

# Stressed Out – Therapeutic Implications of ER Stress Related Cancer Research

Randal Riha<sup>1</sup>, Pooja Gupta-Saraf<sup>1</sup>, Payel Bhanja<sup>1</sup>, Samyak Badkul<sup>1</sup>, Subhrajit Saha<sup>1, 2</sup>✉

1. Department of Radiation Oncology, University of Kansas Medical Center, Kansas 66160, USA;
2. Department of Cancer Biology, University of Kansas Medical Center, Kansas 66160, USA.

✉ Corresponding author: Subhrajit Saha PhD, Assistant Professor, Department of Radiation Oncology, Department of Cancer Biology, The University of Kansas Medical Center, MS 4033, 3901 Rainbow Boulevard, Kansas City, Kansas 66160; Phone: 913-588-1054; Fax 913-588-3663; Email: ssaha@kumc.edu

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Received: 2017.08.22; Accepted: 2017.09.25; Published: 2017.09.27

## Abstract

The unfolded protein response (UPR) is an established and well-studied cellular response to the stress and serves to relieve the stress and reinstate cellular homeostasis. It occurs in the endoplasmic reticulum (ER), responsible of properly folding and processing of secretory and transmembrane proteins. It is extremely sensitive to alteration in homeostasis caused by various internal or external stressors which leads to accumulation of misfolded or unfolded proteins in the ER lumen. The UPR works by restoring protein homeostasis in the ER, either through the boosting of protein-folding and degradation capability or by assuaging the demands for such effects, and can cause the activation of cell death if unable to do so. Cancer cells have adapted to gain advantage from the UPR and keeping the cell away from apoptosis and promoting survival, including survival of the cancer stem cells and evading the immune system. Several components of the UPR are overexpressed in a malignant cell and are responsible for resistance from various chemotherapy options and radiotherapy, which are also responsible for causing ER stress and activating the UPR. In this review, we discuss the various ways in which UPR can aid different cancers to survive and evade therapy and highlight recent research, which exploits the UPR to confer sensitivity to these cancer cells against various drugs and radiation.

Key words: ER Stress, UPR, Cancer Stem Cells, Radiation, Immunotherapy.

## Introduction

The ability to survive and proliferate in a hostile biological environment is a hallmark of cancer. In the context of rapidly proliferating cells, there is a large demand for protein synthesis. The endoplasmic reticulum (ER) is the key organelle for proper synthesis and folding of these proteins. Many insults are capable of disturbing ER homeostasis, resulting in diminished capacity for proper protein folding, including hypoxia, nutrient starvation, acidosis, redox imbalance, loss of calcium homeostasis, or exposure to drugs or other compounds. The result is a buildup of unfolded and improperly folded proteins, termed ER stress. Cells react to the buildup of these proteins by activating defense pathways termed the unfolded

protein response (UPR), which function to reestablish ER homeostasis, aid in adaptation, as well as initiating apoptosis in the scenario of excessive ER stress. Malignant cells rely on these UPR pathways to adapt to perturbations in ER folding capacity due to the hostile tumor microenvironment as well as the increase in unfolded and misfolded proteins resulting from high synthetic demands. Through study of these pathways in cancer, it is becoming increasingly clear that the UPR can be therapeutically exploited to reduce the survivability of malignant cells, or tip the balance towards apoptosis. In this review, we have discussed studies on UPR signaling in cancer research and their applications in cancer therapy.

## ER Stress and the Unfolded Protein Response

There are three ER transmembrane receptors which govern the UPR. They are pancreatic ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol requiring enzyme 1 (IRE1). These three receptors are maintained in an inactive state during ER homeostasis by interaction with glucose-regulated protein 78 kDa (GRP78), which is also known as binding immunoglobulin protein (BiP) and heat shock 70 kDa protein 5 (HSPA5). GRP78 is a molecular chaperone which functions to bind to and promote folding of unfolded proteins in the ER lumen. These unfolded proteins are thought to compete for GRP78 binding with the ER luminal domains of the stress receptors. GRP78 dissociation from the ER transmembrane receptors subsequently activates or positively regulates components of the UPR [1, 2].

PERK activation results in phosphorylation of eukaryotic translation initiation factor alpha subunit (eIF2 $\alpha$ ), which inhibits protein translation, reducing the ER stress burden [3, 4]. Cap independent mRNAs such as activating transcription factor 4 (ATF4) are preferentially translated leading to transcriptional activation of GRP78, growth arrest and DNA damage-inducible protein transcript 34 (GADD34), and the pro-apoptotic protein C/EBP homologous protein (CHOP) [5-10]. While initially contributing to cellular survival in conditions of ER stress, PERK is considered pro-apoptotic due to strong induction of CHOP in chronic or terminal ER stress. ATF6 dissociates from GRP78 during periods of ER stress, after which, it translocates to the Golgi apparatus where it is cleaved by protease, allowing translocation to the nucleus [2, 11]. ATF6 acts on the promoter region of UPR target genes termed the endoplasmic reticulum stress element (ERSE), activating genes responsible for components of the UPR response, including molecular chaperones GRP78, Grp94, and calreticulin, as well as CHOP, and XBP1 [12-15]. ATF6 is primarily considered pro-survival due to its role in promoting transcription of chaperones and XBP1. IRE1, upon activation, splices XBP1 into the active form, producing a potent transcription factor that translocates to the nucleus, and binds to the ERSE to upregulate transcription of UPR genes, similarly to ATF6. One unique gene activated by XBP1 is that of ER degradation enhancing  $\alpha$ -mannosidase-like protein (EDEM), which locates to the ER and functions as part of the ER associated degradation (ERAD) pathway, targeting misfolded proteins for export into the cytoplasm for degradation via the ubiquitin-proteasome pathway [16-19]. IRE1 is

considered pro-survival, but can contribute to apoptotic signaling through the TRAF2-ASK-JNK pathway [20-22]. The actions of each branch of the UPR can be thought to contribute to a balance between survival and cell death (Figure 1).

## Stem Cells and ER Stress

Stem cells are critical for maintenance of tissues and cell populations throughout the body. In addition to normal human stem cells, cancer stem cells (CSCs) are a population of cells that possess characteristics of normal stem cells, as well as capacity for tumorigenesis which make these cells capable of causing cancer recurrence and metastasis. Though there is substantial controversy regarding CSCs, it is clear, that they play a role in the heterogeneity of cells which make up a cancer. In addition to illuminating a role in the development, survival, recurrence, and metastasis of cancer cells, an understanding of how ER stress effects stem cells may uncover a target against these CSCs. The effect of ER stress on normal stem cell populations must also be considered in the setting of systemic or regional therapy utilizing ER stress inducing agents as there may be a potential for side effects.

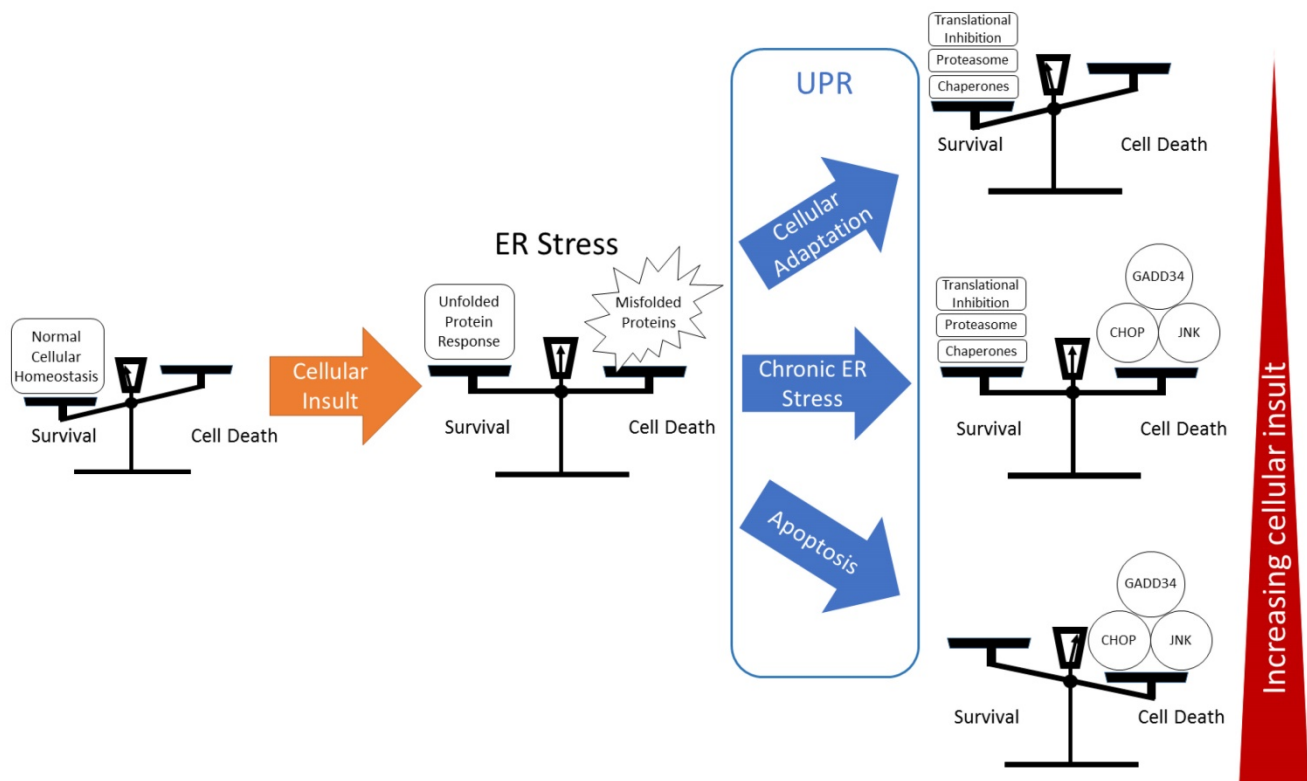
CSCs have been demonstrated to be particularly resistant to traditional cancer therapy. The ability of these cells to seed tumors has led some to theorize that these cells are the origin of cancer relapse and metastasis [23]. Evidence is starting to suggest that the UPR protein GRP78 may have a role in the function and survival of these cells, which is not unexpected, as GRP78 is required for embryonic stem cell survival and maintenance of hematopoietic stem cells [24, 25]. One study showed MDA-468 breast cancer cells with a stem cell/progenitor phenotype having increased expression of UPR proteins GRP78, GRP94, and protein disulfide-isomerase. The presence of these proteins was confirmed to be present in breast cancer patient bone marrow when compared to disease-free control subjects [26]. Another study found GRP78 to play a crucial role in stem cell oncogenesis in MCF-7 breast cancer cells [27]. GRP78 has also been shown to play a critical role in tumorigenesis of prostate adenocarcinoma in mice with PTEN tumor suppressor gene deletion [28]. In human prostate cancer patients, GRP78 is upregulated and is correlated with clinical tumor recurrence and reduced survival [29]. Increased recurrence and reduced survival would be consistent with the presence of a population of treatment resistant CSCs. With the hypoxic niche of the bone marrow as a common site of metastatic cancer growth in breast and prostate cancer, it is intriguing to consider whether increased

expression of GRP78 is a result of hypoxia related ER stress induction of the UPR, or whether GRP78 is induced by other processes, predisposing those cells to survival in that microenvironment. Whether hypoxia drives the processes that establish CSC phenotype is certainly an open question [30].

With the expression of GRP78 in CSCs, it may be a future target for eliminating stem cell populations responsible for recurrence and metastasis. In head and neck cancer initiating cells (HN-CICs), GRP78 has been found to contribute to maintenance of the cancer initiating cell population. By knocking down GRP78, tumor initiating activity was found to be lessened, however, not entirely eliminated [31]. More recently, two unique treatments have been shown promise through targeting GRP78. A monoclonal antibody against GRP78 was shown to suppress signaling through the PI3K/Akt/mTOR pathway, which is responsible for radiation resistance in non-small cell lung cancer and glioblastoma multiforme (GBM). They found that ionizing radiation increased GRP78 expression through the induction of ER stress, and treatment with the monoclonal antibody along with ionizing radiation in mouse xenograph models

showed significant tumor growth delay [32]. The other recent therapeutic model utilized a phage, displaying a ligand specific to GRP78, to deliver the herpes simplex virus thymidine kinase type-1 suicide inducing transgene to mice with MDA-PCa-118b patient derived tumor xenografts. After treatment with the antiviral drug ganciclovir, tumors were reduced by an average of 50% [33]. It is curious to speculate whether ionizing radiation could be employed prior to treatment with the viral phage to upregulate expression of GRP78 to increase the effectiveness of phage targeting of cancer cells.

The effect of ER stress on normal stem cells must be considered in the context of ER stress related therapies. Hematopoietic stem cells have been shown to be predisposed to apoptosis through a strong PERK mediated response to ER stress. This is hypothesized to be a quality control mechanism to ensure clonal integrity of hematopoietic stem cells [34]. Although this indicates that caution should be warranted to avoid loss of this stem cell population, it also provides hope of potential therapy for hematopoietic malignancies.



**Figure 1. Balance between survival and death.** ER stress and the UPR can be considered as a balance between survival and cell death. A cellular insult inducing ER stress is countered by the UPR, resulting in a number of outcomes that can be simplified to three outcomes. A strong UPR, or minor insult results in adaptation, which is often used by cancer cells for survival. With increasing insult, a chronic ER stress can result in a balance that can be tipped towards apoptosis through further induction of ER stress. Finally, a terminal ER stress response ends in apoptotic cell death.

In the development of tumors and corresponding CSCs, mutations arise in a way that is unique to that specific cancer. The role that ER stress plays in the tumorigenesis is expected to vary between different types of cancer. Similarly, potential therapeutic targeting of ER stress and UPR pathways would be expected to differ based upon the underlying biology of each individual cancer.

### Cancer therapy and ER stress

Recent cancer research has illuminated the many roles that the UPR play in the survival and propagation of cancer cells. As the UPR is being linked to survival adaptation in many diverse types of cancer cells, we are learning that there is a similarly diverse variability in activation or upregulation of the three major UPR pathways. As activation and reliance upon the UPR for survival differs by cell type and environmental niche, research into therapeutics to target the UPR are likely to be with specificity to cell type rather than a universal “magic bullet”. The variability of ER stress status and reliance on the UPR can also be considered as a potential biomarker useful for determining the most effective treatments and therapeutics. If a cancer is known to have a significant chronic ER stress, the clinician could choose a therapeutic most likely to result in a terminal ER stress response.

Table 1 illustrates the wide variety of cancer types that have been investigated in the context of the UPR. Figure 2 illustrates potential therapeutics which either induce ER stress or otherwise target the UPR. The following sections will highlight the aspects of ER

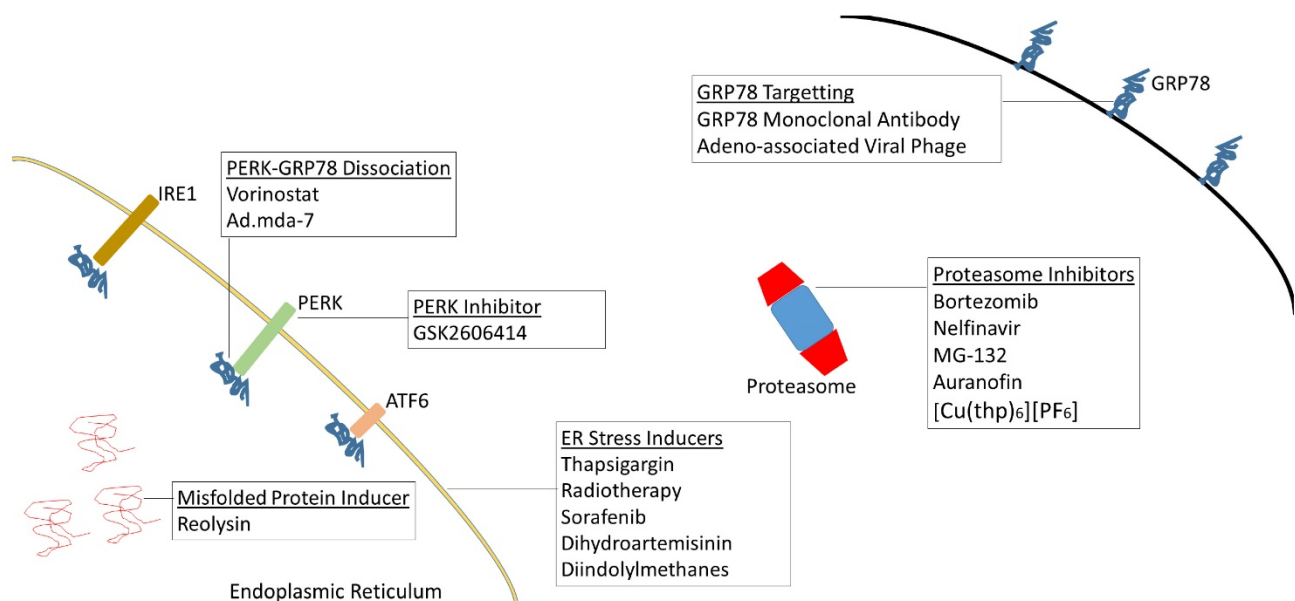
stress and therapeutic implications unique to specific types of malignancies.

### Hodgkin cells

Cells from Hodgkin’s disease have been shown to have a defect in regulation of NF-κB. When treated with proteasome inhibitor MG-132, HD-My-Z Hodgkin cells showed no decrease in expression of NF-κB, likely due to constitutive activity in this cell line. These treated cells, however, underwent apoptosis, and radiosensitivity was noted when compared to intrinsic radiosensitivity in clonogenic survival assays. This radiosensitivity result is intriguing as NF-κB has been implicated in protection from apoptosis following exposure to ionizing radiation [35]. A look into the more recent research, may suggest ER stress and downstream UPR pathways as causal pathways for this increased radiosensitivity.

**Table 1.** Cancers with ER stress related research

Type of Cancer	Research demonstrating UPR for survival or carcinogenesis (references)	Research regarding ER stress in treatment (references)
Hodgkin’s lymphoma	[36]	[37]
Pancreatic Adenocarcinoma	[39]	[41-44]
Hepatocellular Carcinoma	[48, 55]	[57, 60]
Prostate Adenocarcinoma	[29, 102-104]	[105, 106]
Colorectal Adenocarcinoma	[107]	[108-111]
Non-small Cell Lung Cancer	[112, 113]	[114, 115]
Ductal Carcinoma	[116, 117]	[118-120]



**Figure 2. Targets for therapy.** ER stress and the UPR can be targeted at multiple sites. The potential implication is effective therapeutics for various types of malignancies

Hodgkin cells have been shown to have upregulated levels of UPR signaling molecules, specifically those that are associated with survival, including ATF6, GRP78, and XBP1 (both spliced and un-spliced). Conversely, pro-apoptotic signaling molecules associated with the UPR, including pASK1 and CHOP were both noted to be attenuated. This state of ER stress signaling is likely contributive to the ability of Hodgkin cells to survive in a chronic ER stress environment from accumulation of viral proteins, as Hodgkin cells are closely associated with the Epstein-Barr virus [36].

Furthermore, a more recent study of tyrosine kinase inhibitors (TKIs) on Hodgkin cells has demonstrated an increase in ER stress and ER stress-induced apoptosis. L-428, L-1236, and KM-H2 cells were treated *in vitro* with the TKI sorafenib and monitored for markers of the UPR. Phosphorylation of PERK was noted, with a corresponding decrease in total intracellular signaling attributed to global protein translation inhibition via phosphorylation of eIF2 $\alpha$ . In addition, pro-apoptotic signaling molecules GADD34 and CHOP were noted to be upregulated after incubation with sorafenib [37].

The ER stress response is clearly important to the survival of Hodgkin cells, and it has been shown that the UPR can likely be exploited through therapeutic means for treatment. The question remains as to whether the mechanism of action in proteasome inhibitors in Hodgkin disease is related to ER stress and the UPR, and if so, whether the increased UPR is responsible for radiosensitization. It is known that XBP1 is upregulated in these cells, and a downstream product of XBP1 is EDEM, a critical protein for the targeting of misfolded glycoproteins for degradation by the proteasome. Does the inhibition of the proteasome result in an increased concentration of misfolded glycoproteins in the ER of Hodgkin cells, leading to a terminal ER stress response? Furthermore, the mechanistic behind the increased radiosensitivity of proteasome inhibitor treated Hodgkin cells could provide a basis for research into potential future chemoradiotherapeutic regimens.

## Pancreatic Cancer

Most pancreatic cancers are pancreatic ductal adenocarcinomas (PDACs), those that arise from the epithelium of pancreatic ducts. These cancers have an extremely poor prognosis, with a 1 year relative survival of 20% according to the American Cancer Society. As diagnosis is often late in the disease process, it is often difficult or impossible to surgically resect with clear margins. In this clinical context, novel approaches to chemotherapeutic treatment

have the potential to improve outcomes.

Pancreatic cancers have been shown to become significantly hypoxic as they grow, with large volumes of the tumor at only a small fraction at the oxygenation levels of the surrounding normal pancreas [38]. In these conditions, one would expect high levels of ER stress due not only to hypoxia, but likely glucose depletion as well. Cellular adaptation to this environment is certainly linked to the survival and proliferation of malignant cells. Choe *et al.* showed that in pancreatic cancer cells, activation of the PERK and IRE1 arms of the UPR are delayed in the presence of ER stressors, compared to normal pancreatic cells. This was attributed to an abundance of protein folding machinery, such as chaperones. Additionally, once activated, the pro-survival XBP1 was noted to be activated for a longer time period in cancer cells when compared to normal cells [39]. As well as adapting to chronic ER stress, it has been recently postulated that anterior-gradient 2 (AGR2) may contribute to the initiation and development of PDAC [40]. As AGR2 is a gene associated with protein folding and maturation which is expressed in pancreatic cells under ER stress conditions, it may become a key piece in our understanding of how PDACs arise, as well as a potential future therapeutic target.

As adaptation, to an environment conducive to ER stress, is essential for survival and propagation of pancreatic cancer cells, ER stress and the UPR may provide a future target for therapeutics. *In vitro* studies of diindolylmethane derivatives have been shown to be similar in effect to thapsigargin in inducing ER stress, followed by subsequent apoptosis via death receptor 5 (DR5) through induction by CHOP [41]. Proteasome inhibitors have seen interest as a potential treatment for pancreatic cancer due to their unique effects. Bortezomib has been shown to increase GRP78, CHOP, and c-Jun NH<sub>2</sub> terminal kinase (JNK) in L3.6pl pancreatic cancer cells, yet interestingly blocks PERK autophosphorylation, preventing phosphorylation of eIF2 $\alpha$  and the subsequent translational inhibition [42, 43]. Without the suppression of translation, ubiquitinated proteins will accumulate, leading to a terminal ER stress response. More recently, a reovirus variant known as Reolysin has been shown to grow preferentially in cells with RAS activating mutations (found in about 90% of all pancreatic ductal adenocarcinomas). In pancreatic cancer cells, the virus causes an accumulation of viral protein products and accompanying ER stress and apoptosis. Adding bortezomib increases the rate of ER stress mediated apoptosis, as it would be expected after inhibiting a

major pathway for protein degradation in an already stressed cell [44]. Treatments like these carry future hope for a disease which is commonly accompanied by a bleak prognosis.

### Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is a cancer of liver hepatocytes. In the United States, it is the second most lethal cancer after PDAC, with an 8.9% 5-year survival. Etiology is broad, with risk factors including hepatitis B and C viruses, chronic alcohol consumption, diabetes, non-alcoholic fatty liver disease, aflatoxin-B1, as well as a variety of other liver diseases [45]. Common to all the listed etiologies is the capacity to induce ER stress [46-53]. From the turn of the 21<sup>st</sup> century, hepatitis C has become the primary associated risk factor for HCC in the US, with approximately 60% seropositivity [54].

A study of human tissue infected with hepatitis C virus found morphological evidence of ER stress as a dilated and dysfunctional phenotype. Phosphorylated PERK, activated ATF6, and spliced XBP1 was increased compared to uninfected tissue, indicating that there was activation of the UPR response proteins, however, downstream UPR genes such as those encoding for XBP1, ATF6, GADD34, molecular chaperones, and calcium pump ATP2A2 were not induced. GRP78, however was found to be accumulated, suggesting a post-transcriptional mechanism of upregulation that may serve to increase protein folding capacity [55]. GRP78 is also found constitutively overexpressed in HCC cells, which may confer chemotherapeutic resistance [48].

As GRP78 appears to confer resistance to chemotherapy, it may be a future therapeutic target. The histone deacetylase inhibitor vorinostat was reported to acetylate GRP78, causing dissociation from, and subsequent activation of PERK [56]. In Huh7 HCC cells treated with vorinostat, phosphorylation of PERK and eIF2 $\alpha$  was observed. Autophagy was induced, reportedly through downregulation of the Akt/mTOR pathway [57]. The UPR may be culpable, as ER stress has been shown to suppress the AKT/mTOR pathway [58].

As HCC is resistant to most chemotherapeutic agents, reducing this resistance may prove effective as a novel agent. Doxorubicin is widely used to treat HCC, although there is no clear survival benefit [59]. Doxorubicin resistance has been shown to be conferred through the UPR in multiple studies by pretreating with the known ER stress inducer tunicamycin [60, 61]. Researchers have found that by treating HepG2 HCC cells with the plant derived phenolic compound paeonol, doxorubicin resistance

was abrogated by preventing ER stress induced activation of COX-2 and the downstream PI3K/Akt pathway [60]. Further research will be needed to determine if these pathways are effective *in vivo*, and ultimately in clinical cancer treatment.

### Radiotherapy as an Inducer of ER Stress

Radiotherapy is accepted as a mainstay treatment against a wide variety of malignancies, including some of the most common cancers such as lung, breast, and prostate cancers. It has the advantage of being focal, such that the damage can be localized around the target tissue, avoiding systemic toxicity. Radiotherapy is not without risk however, as it can result in toxicity to any tissues in the beam path, and it can result in secondary malignancies. The classical understanding of the effects of radiotherapy centers around the generation of DNA single and double strand breaks either directly through DNA ionization or through interaction with generated reactive oxygen species. Unsuccessful repair of these breaks results in cell death [62]. A more complicated picture of the effects of radiotherapy has become apparent, with activation of multiple cell signaling cascades such as activation of membrane sphingomyelinase, activation of p53, activation of cell death receptors, and through induction of ER stress [63-65]. As ER stress plays a key role in cell survival as well as apoptosis, induction of ER stress or pharmacological manipulation of downstream UPR pathways may provide a key to improving the therapeutic ratio, resulting in increased tumor cell killing and reduction of toxicity to surrounding tissue.

Many studies have associated IR with ER stress and activation of the UPR in both cancer cells as well as normal cells [66-71]. In MCF-7 breast cancer cells, IR caused activation of the PERK-eIF2 $\alpha$  pathway. This was related to cell death, as PERK knockdown through siRNA conferred survival [68]. In another study of MDA-MB-231 cancer cells, similar results were found, in that PERK knockdown conferred survival to IR. Expression of downstream proteins GRP78 and pro-apoptotic CHOP however, were not increased despite phosphorylation of eIF2 $\alpha$  by PERK. This may suggest that IR induced ER stress acts in a CHOP independent manner [69]. Not all research indicates that PERK knockdown increases survival. Another group found that siRNA interference of PERK, ATF4, LAMP3 as well as pharmacological inhibition of PERK leads to decreased clonogenic survival to IR [72].

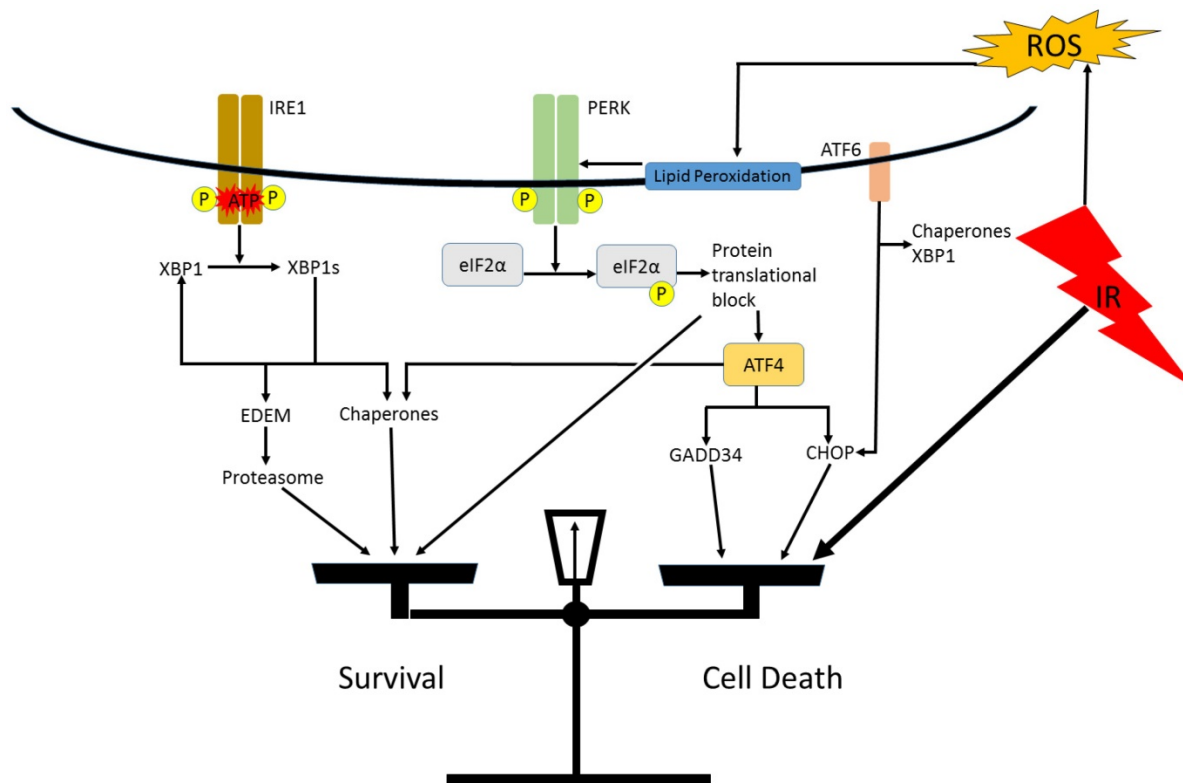
Research in normal tissues shows activation of the UPR in response to IR. Pulmonary artery endothelial cells were found to respond to IR doses of

up to 50 Gy by activation of UPR signal transduction pathways associated with PERK and ATF6, although IRE1 splicing of XBP1 was not detected [66]. Normal rat intestinal epithelial (IEC-6 line) cells treated with 10 Gy of IR was reported to result in ER stress. UPR pathways associated with PERK and IRE1 were activated, including detection of XBP1 splicing, but the ATF6 pathway was not involved [67]. In human vascular endothelial cells, only the PERK- eIF2 $\alpha$  pathway was activated in response to IR. These researchers postulated that ER stress may not be induced in this cell type, rather the PERK arm of the UPR may be activated due to interaction with lipid peroxidation products in the ER membrane (Figure 3) [70].

Current literature suggests that IR can result in ER stress and/or activation of the UPR in at least certain cell types *in vitro*, however, research has not yet elucidated the mechanism by which this occurs. Zhang et al. postulates that reactive oxygen species resulting from IR activates ER membrane receptors associated with the UPR [67]. This theory is consistent with other research showing ER stress induction by chemical induction of reactive oxygen species (ROS) [73, 74]. There has been little *in vivo* research on ER stress human subjects after radiotherapy, but one recent study found evidence of UPR activation via a

significant upregulation of UPR genes (an average of more than 4-fold increase) including ATF4, CHOP, XBP1, GRP78, LC3, and ATG5 in peripheral lymphocytes after exposure to as little as 1 Gy when compared to non-irradiated control subjects [75]. Further research is necessary to determine how IR activates UPR effectors, by what mechanism, and whether it is dependent on cell type, or another undiscovered factor.

As the UPR clearly plays a role in the response to radiotherapy, pharmacological manipulation of the UPR or induction of ER stress could be utilized to radiosensitize cancer cells. As cancer cells may rely on the UPR more heavily for survival, this type of treatment could preferentially affect cancer cells while sparing normal cells. The most basic method would be administration of an agent known to invoke ER stress, followed by administration of radiotherapy. The HIV protease inhibitor, nelfinavir, has been shown to inhibit the proteasome. As the proteasome is critical for degradation of misfolded proteins, inhibition is hypothesized to result in the buildup of misfolded proteins in the ER causing ER stress. After administration of nelfinavir to SQ20B head and neck cancer cells, phosphorylation of PERK and eIF2 $\alpha$  was noted, along with increased expression of the downstream protein GADD34. These treated cells



**Figure 3. A hypothetical model of IR induced ER stress and activation of the UPR.** IR generated reactive oxygen products react with lipids in the ER membrane. These peroxidation products activate the PERK arm of the UPR. If a strong enough ER stress response is invoked, the balance will be tipped towards apoptosis via the pro-apoptotic UPR genes.

showed decreased surviving fraction on clonogenic assay after administration of IR, indicating radiosensitization [76]. Research on the proteasome inhibitor, bortezomib, has shown similar radiosensitization, although they have focused on pathways other than the UPR [77]. Other inducers of ER stress such as celecoxib and monascuspiloin have been demonstrated as radiosensitizers *in vitro* [78, 79].

A recent novel research pharmaceutical, GSK2606414, has been identified to selectively inhibit PERK, which could act as a radiosensitizer. It has been shown to be orally bioavailable, and inhibit tumor growth *in vivo* [80]. When combined with exposure to radiotherapy in glioblastoma cells, a 70-80% reduction in the genes downstream PERK was noted [81]. Further radiobiology research should certainly explore the effect of this drug along with radiation on various cancer cell lines. Future drugs like this could elucidate the mechanism of radiation resistance and possibly lead to a potent radiosensitizing therapeutic regimen.

Radiotherapy, along with surgery and chemotherapy make up the classic triad in the treatment of cancer. When these fail due to lack of efficacy or excessive side effects, there is the promise of the rapidly growing field of immunotherapy, made up of treatments such as interferons, cytokines, monoclonal antibodies, checkpoint inhibitors, dendritic cell therapy, T-cell engaging antibodies, and autologous vaccines.

### Immune Modulation by ER Stress

ER stress is becoming recognized as having a role in the fundamental state of immune system, allowing and often promoting propagation of cancer cells. Furthermore, ER stress is recognized as playing an integral role, in cancer cells evading the immune system, through dysfunction in professional antigen presenting cells (APC), or modulation of antigen presentation through major histocompatibility complex (MHC) molecules. Research into these pathways may lead to future cancer immunotherapies that allow the immune system to identify these cancer cells.

Besides its role in the survival of tumor cells which have a large burden of misfolded proteins, the UPR is involved in producing the inflammatory microenvironment conducive to tumor growth. Cancer cells under ER stress have been shown to transmit ER stress to surrounding macrophages, causing upregulation of GRP78, GADD34, CHOP, and spliced XBP1 [82]. These macrophages in turn release pro-inflammatory cytokines, chemokines, and metalloproteases. Two specific secreted cytokines,

IL-6 and IL-23, have been implicated in tumor growth [83, 84]. Dendritic cells (DCs) are also responsive to transmitted ER stress in the tumor environment, releasing not only pro-inflammatory, pro-tumorigenic cytokines in response to transmitted ER stress, but also arginase, which effects T cells in a suppressive manner [85]. DCs have also been shown to increase IL-23 transcription and secretion upon the induction of ER stress, with CHOP as the responsible transcription factor, thus linking the UPR to specific DC released tumor growth factors [86]. Even in a pro-tumorigenic environment, cancer cells must evade the immune system to proliferate.

Antigen presentation is key to the immune system's ability to mount an effective response to malignant cells. Antigens are presented on cell surface MHC class I from a sampling of cytosolic and ER contents. As ER stress changes the distribution of proteins in the cytosol and ER via PERK-mediated protein translational attenuation, and causes upregulation of molecular chaperones and protein degradation via the ERAD system and proteasome, it would be expected that the UPR would influence antigen presentation. Evidence suggests that epitopes derived from defective proteins are preferentially presented on MHC class I molecules, likely due to increased degradation rate of these problem proteins [87, 88]. This does not necessarily translate into increased presentation of antigens on the surface of cells undergoing ER stress. Cells under ER stress have been shown to have decreased MHC class I antigen presentation, hypothetically believed to be due to global protein translational attenuation [89]. Additionally, the role of a micro-RNA, miRNA-346, has been implicated in reduced antigen presentation of cells under ER stress. Spliced XBP1 induces miRNA-346, resulting in a decrease in antigen presentation through attenuation of MHC class I, interferon induced genes, and transporter associated with antigen processing 1 (TAP1) [90]. This pathway can be connected to human malignancy, as miRNA-346 has been identified to be significantly overexpressed in follicular thyroid carcinoma [91].

DCs can be looked upon as the master controllers of adaptive immunity, and are necessary for T-cell dependent immunity against cancer cells. UPR associated protein XBP1 has been reported to be constitutively active in DCs, and identified as necessary for development and differentiation [92, 93]. IRE1, upstream of XBP1 has been implicated in the control of DC antigen processing and cell to cell contact in addition to maintaining ER homeostasis [93]. Some tumor types, such as ovarian carcinoma, can suppress dendritic cell mediated T cell immunity



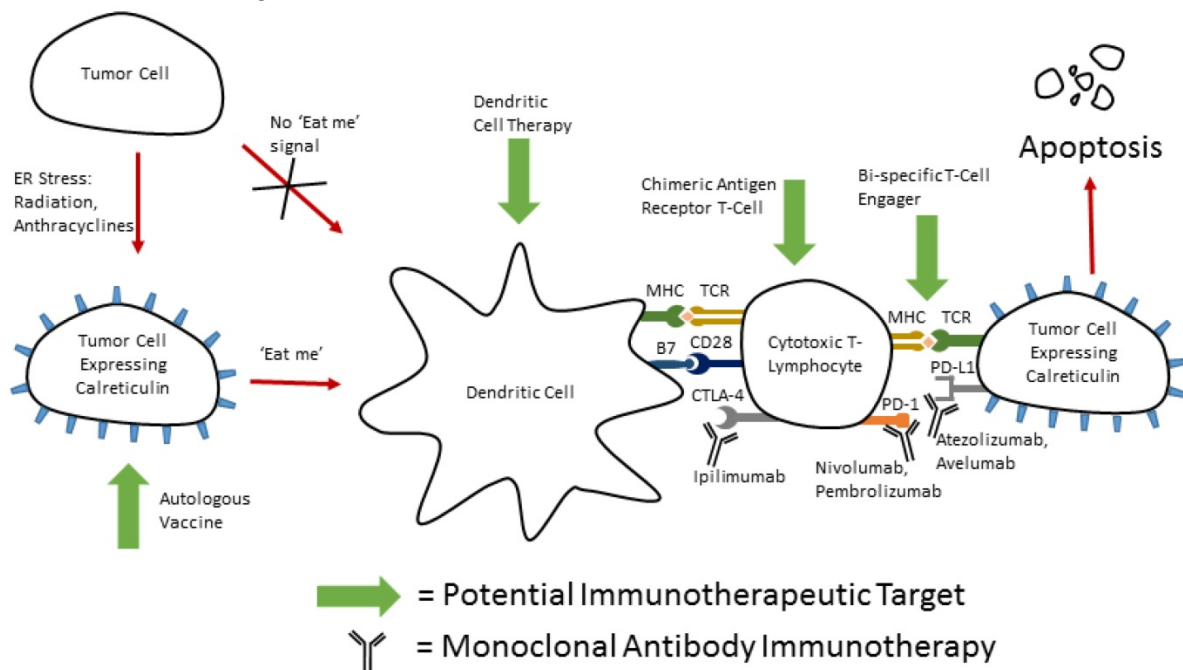
through a type of dendritic cell dysfunction. Lipid peroxide products lead to chronic ER stress in DCs, causing a high constitutive activation of XBP1, leading to triglyceride biosynthesis and accumulation [94]. This causes a dysfunction that can be alleviated through the suppression of XBP1, restoring the anti-tumor immune response. One might wonder if immune function could be restored by pharmacological inhibition of IRE1, such as through the proteasome inhibitor, bortezomib, which has been shown to suppress IRE1 and subsequent XBP1 splicing [95].

Functional DCs can lead to activation of cytotoxic T lymphocytes to mount an immune response to cancer cells under the correct conditions. Before they can activate these T cells, they must internalize antigen associated with the target cells. Calreticulin (CRT), an ER resident molecular chaperone has been shown to function as an “eat me” signal to DCs when exposed on the cell surface. CRT is presented on the cell surface after induction of ER stress in a PERK dependent fashion, reliant upon phosphorylation of eIF2 $\alpha$  [96-98]. Agents capable of inducing CRT exposure include gamma radiation, UVC light, and chemotherapeutic agents such as anthracyclines, and oxaliplatin [96, 99, 100]. Recent research hints that CRT may have a role in clinical oncology. In human patients with non-small cell lung cancer, there was a strong correlation between CRT

expression on the surface of tumor cells, and not only an increase in local DC and effector memory T-cells, but a better prognosis as well [101]. It is not understood, however, why some of these tumors have low CRT expression or whether treatment could be directed to increase expression. It is therefore a relevant question to ask whether we can invoke ER stress therapeutically to cause CRT expression and trigger immunogenic cell death. A fascinating potential therapy could be realized by combining inducers of CRT and antigen presentation with immunotherapy or cancer vaccines (Figure 4).

### Conclusion

The UPR is critically important for the survival of cells exposed to conditions of ER stress. Due to local tumor microenvironments, coupled with high protein synthesis demands, cancer cells are uniquely reliant on the UPR for survival in a way that normal cells are not. Future therapeutic targets could target specific UPR pathways that cancer cells rely upon, while leaving normal cells relatively intact. In cancer cells under chronic ER stress, therapy could be targeted to tip the balance towards apoptosis through the induction of additional ER stress through chemotherapy, radiotherapy as well as through more exotic means such as engineered viruses. An essential question is how the normal tissues will survive in the



**Figure 4. Bringing therapies together for multi-directional attack.** Potential therapeutic combination of immunotherapy and inducers of ER stress (radiation, or anthracyclines). These ER stressors induce the expression of calreticulin on the surface of tumor cells. This acts as a signal for phagocytosis of injured cells by dendritic cells, and subsequent activation of cell mediated immunity. Cells expression calreticulin from ER stress inducers such as ionizing radiation have been shown to have increased sensitivity to cytotoxic T-lymphocyte lysis [123].

presence of ER stress inducing treatment. It may be that without the existing insult of oxygen and nutrient starvation, upregulated UPR machinery and high protein demand, that ER stress is maintained at sub-lethal levels in comparison with rapidly dividing malignant cells. Future research should not only illuminate whether ER stress and the UPR can be harnessed against malignancies, but also elucidate the consequence to normal tissues of an insult great enough to drive malignant cells to a terminal ER stress response.

## Acknowledgement

This work was supported by grants from NIDDK K01DK096032 (S.S.).

## Competing Interests

The authors have declared that no competing interest exists.

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