–5791. [PubMed: 15994954]
75. Glozak MA, Seto E. Histone deacetylases and cancer. *Oncogene.* 2007; 26:5420–5432. [PubMed: 17694083]
76. Baumeister P, Dong D, Fu Y, Lee AS. Transcriptional induction of GRP78/BiP by histone deacetylase inhibitors and resistance to histone deacetylase inhibitor-induced apoptosis. *Mol Cancer Ther.* 2009; 8:1086–1094. [PubMed: 19417144]
77. Grandage VL, Gale RE, Linch DC, Khwaja A. PI3-kinase/Akt is constitutively active in primary acute myeloid leukaemia cells and regulates survival and chemoresistance via NF-kappaB, Mapkinase and p53 pathways. *Leukemia.* 2005; 19:586–594. [PubMed: 15703783]
78. Ermakova SP, Kang BS, Choi BY, Choi HS, Schuster TF, Ma WY, et al. (-)-Epigallocatechin gallate overcomes resistance to etoposide-induced cell death by targeting the molecular chaperone glucose-regulated protein 78. *Cancer Res.* 2006; 66:9260–9269. [PubMed: 16982771]
79. Ni M, Zhou H, Wey S, Baumeister P, Lee AS. Regulation of PERK signaling and leukemic cell survival by a novel cytosolic isoform of the UPR regulator GRP78/BiP. *PLoS One.* 2009; 4:e6868. [PubMed: 19718440]
80. Yu DH, Macdonald J, Liu G, Lee AS, Ly M, Davis T, et al. Pyrvinium targets the unfolded protein response to hypoglycemia and its anti-tumor activity is enhanced by combination therapy. *PLoS One.* 2008; 3:e3951. [PubMed: 19079611]
81. Backer JM, Krivoshein AV, Hamby CV, Pizzonia J, Gilbert KS, Ray YS, et al. Chaperone-targeting cytotoxin and endoplasmic reticulum stress-inducing drug synergize to kill cancer cells. *Neoplasia.* 2009; 11:1165–1173. [PubMed: 19881952]
82. Li M, Wang J, Jing J, Hua H, Luo T, Xu L, et al. Synergistic promotion of breast cancer cells death by targeting molecular chaperone GRP78 and heat shock protein 70. *J Cell Mol Med.* 2009; 13:4540–4550. [PubMed: 19017364]
83. Kim JY, Hwang JH, Cha MR, Yoon MY, Son ES, Tomida A, et al. Arctigenin blocks the unfolded protein response and shows therapeutic antitumor activity. *J Cell Physiol.* 2010; 224:33–40. [PubMed: 20232300]
84. Martin S, Hill DS, Paton JC, Paton AW, Birch-Machin MA, Lovat PE, et al. Targeting GRP78 to enhance melanoma cell death. *Pigment Cell Melanoma Res.* 2010; 23:675–682. [PubMed: 20546536]
85. Roue G, Perez-Galan P, Mozos A, Lopez-Guerra M, Xargay-Torrent S, Rosich L, et al. The Hsp90 inhibitor IPI-504 overcomes bortezomib resistance in mantle cell lymphoma in vitro and in vivo by down-regulation of the prosurvival ER chaperone BiP/Grp78. *Blood.* 2011; 117:1270–1279. [PubMed: 21106982]
86. Arap MA, Lahdenranta J, Mintz PJ, Hajitou A, Sarkis AS, Arap W, et al. Cell surface expression of the stress response chaperone GRP78 enables tumor targeting by circulating ligands. *Cancer Cell.* 2004; 6:275–284. [PubMed: 15380518]
87. Kim Y, Lillo AM, Steiniger SC, Liu Y, Ballatore C, Anichini A, et al. Targeting heat shock proteins on cancer cells: selection, characterization, and cell-penetrating properties of a peptidic GRP78 ligand. *Biochemistry.* 2006; 45:9434–9444. [PubMed: 16878978]
88. Liu Y, Steiniger SC, Kim Y, Kaufmann GF, Felding-Habermann B, Janda KD. Mechanistic studies of a peptidic GRP78 ligand for cancer cell-specific drug delivery. *Mol Pharm.* 2007; 4:435–447. [PubMed: 17373820]
89. Sato M, Yao VJ, Arap W, Pasqualini R. GRP78 signaling hub a receptor for targeted tumor therapy. *Adv Genet.* 2010; 69:97–114. [PubMed: 20807604]
90. Jakobsen CG, Rasmussen N, Laenkholtm AV, Ditzel HJ. Phage display derived human monoclonal antibodies isolated by binding to the surface of live primary breast cancer cells recognize GRP78. *Cancer Res.* 2007; 67:9507–9517. [PubMed: 17909061]
91. Rauschert N, Brandlein S, Holzinger E, Hensel F, Muller-Hermelink HK, Vollmers HP. A new tumor-specific variant of GRP78 as target for antibody-based therapy. *Lab Invest.* 2008; 88:375–386. [PubMed: 18268478]

92. Johnson S, Michalak M, Opas M, Eggleton P. The ins and outs of calreticulin: from the ER lumen to the extracellular space. *Trends Cell Biol.* 2001; 11:122–129. [PubMed: 11306273]
93. Gelebart P, Opas M, Michalak M. Calreticulin, a Ca²⁺-binding chaperone of the endoplasmic reticulum. *Int J Biochem Cell Biol.* 2005; 37:260–266. [PubMed: 15474971]
94. Caramelo JJ, Parodi AJ. Getting in and out from calnexin/calreticulin cycles. *J Biol Chem.* 2008; 283:10221–10225. [PubMed: 18303019]
95. Michalak M, Groenendyk J, Szabo E, Gold LI, Opas M. Calreticulin, a multi-process calcium-buffering chaperone of the endoplasmic reticulum. *Biochem J.* 2009; 417:651–666. [PubMed: 19133842]
96. Gold LI, Eggleton P, Sweetwyne MT, Van Duyn LB, Greives MR, Naylor SM, et al. Calreticulin: non-endoplasmic reticulum functions in physiology and disease. *FASEB J.* 2010; 24:665–683. [PubMed: 19940256]
97. Obeid M, Panaretakis T, Joza N, Tufi R, Tesniere A, van Endert P, et al. Calreticulin exposure is required for the immunogenicity of gamma-irradiation and UVC light-induced apoptosis. *Cell Death Differ.* 2007; 14:1848–1850. [PubMed: 17657249]
98. Martins I, Kepp O, Galluzzi L, Senovilla L, Schlemmer F, Adjemian S, et al. Surface-exposed calreticulin in the interaction between dying cells and phagocytes. *Ann N Y Acad Sci.* 2010; 1209:77–82. [PubMed: 20958319]
99. Obeid M, Panaretakis T, Tesniere A, Joza N, Tufi R, Apetoh L, et al. Leveraging the immune system during chemotherapy: moving calreticulin to the cell surface converts apoptotic death from “silent” to immunogenic. *Cancer Res.* 2007; 67:7941–7944. [PubMed: 17804698]
100. Chao MP, Jaiswal S, Weissman-Tsukamoto R, Alizadeh AA, Gentles AJ, Volkmer J, et al. Calreticulin is the dominant pro-phagocytic signal on multiple human cancers and is counterbalanced by CD47. *Sci Transl Med.* 2010; 2:63ra94.
101. Wemeau M, Kepp O, Tesniere A, Panaretakis T, Flament C, De Botton S, et al. Calreticulin exposure on malignant blasts predicts a cellular anticancer immune response in patients with acute myeloid leukemia. *Cell Death Dis.* 2010; 1:e104. [PubMed: 21368877]
102. Obeid M, Tesniere A, Panaretakis T, Tufi R, Joza N, van Endert P, et al. Ecto-calreticulin in immunogenic chemotherapy. *Immunol Rev.* 2007; 220:22–34. [PubMed: 17979837]
103. Martins I, Kepp O, Schlemmer F, Adjemian S, Tailler M, Shen S, et al. Restoration of the immunogenicity of cisplatin-induced cancer cell death by endoplasmic reticulum stress. *Oncogene.* 2011; 30:1147–1158. [PubMed: 21151176]
104. Panaretakis T, Kepp O, Brockmeier U, Tesniere A, Bjorklund AC, Chapman DC, et al. Mechanisms of pre-apoptotic calreticulin exposure in immunogenic cell death. *EMBO J.* 2009; 28:578–590. [PubMed: 19165151]
105. Obeid M. ERP57 membrane translocation dictates the immunogenicity of tumor cell death by controlling the membrane translocation of calreticulin. *J Immunol.* 2008; 181:2533–2543. [PubMed: 18684944]
106. Panaretakis T, Joza N, Modjtahedi N, Tesniere A, Vitale I, Durchschlag M, et al. The co-translocation of ERp57 and calreticulin determines the immunogenicity of cell death. *Cell Death Differ.* 2008; 15:1499–1509. [PubMed: 18464797]
107. Obeid M, Tesniere A, Ghiringhelli F, Fimia GM, Apetoh L, Perfettini JL, et al. Calreticulin exposure dictates the immunogenicity of cancer cell death. *Nat Med.* 2007; 13:54–61. [PubMed: 17187072]
108. Ng FW, Nguyen M, Kwan T, Branton PE, Nicholson DW, Cromlish JA, et al. p28 Bap31, a Bcl-2/Bcl-XL- and procaspase-8-associated protein in the endoplasmic reticulum. *J Cell Biol.* 1997; 139:327–338. [PubMed: 9334338]
109. Podack ER, Raez LE. Allogeneic tumor-cell-based vaccines secreting endoplasmic reticulum chaperone gp96. *Expert Opin Biol Ther.* 2007; 7:1679–1688. [PubMed: 17961091]
110. Strbo N, Podack ER. Secreted heat shock protein gp96-Ig: an innovative vaccine approach. *Am J Reprod Immunol.* 2008; 59:407–416. [PubMed: 18405311]
111. di Pietro A, Tosti G, Ferrucci PF, Testori A. The immunological era in melanoma treatment: new challenges for heat shock protein-based vaccine in the advanced disease. *Expert Opin Biol Ther.* 2011; 11:1395–1407. [PubMed: 21801084]

112. Baker-LePain JC, Sarzotti M, Fields TA, Li CY, Nicchitta CV. GRP94 (gp96) and GRP94 N-terminal geldanamycin binding domain elicit tissue nonrestricted tumor suppression. *J Exp Med.* 2002; 196:1447–1459. [PubMed: 12461080]
113. Liu S, Wang H, Yang Z, Kon T, Zhu J, Cao Y, et al. Enhancement of cancer radiation therapy by use of adenovirus-mediated secretable glucose-regulated protein 94/gp96 expression. *Cancer Res.* 2005; 65:9126–9131. [PubMed: 16230366]
114. Schreiber TH, Deyev VV, Rosenblatt JD, Podack ER. Tumor-induced suppression of CTL expansion and subjugation by gp96-Ig vaccination. *Cancer Res.* 2009; 69:2026–2033. [PubMed: 19223534]
115. Qian J, Hong S, Wang S, Zhang L, Sun L, Wang M, et al. Myeloma cell line-derived, pooled heat shock proteins as a universal vaccine for immunotherapy of multiple myeloma. *Blood.* 2009; 114:3880–3889. [PubMed: 19654406]
116. Pakravan N, Hassan ZM. Comparison of adjuvant activity of N- and C-terminal domain of gp96 in a Her2-positive breast cancer model. *Cell Stress Chaperones.* 2011; 16:449–457. [PubMed: 21359667]
117. Pakravan N, Hashemi SM, Hassan ZM. Adjuvant activity of GP96 C-terminal domain towards Her2/neu DNA vaccine is fusion direction-dependent. *Cell Stress Chaperones.* 2011; 16:41–48. [PubMed: 20730610]
118. Oki Y, McLaughlin P, Fayad LE, Pro B, Mansfield PF, Clayman GL, et al. Experience with heat shock protein-peptide complex 96 vaccine therapy in patients with indolent non-Hodgkin lymphoma. *Cancer.* 2007; 109:77–83. [PubMed: 17133412]
119. Testori A, Richards J, Whitman E, Mann GB, Lutzky J, Camacho L, et al. Phase III comparison of vitespen, an autologous tumor-derived heat shock protein gp96 peptide complex vaccine, with physician's choice of treatment for stage IV melanoma: the C-100–21 Study Group. *J Clin Oncol.* 2008; 26:955–962. [PubMed: 18281670]
120. Wood C, Srivastava P, Bukowski R, Lacombe L, Gorelov AI, Gorelov S, et al. An adjuvant autologous therapeutic vaccine (HSPPC-96; vitespen) versus observation alone for patients at high risk of recurrence after nephrectomy for renal cell carcinoma: a multicentre, open-label, randomised phase III trial. *Lancet.* 2008; 372:145–154. [PubMed: 18602688]
121. Eton O, Ross MI, East MJ, Mansfield PF, Papadopoulos N, Ellerhorst JA, et al. Autologous tumor-derived heat-shock protein peptide complex-96 (HSPPC-96) in patients with metastatic melanoma. *J Transl Med.* 2010; 8:9. [PubMed: 20109236]
122. Tamura Y, Hirohashi Y, Kutomi G, Nakanishi K, Kamiguchi K, Torigoe T, et al. Tumor-produced secreted form of binding of immunoglobulin protein elicits antigen-specific tumor immunity. *J Immunol.* 2011; 186:4325–4330. [PubMed: 21339366]
123. Gong J, Zhang Y, Durfee J, Weng D, Liu C, Koido S, et al. A heat shock protein 70-based vaccine with enhanced immunogenicity for clinical use. *J Immunol.* 2010; 184:488–496. [PubMed: 19949080]
124. Ren F, Xu Y, Mao L, Ou R, Ding Z, Zhang X, et al. Heat shock protein 110 improves the antitumor effects of the cytotoxic T lymphocyte epitope E7(49–57) in mice. *Cancer Biol Ther.* 2010; 9:134–141. [PubMed: 19901562]
125. Wang XY, Sun X, Chen X, Facciponte J, Repasky EA, Kane J, et al. Superior antitumor response induced by large stress protein chaperoned protein antigen compared with peptide antigen. *J Immunol.* 2010; 184:6309–6319. [PubMed: 20439916]
126. Qian J, Yi H, Guo C, Yu X, Zuo D, Chen X, et al. CD204 suppresses large heat shock protein-facilitated priming of tumor antigen gp100-specific T cells and chaperone vaccine activity against mouse melanoma. *J Immunol.* 2011; 187:2905–2914. [PubMed: 21832164]
127. Guo QY, Yuan M, Peng J, Cui XM, Song G, Sui X, et al. Antitumor activity of mixed heat shock protein/peptide vaccine and cyclophosphamide plus interleukin-12 in mice sarcoma. *J Exp Clin Cancer Res.* 2011; 30:24. [PubMed: 21352555]
128. Noiva R. Protein disulfide isomerase: the multifunctional redox chaperone of the endoplasmic reticulum. *Semin Cell Dev Biol.* 1999; 10:481–493. [PubMed: 10597631]
129. Ellgaard L, Ruddock LW. The human protein disulphide isomerase family: substrate interactions and functional properties. *EMBO Rep.* 2005; 6:28–32. [PubMed: 15643448]

130. Gruber CW, Cemazar M, Heras B, Martin JL, Craik DJ. Protein disulfide isomerase: the structure of oxidative folding. *Trends Biochem Sci.* 2006; 31:455–464. [PubMed: 16815710]
131. Turano C, Coppari S, Altieri F, Ferraro A. Proteins of the PDI family: unpredicted non-ER locations and functions. *J Cell Physiol.* 2002; 193:154–163. [PubMed: 12384992]
132. Ko HS, Uehara T, Nomura Y. Role of ubiquilin associated with protein-disulfide isomerase in the endoplasmic reticulum in stress-induced apoptotic cell death. *J Biol Chem.* 2002; 277:35386–35392. [PubMed: 12095988]
133. Uehara T, Nakamura T, Yao D, Shi ZQ, Gu Z, Ma Y, et al. S-nitrosylated protein-disulphide isomerase links protein misfolding to neurodegeneration. *Nature.* 2006; 441:513–517. [PubMed: 16724068]
134. Lovat PE, Corazzari M, Armstrong JL, Martin S, Pagliarini V, Hill D, et al. Increasing melanoma cell death using inhibitors of protein disulfide isomerases to abrogate survival responses to endoplasmic reticulum stress. *Cancer Res.* 2008; 68:5363–5369. [PubMed: 18593938]
135. Goplen D, Wang J, Enger PO, Tysnes BB, Terzis AJ, Laerum OD, et al. Protein disulfide isomerase expression is related to the invasive properties of malignant glioma. *Cancer Res.* 2006; 66:9895–9902. [PubMed: 17047051]
136. Haefliger S, Klebig C, Schaubitzer K, Schardt J, Timchenko N, Mueller BU, et al. Protein disulfide isomerase blocks CEBPA translation and is up-regulated during the unfolded protein response in AML. *Blood.* 2011; 117:5931–5940. [PubMed: 21471526]
137. Fonseca C, Soiffer R, Ho V, Vanneman M, Jinushi M, Ritz J, et al. Protein disulfide isomerases are antibody targets during immune-mediated tumor destruction. *Blood.* 2009; 113:1681–1688. [PubMed: 19008459]
138. Schroder M, Kaufman RJ. Divergent roles of IRE1alpha and PERK in the unfolded protein response. *Curr Mol Med.* 2006; 6:5–36. [PubMed: 16472110]
139. Harding HP, Zhang Y, Bertolotti A, Zeng H, Ron D. Perk is essential for translational regulation and cell survival during the unfolded protein response. *Mol Cell.* 2000; 5:897–904. [PubMed: 10882126]
140. Cullinan SB, Zhang D, Hannink M, Arvisais E, Kaufman RJ, Diehl JA. Nrf2 is a direct PERK substrate and effector of PERK-dependent cell survival. *Mol Cell Biol.* 2003; 23:7198–7209. [PubMed: 14517290]
141. Cullinan SB, Diehl JA. PERK-dependent activation of Nrf2 contributes to redox homeostasis and cell survival following endoplasmic reticulum stress. *J Biol Chem.* 2004; 279:20108–20117. [PubMed: 14978030]
142. Zhang P, McGrath B, Li S, Frank A, Zambito F, Reinert J, et al. The PERK eukaryotic initiation factor 2 alpha kinase is required for the development of the skeletal system, postnatal growth, and the function and viability of the pancreas. *Mol Cell Biol.* 2002; 22:3864–3874. [PubMed: 11997520]
143. Wei J, Sheng X, Feng D, McGrath B, Cavener DR. PERK is essential for neonatal skeletal development to regulate osteoblast proliferation and differentiation. *J Cell Physiol.* 2008; 217:693–707. [PubMed: 18683826]
144. Harding HP, Zeng H, Zhang Y, Jungries R, Chung P, Plesken H, et al. Diabetes mellitus and exocrine pancreatic dysfunction in *perk*^{-/-} mice reveals a role for translational control in secretory cell survival. *Mol Cell.* 2001; 7:1153–1163. [PubMed: 11430819]
145. Zhang W, Feng D, Li Y, Iida K, McGrath B, Cavener DR. PERK EIF2AK3 control of pancreatic beta cell differentiation and proliferation is required for postnatal glucose homeostasis. *Cell Metab.* 2006; 4:491–497. [PubMed: 17141632]
146. Bi M, Naczki C, Koritzinsky M, Fels D, Blais J, Hu N, et al. ER stress-regulated translation increases tolerance to extreme hypoxia and promotes tumor growth. *EMBO J.* 2005; 24:3470–3481. [PubMed: 16148948]
147. Blais JD, Addison CL, Edge R, Falls T, Zhao H, Wary K, et al. Perk-dependent translational regulation promotes tumor cell adaptation and angiogenesis in response to hypoxic stress. *Mol Cell Biol.* 2006; 26:9517–9532. [PubMed: 17030613]

148. Gupta S, McGrath B, Cavener DR. PERK regulates the proliferation and development of insulin-secreting beta-cell tumors in the endocrine pancreas of mice. *PLoS One*. 2009; 4:e8008. [PubMed: 19956728]
149. Hamanaka RB, Bennett BS, Cullinan SB, Diehl JA. PERK and GCN2 contribute to eIF2alpha phosphorylation and cell cycle arrest after activation of the unfolded protein response pathway. *Mol Biol Cell*. 2005; 16:5493–5501. [PubMed: 16176978]
150. Liu Y, Laszlo C, Liu W, Chen X, Evans SC, Wu S. Regulation of G(1) arrest and apoptosis in hypoxia by PERK and GCN2-mediated eIF2alpha phosphorylation. *Neoplasia*. 2010; 12:61–68. [PubMed: 20072654]
151. Ye J, Kumanova M, Hart LS, Sloane K, Zhang H, De Panis DN, et al. The GCN2-ATF4 pathway is critical for tumour cell survival and proliferation in response to nutrient deprivation. *EMBO J*. 2010; 29:2082–2096. [PubMed: 20473272]
152. Rouschop KM, van den Beucken T, Dubois L, Niessen H, Bussink J, Savelkoul K, et al. The unfolded protein response protects human tumor cells during hypoxia through regulation of the autophagy genes MAP1LC3B and ATG5. *J Clin Invest*. 2010; 120:127–141. [PubMed: 20038797]
153. Rzymiski T, Milani M, Pike L, Buffa F, Mellor HR, Winchester L, et al. Regulation of autophagy by ATF4 in response to severe hypoxia. *Oncogene*. 2010; 29:4424–4435. [PubMed: 20514020]
154. Avivar-Valderas A, Salas E, Bobrovnikova-Marjon E, Diehl JA, Nagi C, Debnath J, et al. PERK integrates autophagy and oxidative stress responses to promote survival during extracellular matrix detachment. *Mol Cell Biol*. 2011; 31:3616–3629. [PubMed: 21709020]
155. Schewe DM, Aguirre-Ghiso JA. Inhibition of eIF2alpha dephosphorylation maximizes bortezomib efficiency and eliminates quiescent multiple myeloma cells surviving proteasome inhibitor therapy. *Cancer Res*. 2009; 69:1545–1552. [PubMed: 19190324]
156. Donze O, Jagus R, Koromilas AE, Hershey JW, Sonenberg N. Abrogation of translation initiation factor eIF-2 phosphorylation causes malignant transformation of NIH 3T3 cells. *EMBO J*. 1995; 14:3828–3834. [PubMed: 7641700]
157. Perkins DJ, Barber GN. Defects in translational regulation mediated by the alpha subunit of eukaryotic initiation factor 2 inhibit antiviral activity and facilitate the malignant transformation of human fibroblasts. *Mol Cell Biol*. 2004; 24:2025–2040. [PubMed: 14966282]
158. Sequeira SJ, Ranganathan AC, Adam AP, Iglesias BV, Farias EF, Aguirre-Ghiso JA. Inhibition of proliferation by PERK regulates mammary acinar morphogenesis and tumor formation. *PLoS ONE*. 2007; 2:e615. [PubMed: 17637831]
159. Ranganathan AC, Ojha S, Kourtidis A, Conklin DS, Aguirre-Ghiso JA. Dual function of pancreatic endoplasmic reticulum kinase in tumor cell growth arrest and survival. *Cancer Res*. 2008; 68:3260–3268. [PubMed: 18451152]
160. Bobrovnikova-Marjon E, Grigoriadou C, Pytel D, Zhang F, Ye J, Koumenis C, et al. PERK promotes cancer cell proliferation and tumor growth by limiting oxidative DNA damage. *Oncogene*. 2010; 29:3881–3895. [PubMed: 20453876]
161. Yorimitsu T, Nair U, Yang Z, Klionsky DJ. Endoplasmic reticulum stress triggers autophagy. *J Biol Chem*. 2006; 281:30299–30304. [PubMed: 16901900]
162. Hoyer-Hansen M, Jaattela M. Connecting endoplasmic reticulum stress to autophagy by unfolded protein response and calcium. *Cell Death Differ*. 2007; 14:1576–1582. [PubMed: 17612585]
163. Kim KW, Moretti L, Mitchell LR, Jung DK, Lu B. Endoplasmic reticulum stress mediates radiation-induced autophagy by perk-eIF2alpha in caspase-3/7-deficient cells. *Oncogene*. 2010; 29:3241–3251. [PubMed: 20348950]
164. Milani M, Rzymiski T, Mellor HR, Pike L, Bottini A, Generali D, et al. The role of ATF4 stabilization and autophagy in resistance of breast cancer cells treated with Bortezomib. *Cancer Res*. 2009; 69:4415–4423. [PubMed: 19417138]
165. Koong AC, Chauhan V, Romero-Ramirez L. Targeting XBP-1 as a novel anti-cancer strategy. *Cancer Biol Ther*. 2006; 5:756–759. [PubMed: 16861911]
166. Yoshida H. Unconventional splicing of XBP-1 mRNA in the unfolded protein response. *Antioxid Redox Signal*. 2007; 9:2323–2333. [PubMed: 17979529]

167. Glimcher LH. XBP1: the last two decades. *Ann Rheum Dis.* 2010; 69 (Suppl 1):i67–71. [PubMed: 19995749]
168. Zhang K, Wong HN, Song B, Miller CN, Scheuner D, Kaufman RJ. The unfolded protein response sensor IRE1 α is required at 2 distinct steps in B cell lymphopoiesis. *J Clin Invest.* 2005; 115:268–281. [PubMed: 15690081]
169. Fujimoto T, Yoshimatsu K, Watanabe K, Yokomizo H, Otani T, Matsumoto A, et al. Overexpression of human X-box binding protein 1 (XBP-1) in colorectal adenomas and adenocarcinomas. *Anticancer Res.* 2007; 27:127–131. [PubMed: 17352224]
170. Davies MP, Barraclough DL, Stewart C, Joyce KA, Eccles RM, Barraclough R, et al. Expression and splicing of the unfolded protein response gene XBP-1 are significantly associated with clinical outcome of endocrine-treated breast cancer. *Int J Cancer.* 2008; 123:85–88. [PubMed: 18386815]
171. Maestre L, Tooze R, Canamero M, Montes-Moreno S, Ramos R, Doody G, et al. Expression pattern of XBP1(S) in human B-cell lymphomas. *Haematologica.* 2009; 94:419–422. [PubMed: 19176362]
172. Romero-Ramirez L, Cao H, Nelson D, Hammond E, Lee AH, Yoshida H, et al. XBP1 is essential for survival under hypoxic conditions and is required for tumor growth. *Cancer Res.* 2004; 64:5943–5947. [PubMed: 15342372]
173. Gomez BP, Riggins RB, Shajahan AN, Klimach U, Wang A, Crawford AC, et al. Human X-box binding protein-1 confers both estrogen independence and antiestrogen resistance in breast cancer cell lines. *FASEB J.* 2007; 21:4013–4027. [PubMed: 17660348]
174. Hsiao JR, Chang KC, Chen CW, Wu SY, Su IJ, Hsu MC, et al. Endoplasmic reticulum stress triggers XBP-1-mediated up-regulation of an EBV oncoprotein in nasopharyngeal carcinoma. *Cancer Res.* 2009; 69:4461–4467. [PubMed: 19435892]
175. Carrasco DR, Sukhdeo K, Protopopova M, Sinha R, Enos M, Carrasco DE, et al. The differentiation and stress response factor XBP-1 drives multiple myeloma pathogenesis. *Cancer Cell.* 2007; 11:349–360. [PubMed: 17418411]
176. Thorpe JA, Schwarze SR. IRE1 α controls cyclin A1 expression and promotes cell proliferation through XBP-1. *Cell Stress Chaperones.* 2010; 15:497–508. [PubMed: 20013084]
177. Auf G, Jabouille A, Guerit S, Pineau R, Delugin M, Boucheccareilh M, et al. Inositol-requiring enzyme 1 α is a key regulator of angiogenesis and invasion in malignant glioma. *Proc Natl Acad Sci U S A.* 2010; 107:15553–15558. [PubMed: 20702765]
178. Drogat B, Auguste P, Nguyen DT, Boucheccareilh M, Pineau R, Nalbantoglu J, et al. IRE1 signaling is essential for ischemia-induced vascular endothelial growth factor-A expression and contributes to angiogenesis and tumor growth *in vivo*. *Cancer Res.* 2007; 67:6700–6707. [PubMed: 17638880]
179. Romero-Ramirez L, Cao H, Regalado MP, Kambham N, Siemann D, Kim JJ, et al. X box-binding protein 1 regulates angiogenesis in human pancreatic adenocarcinomas. *Transl Oncol.* 2009; 2:31–38. [PubMed: 19252749]
180. Han D, Lerner AG, Vande Walle L, Upton JP, Xu W, Hagen A, et al. IRE1 α kinase activation modes control alternate endoribonuclease outputs to determine divergent cell fates. *Cell.* 2009; 138:562–575. [PubMed: 19665977]
181. Hollien J, Weissman JS. Decay of endoplasmic reticulum-localized mRNAs during the unfolded protein response. *Science.* 2006; 313:104–107. [PubMed: 16825573]
182. Hollien J, Lin JH, Li H, Stevens N, Walter P, Weissman JS. Regulated Ire1-dependent decay of messenger RNAs in mammalian cells. *J Cell Biol.* 2009; 186:323–331. [PubMed: 19651891]
183. Papandreou I, Denko NC, Olson M, Van Melckebeke H, Lust S, Tam A, et al. Identification of an Ire1 α endonuclease specific inhibitor with cytotoxic activity against human multiple myeloma. *Blood.* 2011; 117:1311–1314. [PubMed: 21081713]
184. Mahoney DJ, Lefebvre C, Allan K, Brun J, Sanaei CA, Baird S, et al. Virus-tumor interactome screen reveals ER stress response can reprogram resistant cancers for oncolytic virus-triggered caspase-2 cell death. *Cancer Cell.* 2011; 20:443–456. [PubMed: 22014571]

185. Sinkovics JG, Horvath JC. Natural and genetically engineered viral agents for oncolysis and gene therapy of human cancers. *Arch Immunol Ther Exp (Warsz)*. 2008; 56 (Suppl 1):3s–59s. [PubMed: 19104757]

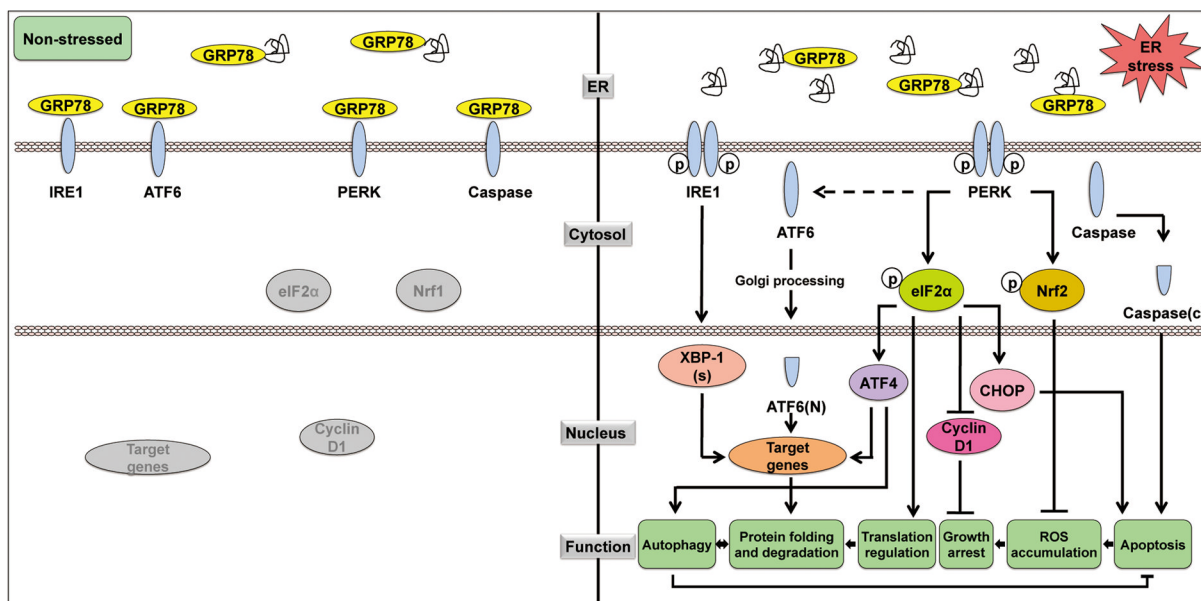


Figure 1.

Unfolded protein response and its regulation on cell activities. *Left panel:* Under non-stress condition, ER luminal GRP78, in addition to folding proteins, binds to IRE1, ATF6, PERK and caspase-12 and -7, quenching their activation. *Right panel:* When cells are under ER stress, GRP78 is titrated away through binding to the malformed proteins, resulting in activation of the IRE1, ATF6 and PERK signaling pathway. IRE1 activates its RNase activity to cleave the mRNA of XBP1, resulting in a spliced form of XBP1 (XBP1-s). ATF6 translocates from the ER to the Golgi apparatus, where it is cleaved into the active nuclear form ATF6(N). PERK dimerizes and autophosphorylates, and thereby phosphorylates its two major substrates eukaryotic translation initiation factor 2 (eIF2) and nuclear factor-like 2 (Nrf2). eIF2 phosphorylation attenuates global protein synthesis, and inhibits cyclin D1 translation through which contributes to cell cycle arrest. The phosphorylation of eIF2 also activates the transcription of ATF4. XBP1(s), ATF6(N) and ATF4 act in concert to induce transcription of target genes mediating protein folding and degradation. Autophagy is also triggered. Another function of phosphorylated eIF2 is to activate CHOP which promotes apoptosis. The phosphorylation of Nrf2 activates the expression of enzymes required for ROS quenching, and thereby inhibits ROS accumulation. Procaspase-12 and -7, upon released from GRP78, are cleaved into their activated forms triggering apoptosis.

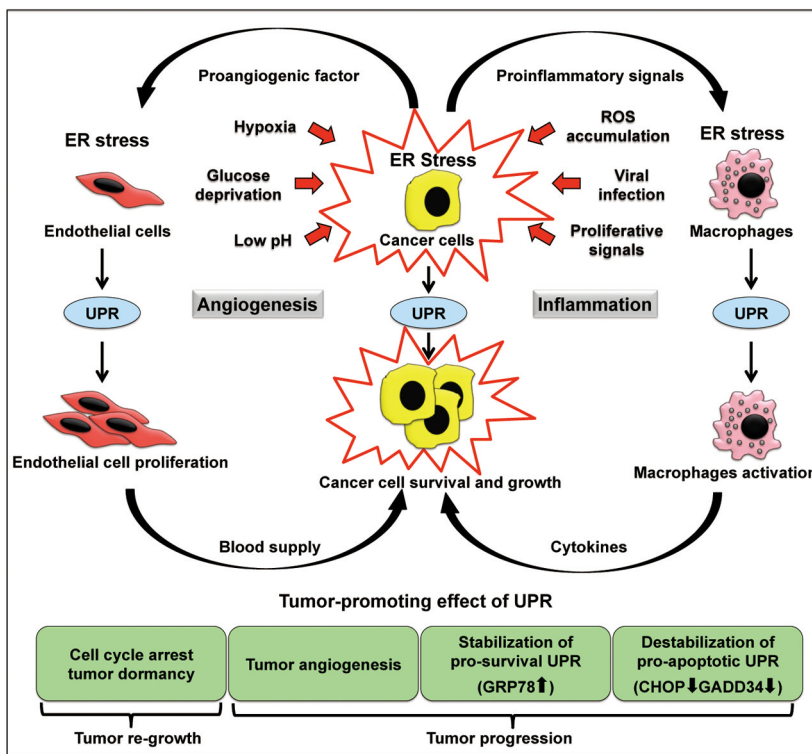


Figure 2. The vicious circle of ER stress and unfolded protein response in promoting tumor progression. Cancer cells are under ER stress due to the growth signaling and factors from the microenvironments. Adaptive UPR is activated to support tumor cell survival and growth. Cancer cells under ER stress secrete proangiogenic factors to stimulate the proliferation of endothelial cells, which in return promotes cancer cell survival and tumor growth. Cancer cells under ER stress also secrete proinflammatory signals to the stromal cells in the vicinity, mostly tumor-associated macrophages, which in turn are activated and secrete inflammatory cytokines that promote tumor growth, angiogenesis, invasion and metastasis.

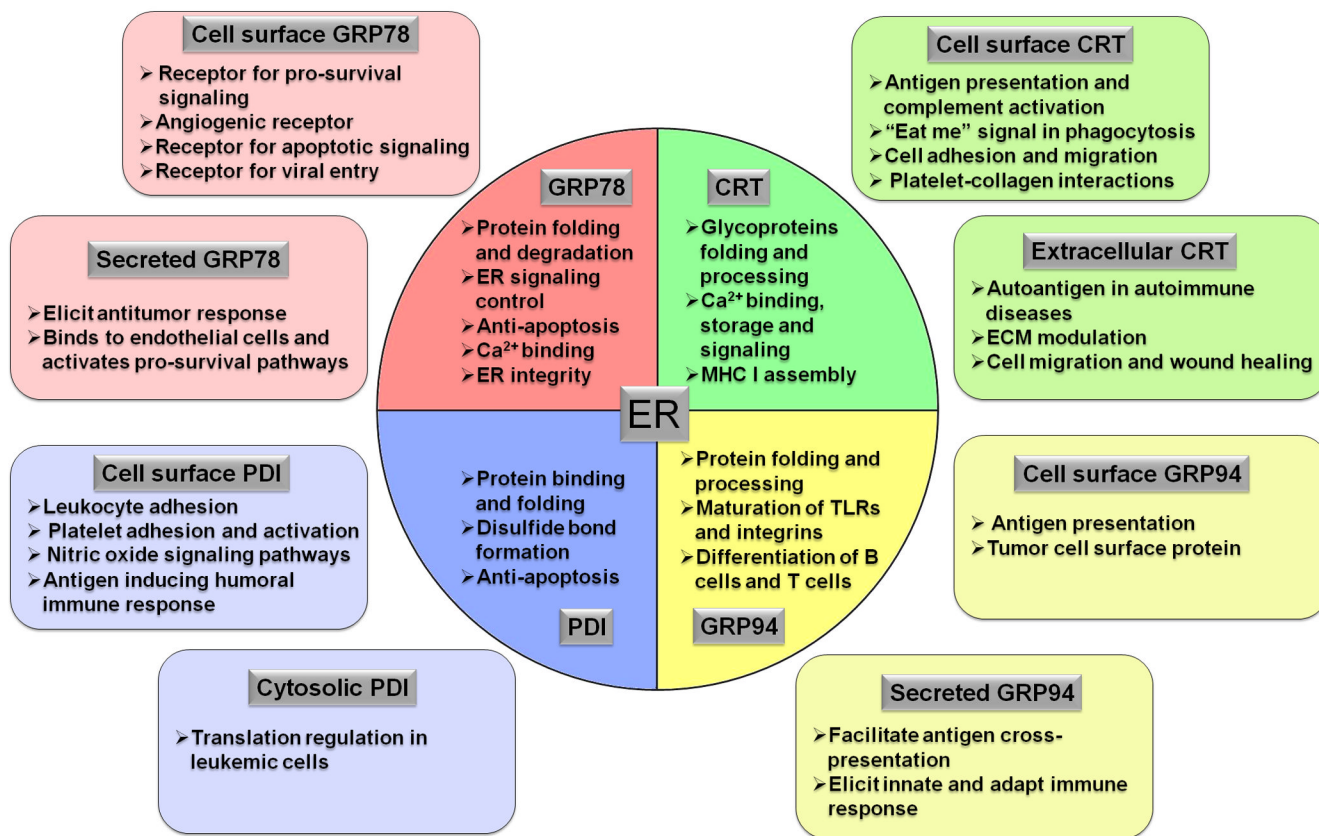


Figure 3. The different localizations and functions of major ER chaperones. The circle in the center indicates ER, where the majority of ER chaperones are located. The squares outside of ER indicate non-traditional localization of these chaperones and their functions.