Tumor progression and the Different Faces of the PERK kinase

Dariusz Pytel¹,³, Ireneusz Majsterek², and J. Alan Diehl¹,³

¹Department of Biochemistry and Molecular Biology, Hollings Cancer Center, Medical University of South Carolina, Charleston, SC 29425 ²Department of Clinical Chemistry and Biochemistry, Medical University of Lodz, Hallera 1, 90-647 Lodz, Poland

Abstract

The serine/threonine endoplasmic reticulum (ER) kinase, protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK), is a pro-adaptive protein kinase whose activity is regulated indirectly by protein misfolding within the ER. Since the oxidative folding environment in the ER is sensitive to a variety of cellular stresses, many of which occur during neoplastic transformation and in the tumor microenvironment, there has been considerable interest in defining whether PERK positively contributes to tumor progression and whether it represents a significant therapeutic target. Herein, we review the current knowledge of PERK-dependent signaling pathways, the contribution of downstream substrates including recently characterized new PERK substrates transcription factors FOXO (Forkhead box O protein) and diacylglycerol (DAG) a lipid signaling second messenger, and efforts to develop small molecule PERK inhibitors.

Keywords

PERK; UPR; ER stress; lipids; micro-RNA

Introduction

Proteins destined for secretion often require significant post-translational modifications necessary for proper folding and function. Secretory proteins are co-translationally imported into the endoplasmic reticulum (ER), where they undergo maturation and folding. The ER provides a chaperone rich, oxidizing environment where protein glycosylation and disulfide bond formation can be achieved in an orderly fashion prior to secretion. ER homeostasis depends upon balanced protein import and folding which is in turn dependent upon ER resident chaperones, ATP, and maintenance of the oxidative nature of the ER. Perturbation of this environment results in reduced protein folding and an accumulation of misfolded...
proteins within the ER. This accumulation of mis- or unfolded proteins provides a significant barrier in the secretory apparatus and is detrimental to cell and organismal homeostasis. As such, an evolutionarily conserved cell checkpoint mechanism termed the Unfolded Protein Response (UPR) functions to sense and facilitate adaption or cell execution in response to unfolded proteins. The mammalian UPR is composed of three main effectors of protein misfolding: PERK, Ire1α/β, and ATF6α/β. These three signal transducers collectively determine cellular fate in response to the accumulation of unfolded proteins.\textsuperscript{1–4}

Ire1 (α, ubiquitously expressed; β tissue restricted) is composed of a luminal domain that senses stress, a single transmembrane domain, and a cytosolic tail that contains both a protein kinase domain and an RNase domain.\textsuperscript{5,6} Ire1 regulates expression of numerous ER chaperones through activation of the X-box binding protein 1 (Xbp1) transcription factor.\textsuperscript{7} Activation of Xbp1 is mediated by the RNase function of Ire1, which triggers a splicing event that generates a shorter Xbp1 mRNA that is more efficiently translated.\textsuperscript{8,9} Activated IRE1 excises a 26-nucleotide intron from XBP1u mRNA (ubiquitously expressed, unspliced form, which encodes 267 amino acids, 33 kDa) and induces a frame shift resulting in a new translation product, XBP1s (spliced form of XBP1 mRNA encoding 371 amino acids, 54 kDa). XBP1s translocates to the nucleus and serves as a potent transcriptional activator. Xbp1s consist of the original amino-terminal DNA binding domain and a C-terminal transactivation domain. IRE1 through its RNA activity also regulates IRE1-dependent decay of mRNA (RIDD). This serves to reduce the load of proteins in the ER lumen, thus maintaining ER homeostasis. During chronic ER stress, RIDD triggers apoptosis by increasing caspase 2 translation following the cleavage of micro-RNAs such as miR-17, 34q, 96 and 125b.\textsuperscript{10} Xbp1 is also a transcriptional target of ATF6, an ER bound transcription factor induced by ER stress.\textsuperscript{9} While normally tethered to the ER, upon stress, ATF6 migrates to the trans-Golgi, where it is processed by S1P and S2P proteases to release the N-terminal DNA-binding transcription factor domain.\textsuperscript{11–13}

PERK (protein kinase R (PKR)-like endoplasmic reticulum kinase) or EIF2AK3 (eukaryotic translation initiation factor 2-alpha kinase 3), analogous with Ire1, is a serine/threonine transmembrane endoplasmic reticulum (ER) kinase. Established PERK substrates include the translation initiation factor eIF2α\textsuperscript{14,15} and the transcription factor Nrf2.\textsuperscript{16} Recent studies have identified new PERK substrates that include protein substrates such as FOXO\textsuperscript{17} and a lipid signaling second messenger diacylglycerol (DAG).\textsuperscript{18} PERK, Ire1 and ATF6 serve as a UPR control system in the ER to monitor cell homeostasis. Following stress, the UPR restores homeostasis via mechanisms that reduce ER protein load (eg. via RIDD, or eIF2α-mediated inhibition of translation), by increasing protein folding capacity (eg. transcriptional regulation of chaperones) and by activation of degradation pathways to remove unfolded proteins (ERAD, autophagy).

While the UPR can be triggered experimentally by agents that reduce the folding capacity of the ER, (eg. tunicamycin which inhibits glycosylation of asparagine residues; thapsigargin, inhibits SERCA sarco-/endoplasmic reticulum Ca2+-ATPase, and thereby depletes ER calcium), the UPR is activated by physiologically relevant stresses such as glucose or oxygen restriction,\textsuperscript{19,24} viral infection,\textsuperscript{25,27} proteotoxicity\textsuperscript{28,29} and alterations in membrane lipid composition\textsuperscript{30–34} (Figure 1). Since such stresses are prevalent in human diseases such
as cancer, obesity and neurodegenerative disorders and UPR signal transducers regulate cell fate in response to such stress, significant efforts have been made to develop small molecule inhibitors that might be useful in a clinical setting. While PERK was initially considered to harbor the strongest pro-survival function, it is now clear that all three transducers contribute to cell fate following stress. Thus far, highly selective small molecule inhibitors have been identified for both PERK and Ire1. The focus of this review will be on PERK and our current understanding of its contribution to cell homeostasis.

**UPR, PERK and checkpoint function**

Activation of the UPR is characterized in part by increased transcription of genes encoding ER molecular chaperones including BiP/GRP78 and GRP94, protein disulfide isomerase (PDI), and CHOP (C/EBP homologous protein), a transcription factor also known as growth arrest and DNA damage gene-153 (GADD153). Induction of ER chaperones function to correct protein misfolding and restore assembly within the ER. This is in turn coordinated with a marked decrease in the rate of overall protein synthesis and arrest in the G1 phase of the cell cycle, thereby limiting cell growth and expansion. Inhibition of protein synthesis lowers the overall rate of protein traffic into the ER. That this process is counterbalanced by increased synthesis of ER chaperones highlights the specificity of the UPR. ER stress-induced growth arrest occurs as a result of reduced translation of the critical G1/S-specific cyclin-D1. This system provides a checkpoint that prevents cells from continuing cell division under conditions in which the proper folding and assembly of proteins is significantly compromised. The failure of the UPR to reestablish proper homeostatic balance results in cell death via apoptosis.

While all UPR components contribute to cell homeostasis, PERK directly contributes to checkpoint function and cell survival through its capacity to regulate cell division. In general, cell cycle progression requires the activity of regulatory cyclins and their catalytic partners, the cyclin-dependent kinases (CDKs). Progression through G1 phase specifically requires the activities of the D-type cyclins (D1, D2, D3) in association with either CDK4 or CDK6 followed by activation of the cyclin E- and A-dependent kinase CDK2, as cells are near the G1/S transition. Cell cycle arrest is achieved through degradation of unstable cyclin subunits, by specific post-translational modifications of the CDK subunits, or via association of active cyclin-bound CDKs with polypeptide CDK inhibitors (CKIs). The Cip/Kip family of CKIs (including p21Cip1, p27Kip1, and p57Kip2) act as potent inhibitors of cyclin E-CDK2 and cyclin A-CDK2, they are positive regulators of cyclin D-CDK assembly and remain stably bound to catalytically active cyclin D-CDK complexes. In proliferating fibroblasts, most of the p21Cip1 and p27Kip1 molecules are found as components of active cyclin D-dependent holoenzymes. For example, withdrawal of growth factors inhibits cyclin D synthesis/translation, accelerates cyclin D turnover, and leads to the rapid disassembly of cyclin D-dependent kinases, thereby mobilizing sequestered Cip/Kip proteins from the latent pool and allowing the formation of inhibitory complexes of Cip/Kip with cyclin E- and A-CDK2. Coordinated inhibition of these cyclin-dependent kinases prevents entry into S phase, resulting in G1 phase arrest usually within a single cell cycle. PERK activation triggers an analogous response wherein activation of PERK results in the specific loss of cyclin D1 through inhibition of cyclin D1 protein.

*Oncogene.* Author manuscript; available in PMC 2016 May 18.
synthesis rather than any acceleration in protein degradation. This loss of cyclin D1 triggers cell cycle arrest in normal cells; importantly in tumor cells deficient for retinoblastoma protein (Rb), cyclin D1 is no longer required for proliferation and these cells are refractory to cell cycle regulation by the UPR that no longer require cyclin D1 for proliferation due to loss of its key downstream substrate. As discussed subsequently, PERK-dependent regulation of protein translation by direct phosphorylation of protein translation machinery is essential for this cellular response.

**PERK substrates**

**eIF2α and regulation of translation initiation**

Under homeostatic conditions, PERK exists as an inactive monomer associated with BiP (binding immunoglobulin protein) also known as GRP-78 (glucose-regulated protein). Following exposure of cells to ER stress, BiP is released from PERK, thereby permitting PERK oligomerization and activation.

The best characterized PERK substrate is eIF2α (Figure 2). EIF2, or translation initiation factor 2, is a heterotrimer composed of alpha, beta, and gamma (GTP-binding) subunits that regulate and coordinate the recruitment of the initial methionyl tRNA in a GTP-dependent manner. EIF2α, or the alpha subunit of the eIF2 complex, mediates the binding of the methionyl tRNA to the ribosome. PERK-dependent phosphorylation of eIF2α on serine 51 increases the affinity of eIF2α for the eIF2B guanine nucleotide exchange factor, thereby inhibiting exchange of GDP for GTP and ultimately reducing translation initiation. PERK is one of at least 4 distinct eIF2α protein kinases which include the heme-regulated kinase (HRI) also known as EIF2AK1 kinase, the interferon-inducible, RNA-dependent protein kinase (PKR) known as EIF2AK2 kinase and GCN2 known as EIF2AK4.

EIF2α phosphorylation inhibits translation of many cellular mRNAs (global translation inhibition); those with short half-lives, such cyclin D1, are rapidly depleted from the cell. Strikingly, eIF2α can also increase translational efficiency of select transcripts. Such examples include Activating Transcription Factor 4 (ATF4) and cellular inhibitor of apoptosis 1 and 2 (CIAP1/2). The noted increase in translation efficiency reflects the presence of a short uORF (upstream open reading frame) located in 5′ untranslated region (UTR). ATF4 translation is increased in response to variety of stresses including hypoxia, nutritional deprivation (amino acid limitation and glucose deprivation) and viral infection. ATF4 belongs to the cAMP-responsive element-binding protein (CREB) family of basic zipper-containing proteins. ATF4 regulates downstream expression of the proapoptotic protein, CHOP, during chronic stress to trigger apoptosis and cell death. Also, ATF4 and/or CHOP can regulate autophagy, a major cytoprotective mechanism, by transcriptional activation of the autophagy genes (p62, Nbr1, Atg3, Atg5, Atg7, Atg10, Atg12, Atg16l1, Beclin1, Map1lc3b, Gabarap, Gabarapl2. These genes are involved in the formation, elongation and function of the autophagosome. Induction of autophagy resembles another potent pro-survival pathway from which tumor cells can benefit (e.g. Myc-dependent activation of PERK/eIF2α/ATF4 pathways promotes transformation and
tumor growth and inhibition of PERK reduces Myc-induced autophagy and tumor formation). 

Nrf2 and redox homeostasis

UPR induction is associated with the generation of reactive oxygen species (ROS). The accumulation of ROS to high levels can trigger severe cell/tissue damage. Reactive oxygen can oxidize DNA, lipids, and proteins. To alleviate ROS induced stress, cells rely on signaling pathways that rapidly quench ROS and thereby limit damage. One such pathway is mediated by the Nrf2 (Nuclear factor erythroid-derived 2) transcription factor. Nrf2, a master regulator of redox homeostasis, is constitutively expressed, but its activity is regulated via association with a scaffolding protein, Keap1, that retains Nrf2 in the cytoplasm. Keap1 (Kelch-like ECH-associated protein 1) functions as an E3 ligase adaptor molecule that sequesters Nrf2 in the cytoplasm and targets it for ubiquitin-dependent degradation. Knockdown or knockout of Keap1 is associated with constitutively active Nrf2. While increased Nrf2 function indeed reduces ROS levels and has been considered as a chemoprevention strategy, constitutive Nrf2 activity is also associated with fibrosis and Nrf2 activating mutations have been identified in a variety of human cancers.

Given the ROS burst associated with ER stress, UPR signaling must have a mechanism to alleviate ROS and prevent significant damage. Indeed, PERK can regulate cellular redox homeostasis through activation of Nrf2. PERK phosphorylates Nrf2 on threonine 80 located within the Neh2 domain of Nrf2. Dissociation from Keap1 results in decreased Nrf2 degradation and subsequent increased Nrf2 nuclear import. Nuclear Nrf2 mediates expression of anti-oxidant enzymes through the ARE or anti-oxidant response element. Nrf2 target genes include NAD(P)H:quinone oxidoreductase 1 (NQO1), heme-oxygenase 1 (HO-1), glutathione S-transferase (GST) and glutamylcysteine synthetase ligase (GCLC), rendering Nrf2−/− mice susceptible to oxidative stress. Heterodimeric Nrf2 partners include small Maf proteins and ATF4, whose accumulation is under PERK-dependent translational control. Recent studies show that Nrf2 can be pre-activated in malignant carcinomas (which are typically de-differentiated cells and multidrug resistant (MDR)), via noncanonical PERK-dependent pathway (not activated by oxidation). Constitutive PERK-Nrf2 signaling, reduces ROS levels, increases drug efflux and protects de-differentiated cells from chemotherapy. Treatment with PERK inhibitors, sensitizes MDR cells to chemotherapy.

Forkhead/FOXO transcription factors

The Forkhead or FOXO transcription factor family regulates a diverse set of genes that contribute to organismal homeostasis. Invertebrates such as Drosophila express a single member, FOXO; in contrast, mammalian cells encode four family members; FOXO1, FOXO3, FOXO4 and FOXO6. Among the noted functions of FOXO family proteins is their regulation by Akt and their contribution to metabolic homeostasis. The FOXO transcription factor is typically regulated by Akt-dependent phosphorylation; phosphorylation generates 14-3-3 docking sites within FOXO. Engagement by 14-3-3 sequesters FOXO in the cytoplasm under conditions of high Akt activity. PERK was identified in an RNAi screen for modifiers of reduced FOXO activity in Drosophila. Additional work demonstrated that
PERK phosphorylates FOXO3 at serines 261, 298, 301, 303 and 311 and increase FOXO activity. In addition to the identification of unique eIF2α-independent PERK effectors, this finding has direct implications for the role of PERK and the UPR in the regulation of insulin tolerance. Previous work demonstrated that PERK promotes Akt activation, which in turn reduces FOXO function. The ability of PERK to directly regulate FOXO and potentially override negative regulation by Akt supports a model wherein the UPR and ER stress have the capacity to finely tune signal output downstream of Akt. If the model is correct, it has broad implications for the contribution of PERK to metabolic homeostasis and tumor progression, two systems wherein Akt has vast contributions. FOXO function can be regulated by multiple signaling such as Akt and SGK (many of these pathways are dysregulated in variety of cancers) therefore FOXO may play role in controlling proliferation and apoptosis of tumor cells.

Phosphatidic Acid and lipid biogenesis

Given the focus on the protein kinase activity of PERK and signals resulting from eIF2α phosphorylation, recent reports describing the ability of PERK to utilize certain lipids as a substrate provide a unique twist on PERK function. Investigation of the mechanism whereby PERK can regulate Akt activity resulted in the identification of diacylglycerol (DAG) as a direct PERK substrate. DAG is an important signaling second messenger in cells, contributing to the activity of PKC isoforms among other functions. In addition to its function as a second messenger, DAG is a precursor for phosphatidic acid (PA), which exhibits mitogenic properties contributing to the activation of Ras downstream of receptor tyrosine kinase engagement and thereby contributing to MAPK signaling. PA also triggers mTOR activation through direct binding and activation of Akt (Figure 3). PERK directly phosphorylates multiple DAG species and its kinase activity is induced by direct binding to p85; the regulatory subunit of a better known lipid kinase, phosphatidylinositol-3 kinase (PI3K). In cells, ER stress-dependent generation of PA is PERK-dependent and PA was found to be essential for Akt activation and maintenance of MAPK activity following exposure of cells to ER stress (Figure 2). It is interesting to consider why PERK signaling would coordinate cell cycle arrest while maintaining mitogenic signaling. At first glance, this might seem paradoxical. However, by maintaining MAPK and Akt signaling, PERK can both potentiate cell survival during moderate stress and provide a mechanism for recovery if cells do not commit to an apoptotic fate. Alternatively, the ability of PERK to regulate Akt and or MAPK signaling may contribute to cellular processes that do not reflect the acute stress associated with exposure to agents such as tunicamycin. Consistent with this notion, the DAG kinase activity of PERK plays an important role in adipocyte differentiation.

While PERK-dependent PA generation plays an important role in signal transduction, PERK has a broader impact on lipid biosynthesis and membrane remodeling. PERK signaling through eIF2α also contributes to lipogenic enzyme expression regulation. PERK activation in the developing mouse mammary gland contributes to expression of lipid biosynthetic enzymes such as: fatty acid synthase (FAS), ATP citrate lyase (ACL), and stearyl-CoA desaturase-1 (SCD1). The ability of PERK to induce expression of these genes reflects the translational regulation of Insig1, an inhibitor of sterol regulatory element binding protein.
(SREBP) activity, master regulators of fatty acid and cholesterol biosynthesis. ER stress is associated with the generation of sphingolipids and ceramides through unknown mechanisms. Finally, PERK activity is also sensitive to membrane fluidity, which is a feature of membrane lipid composition. Ultimately, PERK-dependent regulation of lipid biosynthesis not only provides second messengers important for cell fate, but also provides metabolic intermediates necessary for processes such as cell division, autophagy, and secretion, all of which depend upon lipid biosynthesis. Recent studies indicate that NEU3 (plasma membrane-associated sialidase) can interact with PA and play important role in regulation of transmembrane signaling, and promote malignancy in various cancers.

**PERK signaling and micro-RNAs**

The contribution of small noncoding RNAs (microRNAs or miRNAs) to gene expression and protein synthesis has gained considerable traction. Given the decrease in protein synthesis and wide ranging alterations in gene expression patterns observed following engagement of the UPR, the absence of research addressing contributions of miRNAs to cell homeostasis is surprising. During the past several years, several groups have addressed this understudied topic and not surprisingly have found that miRNAs are differentially regulated by the UPR. More specifically, two distinct miRNA families have been noted to respond to PERK. The first, the miR-106b-25 cluster is repressed upon PERK signaling. MiR-106b-25 is dependent upon PERK- activation of Nrf2 and Atf4. Repression of miR-106b-25 permits accumulation of Bim and apoptosis in chronically stressed cells. The second miRNAs to respond to PERK are miR-211 and miR-204. MiR-211 is embedded within an intron of trpm1 while miR-204 is located within intronic sequences of trpm3. Expression of both is coordinated with host gene expression and dependent upon PERK signaling through eIF2α and ATF4. The critical miR-211 target with respect to ER stress is chop/gadd153 a key pro-apoptotic transcription factor. An important aspect of miR-211/204 expression following PERK activation is the transient nature of miRNA accumulation, with maximal accumulation occurring at 5h post stress and a return to basal levels by 8h. This suggests an important role for temporal miR-211/204 function. The identification of chop/gadd153 as the relevant miR-211/204 target emphasizes the importance of temporal regulation of miR-211/204, as their rapid induction antagonizes premature chop/gadd153 expression. In turn, their loss under conditions of chronic stress permits chop/gadd153 accumulation and commitment to cell death in severely damaged cells.

MiR-30c-2-3p is yet another miR that is regulated by PERK signaling. PERK-dependent regulation of miR-30c-2-3p is downstream of NF-κB signaling. NF-κB activation reflects loss of IκB, an inhibitor of NF-κB, and IκB loss is a direct consequence of PERK-dependent inhibition of IκB translation. The relevant miR-30c-2-3p target is Xbp1. Thus, PERK-dependent induction of this micro-RNA serves to limit the transcriptional activity of Xbp1 and thus serves as one point of cross-talk between PERK and Ire1 signaling pathways.

Ire1 signaling has also been linked with micro-RNA accumulation. Unlike PERK where regulation depends upon induction of downstream transcriptional effectors, Ire1 engages
micro-RNAs through its inherent RNase function. Among the key targets of miR-17, miR-34a, miR-96, and miR-125b is caspase. UPR engagement triggers Ire1-dependent cleavage of precursors of miR-17, miR-34a, miR-96 and miR-125b thereby reducing cellular levels of these pro-survival micro-RNAs. Ire1-dependent cleavage occurs at sites distinct from dicer within the precursor molecules and is speculated to reduce the ability of dicer to process a mature micro-RNA. The ability of Ire1 to reduce pro-survival micro-RNAs during ER stress will ultimately help establish the point of no return for cell death.

Given the capacity of both PERK and Ire1 to engage micro-RNA-dependent pathways as a means to establish cell fate following exposure of cells to ER stress, one wonders whether the UPR might also regulate the proteome through long noncoding RNAs (lncRNA). As yet, there is no evidence for differential regulation of lncRNAs during the UPR. However, given our increasing appreciation for the contribution of lncRNAs to gene expression, it seems likely that they will also contribute to cell fate in cells experiencing ER stress.

**Cancer biology and PERK signaling**

PERK function has been linked with cell survival since its identification. Pathophysiologically, tumor progression is closely associated with intrinsic cell and microenvironmental stresses that trigger UPR activation. These include limitation of glucose and oxygen that occur as a result of dysregulated angiogenesis, increased lipid metabolism and improper folding of proteins. Tumor development is also associated with increased levels of reactive oxygen species (ROS) that contribute to cellular DNA damage. From these considerations blossomed the notion that UPR inhibition and more specifically PERK inhibition might elicit anti-tumorigenic effects.

Initial efforts to address the contribution of PERK to tumorigenesis focused on genetic ablation of PERK or expression of dominant negative PERK alleles. In early transformation assays, PERK null fibroblasts were shown to be sensitive to transformation by oncogenes such as K-Ras. However, upon transplantation of transformed PERK−/− fibroblasts into immune compromised mice, a significant inhibition of tumor growth was noted. The reduced growth was attributed to compromised angiogenesis and the sensitivity of PERK deficient cells to the ensuing hypoxic environment. Analogous findings were noted in genetically engineered mice. Intercrossing MMTV-Neu mice with PERK−/− mice revealed no delay in tumor development, but a significant defect in tumor progression and a dramatic reduction in metastatic spread. In contrast to previous work, no alterations were noted in tumor vascularity when comparing PERK+/+ and −/− mice. The reduction in tumor progression was attributed to extensive DNA damage, triggered by increased ROS accumulation. In addition, the pro-survival PERK regulated micro-RNA, miR-211/204, was also reduced in PERK deficient tumors supporting the pro-survival function of this microRNA. While further work is necessary to ascertain the precise contribution of reduced miR-211/204 expression which altered tumor progression, miR-211 expression correlated with gadd153/chop expression in both murine tumors and human lymphomas suggesting it functions to potentiate cell survival both in vitro and in vivo.
The initial focus on the pro-tumorigenic properties of PERK suggested a large therapeutic window, with regard normal tissue toxicity. In contrast, conventional PERK knockout mice exhibit significant developmental defects, generally associated with disruption of secretory tissues as might be expected. Perinatal death associated with embryonic PERK deletion reflected pancreatic failure and a significant disruption of glucose homeostasis. These observations were initially thought to reflect a restricted PERK contribution to developing tissue, as mice where PERK excision was delayed until late embryogenesis were essentially normal. Based upon this later work, it was assumed that PERK function was non-essential in the adult organism. More recently, however, generation of mice wherein PERK can be conditionally deleted with a tamoxifen inducible CRE enzyme definitively demonstrated that PERK excision resulted in destruction of pancreatic tissue, both exocrine and endocrine, independent of age. The importance of PERK function for pancreatic homeostasis represents a significant barrier for the implementation of anti-PERK therapeutic strategies.

Recent work has implicated PERK activity in chronic myeloid leukemia (CML). Imatinib mesylate (STI571), a specific inhibitor of the BCR/ABL, is remarkably effective during initial phases of the disease, but following blast crisis, leukemia cells acquire marked resistance. Increased PERK activation and upregulation of eIF2α pathway have been observed in BCR/ABL positive leukemia cell lines that are resistant to Imatinib, and it has been suggested that PERK inhibition might sensitize CML cells to treatment.

Although much of the published work has focused on tumor intrinsic functions of PERK, there is also evidence for microenvironmental impacts of PERK signaling with regard to tumor progression. For example, UPR and PERK activation is associated with the production of pro-inflammatory cytokines. Conditioned media from tumor cells can induce a UPR like signature in stromal fibroblasts, including TLR4-dependent increased expression of grp78, grp94, gadd153/chop and spliced xbp1. In addition, ER stress and PERK have been implicated in dampening the effects of type 1 interferon. IFN has robust anti-tumor activity in vitro, but limited impact clinically. While as yet untested, it is tempting to speculate that PERK activation may limit IFN activity through direct regulation of the interferon receptor and thus regulates IFN signaling.

PERK and Neurodegenerative disorders

While the potential contribution of PERK to tumorigenesis has garnered considerable attention, PERK is also strongly implicated in the development and progression of neurodegenerative diseases. PERK activation and phosphorylation is observed in Alzheimer’s disease (AD), Parkinson’s disease (PD), amyotrophic lateral sclerosis (ALS), and prion disease (PD). Beta-amyloid plaques (AB) and neurofibrillary tangles (NFT) are prevalent and defining features of Alzheimer disease. The AB plaques are one of several aggregates of misfolded proteins that are observed in affected regions of AD brain. The aggregation of these proteins has been considered a potential protective mechanism that prevents toxicity induced by smaller molecular weight monomers or multimers. Consistent with UPR engagement, increased phosphorylation of eIF2α is observed in the hippocampus of AD patients. In a guinea pig model of AD, UPR activation can induce amyloid
precursor protein (APP, the peptide from which AB fragments are derived) expression in the central nervous system (CNS). Proteolytic processing of APP, including the cleavages that produce AB, largely occurs in the ER and localization is coincident with PERK activity. Based on these findings, we hypothesize that chronic PERK activation in AD neurons leads to excessive accumulation of APP and subsequently AB, thereby contributing to disease progression. Clearly, treatment of this multifaceted disease will require more than a single therapy as well as early diagnosis; however, inhibiting PERK activity is a strong candidate for an intervention that will synergize with other approaches to protect against neuronal and synaptic loss by reducing AB load.

First generation small molecule PERK inhibitors and Concluding Remarks

The recent generation of PERK-specific small molecule inhibitors provides an opportunity to determine how well genetic models that attempt to identify “drugable” targets, such as PERK, predict the clinical behavior of small molecules. The compound GSK2606414 was the first reported small molecular inhibitor of PERK. GSK2606414 is an ATP competitive inhibitor highly specific to PERK (more than 300-fold selectivity for PERK versus other kinases was reported) and has shown very low nanomolar range activity in cell cultures (IC$_{50}$ around 30nM can prevent PERK phosphorylation). In addition, a TR-FRET based high-throughput-screening assay (HTS) was used to screen 79,552 compounds and 2 ATP non-competitive lead compounds exhibiting PERK specificity were identified. Both compounds worked at low micro molar range in both in vitro experiments and cell cultures.

Based on GSK2606414, a second compound, GSK2656157, was developed for preclinical studies. GSK2656157 exhibited promising results in multiple human tumor xenograft models. Consistent with murine genetically engineered mouse models of PERK deficiency, mice receiving GSK2656157 exhibited significant pancreatic toxicity. It may yet be possible to optimize drug dosage or combine with other therapies and thereby limit toxic side effects. Combining PERK inhibitors with current standards of care may provide an avenue to reduce doses and limit potential toxicities associated with either therapy. One possible combination could include combining PERK inhibitors with proteasome inhibitors like Velcade, a current therapy commonly used for treating patients with Multiple Myeloma. Multiple Myeloma affects antibody secreting immune cells. The highly secretory nature of these target cells likely endows this cancer with its sensitivity to a proteasome inhibitor, which is known to trigger ER stress. The use of a PERK inhibitor in this context could sensitize Multiple Myeloma cells to Velcade thereby reducing the dose of Velcade necessary for effective Multiple Myeloma eradication and thus reduce the toxicity of Velcade. A more complete and detailed understanding of PERK downstream signaling is essential for developing such approaches.

As with many molecularly defined targets, the potential efficacy of PERK-based therapy remains unsettled. Genetic approaches have defined both the potential efficacy of such an approach and side toxicities. The advantage of small molecule therapies is their reversibility and the potential to control dose. Both of these issues will need further delineation to subvert potential toxicities and maximize anti-tumor effects.
Acknowledgments

This work was supported by National Institutes of Health grant CA104838 (JAD); Polish National Science Centre (NCN) “HARMONIA 5” grant No. 2013/10/M/N21/00280.

References

1. Schroder M. The unfolded protein response. Mol Biotechnol. 2006; 34:279–290. [PubMed: 17172673]
2. Schroder M. Endoplasmic reticulum stress responses. Cell Mol Life Sci. 2008; 65:862–894. [PubMed: 18038217]
3. Ulianich L, Insabato L. Endoplasmic reticulum stress in endometrial cancer. Front Med (Lausanne). 2014; 1:55. [PubMed: 25593927]
4. Volmer R, Ron D. Lipid-dependent regulation of the unfolded protein response. Curr Opin Cell Biol. 2015; 33C:67–73. [PubMed: 25543896]
5. Tirasesophon W, Welihinda AA, Kaufman RJ. A stress response pathway from the endoplasmic reticulum to the nucleus requires a novel bifunctional protein kinase/endoribonuclease (Ire1p) in mammalian cells. Genes Dev. 1998; 12:1812–1824. [PubMed: 9637683]
6. Yoshida H, Haze K, Yanagi H, Yura T, Mori K. Identification of the cis-acting endoplasmic reticulum stress response element responsible for transcriptional induction of mammalian glucose-regulated proteins. Involvement of basic leucine zipper transcription factors. J Biol Chem. 1998; 273:33741–33749. [PubMed: 9837962]
7. 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]
8. Calfon 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]
9. Yoshida H, Matsui T, Yamamoto A, Okada T, Mori K. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. Cell. 2001; 107:881–891. [PubMed: 11779464]
10. Upton JP, Wang L, Han D, Wang ES, Huskey NE, Lim L, et al. IRE1alpha cleaves select microRNAs during ER stress to derepress translation of proapoptotic Caspase-2. Science. 2012; 338:818–822. [PubMed: 23042294]
11. Haze K, Yoshida H, Yanagi H, Yura T, Mori K. Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress. Mol Biol Cell. 1999; 10:3787–3799. [PubMed: 10564271]
12. Okada T, Haze K, Nanakata S, Yoshida H, Seidah NG, Hirano Y, et al. A serine protease inhibitor prevents endoplasmic reticulum stress-induced cleavage but not transport of the membrane-bound transcription factor ATF6. J Biol Chem. 2003; 278:31024–31032. [PubMed: 12782636]
13. Shen J, Chen X, Hendershot L, Prywes R. ER stress regulation of ATF6 localization by dissociation of BiP/GRP78 binding and unmasking of Golgi localization signals. Dev Cell. 2002; 3:99–111. [PubMed: 12110171]
14. Harding HP, Zhang Y, Ron D. Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. Nature. 1999; 397:271–274. [PubMed: 9930704]
15. Shi Y, Vattem KM, Sood R, An J, Liang J, Stramm L, et al. Identification and characterization of pancreatic eukaryotic initiation factor 2 alpha-subunit kinase, PEK, involved in translational control. Mol Cell Biol. 1998; 18:7499–7509. [PubMed: 9819435]
16. 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]
17. Zhang W, Hietakangas V, Wee S, Lim SC, Gunaratne J, Cohen SM. ER stress potentiates insulin resistance through PERK-mediated FOXO phosphorylation. Genes Dev. 2013; 27:441–449. [PubMed: 23431056]
18. Bobrovnikova-Marjon E, Pytel D, Riese MJ, Vaites LP, Singh N, Koretzky GA, et al. PERK utilizes intrinsic lipid kinase activity to generate phosphatidic acid, mediate Akt activation, and promote adipocyte differentiation. Mol Cell Biol. 2012; 32:2268–2278. [PubMed: 22493067]

19. Blais JD, Filipenko V, Bi M, Harding HP, Ron D, Koumenis C, et al. Activating transcription factor 4 is translationally regulated by hypoxic stress. Mol Cell Biol. 2004; 24:7469–7482. [PubMed: 15314157]

20. Koumenis C, Nazczi K, Koritzinsky M, Rastani S, Diehl A, Sonenberg N, et al. Regulation of protein synthesis by hypoxia via activation of the endoplasmic reticulum kinase PERK and phosphorylation of the translation initiation factor eIF2alpha. Mol Cell Biol. 2002; 22:7405–7416. [PubMed: 12370288]

21. 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]

22. Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol. 2007; 8:519–529. [PubMed: 17565364]

23. Saito S, Furuno A, Sakurai J, Sakamoto A, Park HR, Shin-Ya K, et al. Chemical genomics identifies the unfolded protein response as a target for selective cancer cell killing during glucose deprivation. Cancer Res. 2009; 69:4225–4234. [PubMed: 19435925]

24. de la Cadena SG, Hernandez-Fonseca K, Camacho-Arroyo I, Massieu L. Glucose deprivation induces reticulum stress by the PERK pathway and caspase-7- and calpain-mediated caspase-12 activation. Apoptosis. 2014; 19:414–427. [PubMed: 24185830]

25. Isler JA, Skalet AH, Alwine JC. Human cytomegalovirus infection activates and regulates the unfolded protein response. J Virol. 2005; 79:6890–6899. [PubMed: 15890928]

26. Cox JS, Chapman RE, Walter P. The unfolded protein response coordinates the production of endoplasmic reticulum protein and endoplasmic reticulum membrane. Mol Biol Cell. 1997; 8:1805–1814. [PubMed: 9307975]

27. Kozutsumi Y, Segal M, Normington K, Gething MJ, Sambrook J. The presence of malfolded proteins in the endoplasmic reticulum signals the induction of glucose-regulated proteins. Nature. 1988; 332:462–464. [PubMed: 3352747]

28. Pineau L, Colas J, Dupont S, Beney L, Fleurat-Lessard P, Berjeaud JM, et al. Lipid-induced ER stress: synergistic effects of sterols and saturated fatty acids. Traffic. 2009; 10:673–690. [PubMed: 19302420]

29. Feng B, Yao PM, Li Y, Devlin CM, Zhang D, Harding HP, et al. The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages. Nat Cell Biol. 2003; 5:781–792. [PubMed: 12907943]

30. Karaskov E, Scott C, Zhang L, Teodoro T, Ravazzola M, Volchuk A. Chronic palmitate but not oleate exposure induces endoplasmic reticulum stress, which may contribute to INS-1 pancreatic beta-cell apoptosis. Endocrinology. 2006; 147:3398–3407. [PubMed: 16601139]

31. Kincaid MM, Cooper AA. ERADicate ER stress or die trying. Antioxid Redox Signal. 2007; 9:2373–2387. [PubMed: 17883326]

32. Marciniak SJ, Ron D. Endoplasmic reticulum stress signaling in disease. Physiol Rev. 2006; 86:1133–1149. [PubMed: 17015486]
37. Kim I, Xu W, Reed JC. Cell death and endoplasmic reticulum stress: disease relevance and therapeutic opportunities. Nat Rev Drug Discov. 2008; 7:1013–1030. [PubMed: 19043451]

38. Hoozemans JJ, Veerhuis R, Van Haastert ES, Rozemuller JM, Baas F, Eikelenboom P, et al. The unfolded protein response is activated in Alzheimer’s disease. Acta Neuropathol. 2005; 110:165–172. [PubMed: 15973543]

39. Chang RC, Wong AK, Minthorn E, Zhang SY, Figueroa DJ, Moss K, et al. Characterization of a novel PERK kinase inhibitor with antitumor and antiangiogenic activity. Cancer Res. 2013; 73:1993–2002. [PubMed: 23339398]

40. Atkins C, Liu Q, Minthorn E, Zhang SY, Figueroa DJ, Moss K, et al. Characterization of a novel PERK kinase inhibitor with antitumor and antiangiogenic activity. Cancer Res. 2013; 73:1993–2002. [PubMed: 23339398]

41. Axten JM, Medina JR, Feng Y, Shu A, Romeril SP, Grant SW, et al. Discovery of 7-methyl-5-(1-[[3-(trifluoromethyl)phenyl]acyl]-2,3-dihydro-1H-indol-5-yl]-7H-pyrrolo[2,3-d]pyrimidin-4-amine (GSK2606414), a potent and selective first-in-class inhibitor of protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK). J Med Chem. 2012; 55:7193–7207. [PubMed: 22827572]

42. Axten JM, Romeril SP, Shu A, Ralph J, Medina JR, Feng Y, et al. Discovery of GSK2656157: An Optimized PERK Inhibitor Selected for Preclinical Development. ACS Med Chem Lett. 2013; 4:964–968. [PubMed: 24900593]

43. Pytel D, Seyb K, Liu M, Ray SS, Concannon J, Huang M, et al. Enzymatic Characterization of ER Stress-Dependent Kinase, PERK, and Development of a High-Throughput Assay for Identification of PERK Inhibitors. J Biomol Screen. 2014; 19:1024–1034. [PubMed: 24598103]

44. Papandreou I, Denko NC, Olson M, Van Melckebeke H, Lust S, Tam A, et al. Identification of an Ire1alpha endonuclease specific inhibitor with cytotoxic activity against human multiple myeloma. Blood. 2011; 117:1311–1314. [PubMed: 21081713]

45. Volkmann K, Lucas JL, Vuga D, Wang X, Brumm D, Stiles C, et al. Potent and selective inhibitors of the inositol-requiring enzyme 1 endonuclease. J Biol Chem. 2011; 286:12743–12755. [PubMed: 21303903]

46. Cross BC, Bond PJ, Sadowski PG, Jha BK, Zak J, Goodman JM, et al. The molecular basis for selective inhibition of unconventional mRNA splicing by an IRE1-binding small molecule. Proc Natl Acad Sci U S A. 2012; 109:E869–878. [PubMed: 22315414]

47. Ghosh R, Wang L, Wang ES, Perera BG, Igbiaia A, Morita S, et al. Allosteric inhibition of the Ire1alpha RNase preserves cell viability and function during endoplasmic reticulum stress. Cell. 2014; 158:534–548. [PubMed: 25018104]

48. Brewer JW, Cleveland JL, Hendershot LM. A pathway distinct from the mammalian unfolded protein response regulates expression of endoplasmic reticulum chaperones in non-stressed cells. Embo J. 1997; 16:7207–7216. [PubMed: 9384597]

49. Ma K, vattem KM, Wek RC. Dimerization and release of molecular chaperone inhibition facilitate activation of eukaryotic initiation factor-2 kinase in response to endoplasmic reticulum stress. J Biol Chem. 2002; 277:18728–18735. [PubMed: 11907036]

50. 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]

51. Chikka MR, McCabe DD, Tyra HM, Rutkowski DT. C/EBP Homologous Protein (CHOP) Contributes to Suppression of Metabolic Genes during Endoplasmic Reticulum Stress in the Liver*. J Biol Chem. 2013; 288:4405–4415. [PubMed: 23281479]

52. DeZwaan-McCabe D, Riordan JD, Arensdorf AM, Icardi MS, Dupuy AJ, Rutkowski DT. The Stress-Regulated Transcription Factor CHOP Promotes Hepatic Inflammatory Gene Expression, Fibrosis, and Oncogenesis. PLoS Genet. 2013;9.

53. Brewer JW, Diehl JA. PERK mediates cell-cycle exit during the mammalian unfolded protein response. Proc Natl Acad Sci U S A. 2000; 97:12625–12630. [PubMed: 11035797]

54. Brewer JW, Hendershot LM, Sherr CJ, Diehl JA. Mammalian unfolded protein response inhibits cyclin D1 translation and cell-cycle progression. Proc Natl Acad Sci U S A. 1999; 96:8505–8510. [PubMed: 10411905]
55. 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]

56. Cox JS, Walter P. A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response. Cell. 1996; 87:391–404. [PubMed: 8898193]

57. Zinszner H, Kuroda M, Wang X, Batchvarova N, Lightfoot RT, Remotti H, et al. CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. Genes Dev. 1998; 12:982–995. [PubMed: 9531536]

58. Han J, Back SH, Hur J, Lin YH, Gildersleeve R, Shan J, et al. ER-stress-induced transcriptional regulation increases protein synthesis leading to cell death. Nat Cell Biol. 2013; 15:481–490. [PubMed: 23624402]

59. Sherr CJ. G1 phase progression: cycling on cue. Cell. 1994; 79:551–555. [PubMed: 7954821]

60. Morgan DO. Principles of CDK regulation. Nature. 1995; 374:131–134. [PubMed: 7877684]

61. Sherr CJ, Roberts JM. Inhibitors of mammalian G1 cyclin-dependent kinases. Genes Dev. 1995; 9:1149–1163. [PubMed: 7758941]

62. Cheng M, Olivier P, Diehl JA, Fero M, Roussel MF, Roberts JM, et al. The p21(Cip1) and p27(Kip1) CDK ‘inhibitors’ are essential activators of cyclin D-dependent kinases in murine fibroblasts. Embo J. 1999; 18:1571–1583. [PubMed: 10075928]

63. LaBaer J, Garrett MD, Stevenson LF, Slingerland JM, Sandhu C, Chou HS, et al. New functional activities for the p21 family of CDK inhibitors. Genes Dev. 1997; 11:847–862. [PubMed: 9106657]

64. Blain SW, Montalvo E, Massague J. Differential interaction of the cyclin-dependent kinase (Cdk) inhibitor p27Kip1 with cyclin A-Cdk2 and cyclin D2-Cdk4. J Biol Chem. 1997; 272:25863–25872. [PubMed: 9325318]

65. Hall M, Peters G. Genetic alterations of cyclins, cyclin-dependent kinases, and Cdk inhibitors in human cancer. Adv Cancer Res. 1996; 68:67–108. [PubMed: 8712071]

66. Hamanaka RB, Bobrovnikova-Marjon E, Ji X, Liebhaber SA, Diehl JA. PERK-dependent regulation of IAP translation during ER stress. Oncogene. 2009; 28:910–920. [PubMed: 19029953]

67. Harding HP, Novoa I, Zhang Y, Zeng H, Wek R, Schapira M, et al. Regulated translation initiation controls stress-induced gene expression in mammalian cells. Mol Cell. 2000; 6:1099–1108. [PubMed: 1106749]

68. Miller PF, Hinnebusch AG. cis-acting sequences involved in the translational control of GCN4 expression. Biochim Biophys Acta. 1990; 1050:151–154. [PubMed: 2207139]

69. Yaman I, Fernandez J, Liu H, Caprara M, Komar AA, Koromilas AE, et al. The zipper model of translational control: a small upstream ORF is the switch that controls structural remodeling of an mRNA leader. Cell. 2003; 113:519–531. [PubMed: 12757712]

70. Qian Z, Xuan B, Chapa TJ, Gualberto N, Yu D. Murine cytomegalovirus targets transcription factor ATF4 to exploit the unfolded-protein response. J Virol. 2012; 86:6712–6723. [PubMed: 22496230]

71. B’Chir W, Maurin AC, Carraro V, Averous J, Tiranti V, Zeviani M, et al. The eIF2alpha/ATF4 pathway is essential for stress-induced autophagy gene expression. Nucleic Acids Res. 2013; 41:7683–7699. [PubMed: 23804767]

72. Hart LS, Cunningham JT, Datta T, Dey S, Tameire F, Lehman SL, et al. ER stress-mediated autophagy promotes Myc-dependent transformation and tumor growth. J Clin Invest. 2012; 122:4621–4634. [PubMed: 23143306]

73. Sabharwal SS, Schumacker PT. Mitochondrial ROS in cancer: initiators, amplifiers or an Achilles’ heel? Nat Rev Cancer. 2014; 14:709–721. [PubMed: 25342630]

74. Guzy RD, Hoyos B, Robin E, Chen H, Liu L, Mansfield KD, et al. Mitochondrial complex III is required for hypoxia-induced ROS production and cellular oxygen sensing. Cell Metab. 2005; 1:401–408. [PubMed: 16054089]

75. Brunelle JK, Bell EL, Quesada NM, Vercauteren K, Tiranti V, Zeviani M, et al. Oxygen sensing requires mitochondrial ROS but not oxidative phosphorylation. Cell Metab. 2005; 1:409–414. [PubMed: 16054090]
76. Kobayashi A, Kang MI, Okawa H, Ohitsuji M, Zenke Y, Chiba T, et al. Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. Mol Cell Biol. 2004; 24:7130–7139. [PubMed: 15282312]

77. Cullinan SB, Gordan JD, Jin J, Harper JW, Diehl JA. The Keap1-BTB protein is an adaptor that bridges Nrf2 to a Cul3-based E3 ligase: oxidative stress sensing by a Cul3-Keap1 ligase. Mol Cell Biol. 2004; 24:8477–8486. [PubMed: 15367669]

78. Zhang DD, Lo SC, Cross JV, Templeton DJ, Hannink M. Keap1 is a redox-regulated substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex. Mol Cell Biol. 2004; 24:10941–10953. [PubMed: 15572695]

79. Furukawa M, Xiong Y. BTB protein Keap1 targets antioxidant transcription factor Nrf2 for ubiquitination by the Cullin 3-Roc1 ligase. Mol Cell Biol. 2005; 25:162–171. [PubMed: 15601839]

80. Cullinan SB, Diehl JA. Coordination of ER and oxidative stress signaling: the PERK/Nrf2 signaling pathway. Int J Biochem Cell Biol. 2006; 38:317–332. [PubMed: 16290097]

81. Frohlich DA, McCabe MT, Arnold RS, Day ML. The role of Nrf2 in increased reactive oxygen species and DNA damage in prostate tumorigenesis. Oncogene. 2008; 27:4353–4362. [PubMed: 18372916]

82. Petzer JP, Navamal M, Johnson JK, Kwak MK, Kensler TW, Fishbein JC. Phase 2 enzyme induction by the major metabolite of oltipraz. Chem Res Toxicol. 2003; 16:1463–1469. [PubMed: 14615973]

83. Kanamori M, Higa T, Sonoda Y, Murakami S, Dodo M, Kitamura H, et al. Activation of the Nrf2 pathway and its impact on the prognosis of anaplastic glioma patients. Neuro Oncol. 2014

84. Shibata T, Ohta T, Tong KL, Kokubu A, Odogawa R, Tsuta K, et al. Cancer related mutations in Nrf2 impair its recognition by Keap1-Cul3 E3 ligase and promote malignancy. Proc Natl Acad Sci U S A. 2008; 105:13568–13573. [PubMed: 18757741]

85. 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; 29:3851–3855. [PubMed: 20453876]

86. Alam J, Stewart D, Touchard C, Boinapally S, Choi AM, Cook JL. Nrf2, a Cap’n’Collar transcription factor, regulates induction of the heme oxygenase-1 gene. J Biol Chem. 1999; 274:26071–26078. [PubMed: 10475555]

87. Chan K, Kan YW. Nrf2 is essential for protection against acute pulmonary injury in mice. Proc Natl Acad Sci U S A. 1999; 96:12731–12736. [PubMed: 10535991]

88. Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, et al. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun. 1997; 236:313–322. [PubMed: 920432]

89. Itoh K, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, Engel JD, et al. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. Genes Dev. 1999; 13:76–86. [PubMed: 987101]

90. Nguyen T, Huang HC, Pickett CB. Transcriptional regulation of the antioxidant response element. Activation by Nrf2 and repression by MafK. J Biol Chem. 2000; 275:15466–15473. [PubMed: 1079702]

91. Venugopal R, Jaiswal AK. Nrf1 and Nrf2 positively and c-Fos and Frl negatively regulate the human antioxidant response element-mediated expression of NAD(P)H:quinone oxidoreductase1 gene. Proc Natl Acad Sci U S A. 1996; 93:14960–14965. [PubMed: 8962164]

92. Wild AC, Moinova HR, Mulcahy RT. Regulation of gamma-glutamylcysteine synthetase subunit gene expression by the transcription factor Nrf2. J Biol Chem. 1999; 274:33627–33636. [PubMed: 10595251]

93. Chan JY, Kwong M. Impaired expression of glutathione synthetic enzyme genes in mice with targeted deletion of the Nrf2 basic-leucine zipper protein. Biochim Biophys Acta. 2000; 1517:19–26. [PubMed: 1118612]

94. Hayes JD, Chanas SA, Henderson CJ, McMahon M, Sun C, Moffat GJ, et al. The Nrf2 transcription factor contributes both to the basal expression of glutathione S-transferases in mouse
liver and to their induction by the chemopreventive synthetic antioxidants, butylated hydroxyanisole and ethoxyquin. Biochem Soc Trans. 2000; 28:33–41. [PubMed: 10816095]

95. Leung L, Kwong M, Hou S, Lee C, Chan JY. Deficiency of the Nrf1 and Nrf2 transcription factors results in early embryonic lethality and severe oxidative stress. J Biol Chem. 2003

96. Li W, Yu S, Liu T, Kim JH, Blank V, Li H, et al. Heterodimerization with small Maf proteins enhances nuclear retention of Nrf2 via masking the NESzip motif. Biochim Biophys Acta. 2008; 1783:1847–1856. [PubMed: 18585411]

97. Dhakshinamoorthy S, Jaiswal AK. Small maf (MafG and MafK) proteins negatively regulate antioxidant response element-mediated expression and antioxidant induction of the NAD(P)H:Quinone oxidoreductase1 gene. J Biol Chem. 2000; 275:40134–40141. [PubMed: 11013233]

98. Gong P, Hu B, Stewart D, Ellerbe M, Figueroa YG, Blank V, et al. Cobalt induces heme oxygenase-1 expression by a hypoxia-inducible factor-independent mechanism in Chinese hamster ovary cells: regulation by Nrf2 and MafG transcription factors. J Biol Chem. 2001; 276:27018–27025. [PubMed: 11356853]

99. 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]

100. Del Vecchio CA, Feng Y, Sokol ES, Tillman EJ, Sanduja S, Reinhardt F, et al. De-Differentiation Confers Multidrug Resistance Via Noncanonical PERK-Nrf2 Signaling. PLoS Biol. 2014; 12:e1001945. [PubMed: 25203443]

101. Weigel D, Jurgens G, Kuttner F, Seifert E, Jackle H. The homeotic gene fork head encodes a nuclear protein and is expressed in the terminal regions of the Drosophila embryo. Cell. 1989; 57:645–658. [PubMed: 2566386]

102. Huang H, Tindall DJ. Dynamic FoxO transcription factors. J Cell Sci. 2007; 120:2479–2487. [PubMed: 17646672]

103. Hu P, Han Z, Couvillon AD, Exton JH. Critical role of endogenous Akt/IAPs and MEK1/ERK pathways in countering endoplasmic reticulum stress-induced cell death. J Biol Chem. 2004; 279:49420–49429. [PubMed: 15339911]

104. Carballo-Pescador S, Mauriz JL, Garcia-Palomo A, Gonzalez-Gallego J. FoxO proteins: regulation and molecular targets in liver cancer. Curr Med Chem. 2014; 21:1231–1246. [PubMed: 24372208]

105. Keniry M, Pires MM, Mense S, Lefebvre C, Gan B, Justiano K, et al. Survival factor NFIL3 restricts FOXO-induced gene expression in cancer. Genes Dev. 2013; 27:916–927. [PubMed: 23630076]

106. Fu Z, Tindall DJ. FOXOs, cancer and regulation of apoptosis. Oncogene. 2008; 27:2312–2319. [PubMed: 18391973]

107. Xie L, Ushmorov A, Leithauser F, Guan H, Steidl C, Farbinger J, et al. FOXO1 is a tumor suppressor in classical Hodgkin lymphoma. Blood. 2012; 119:3503–3511. [PubMed: 22343918]

108. Spitzer JA, Deaciu JC, Rodriguez de Turco EB, Roth BL, Hermiller JB, Mehegan JP. Modification of protein kinase C (PKC) activity and diacylglycerol (DAG) accumulation in hepatocytes in continuous endotoxemia. Prog Clin Biol Res. 1989; 308:575–588. [PubMed: 2780715]

109. Poli A, Ramazzotti G, Matteucci A, Manzoli L, Lonetti A, Suh PG, et al. A novel DAG-dependent mechanism links PKCa and Cyclin B1 regulating cell cycle progression. Oncotarget. 2014

110. Szendroedi J, Yoshimura T, Phielix E, Koliaki C, Marcucci M, Zhang D, et al. Role of diacylglycerol activation of PKCtheta in lipid-induced muscle insulin resistance in humans. Proc Natl Acad Sci U S A. 2014; 111:9597–9602. [PubMed: 2497806]

111. Natalini PM, Mateos MV, Ilincheta de Boschero MG, Giusto NM. A novel light-dependent activation of DAGK and PKC in bovine photoreceptor nuclei. Exp Eye Res. 2014; 125:142–155. [PubMed: 24950064]
112. Mor A, Campi G, Du G, Zheng Y, Foster DA, Dustin ML, et al. The lymphocyte function-associated antigen-1 receptor costimulates plasma membrane Ras via phospholipase D2. Nat Cell Biol. 2007; 9:713–719. [PubMed: 17486117]

113. Zhao C, Du G, Skowronek K, Frohman MA, Bar-Sagi D. Phospholipase D2-generated phosphatidic acid couples EGFR stimulation to Ras activation by Sos. Nat Cell Biol. 2007; 9:706–712. [PubMed: 17486115]

114. Rizzo MA, Shome K, Vasudevan C, Stolz DB, Sung TC, Frohman MA, et al. Phospholipase D and its product, phosphatidic acid, mediate agonist-dependent ras-1 translocation to the plasma membrane and the activation of the mitogen-activated protein kinase pathway. J Biol Chem. 1999; 274:1131–1139. [PubMed: 9873061]

115. Fang Y, Vilella-Bach M, Bachmann R, Flanagan A, Chen J. Phosphatidic acid-mediated mitogenic activation of mTOR signaling. Science. 2001; 294:1942–1945. [PubMed: 11729323]

116. Toschi A, Lee E, Xu L, Garcia A, Gadir N, Foster DA. Regulation of mTORC1 and mTORC2 complex assembly by phosphatidic acid: competition with rapamycin. Mol Cell Biol. 2009; 29:1411–1420. [PubMed: 19114562]

117. Lim HK, Choi YA, Park W, Lee T, Ryu SH, Kim SY, et al. Phosphatidic acid regulates systemic inflammatory responses by modulating the Akt-mammalian target of rapamycin-p70 S6 kinase 1 pathway. J Biol Chem. 2003; 278:45117–45127. [PubMed: 12960176]

118. Bobrovnikova-Marjon E, Hatzivassiliou G, Grigoriadou C, Romero M, Cavener DR, Thompson CB, et al. PERK-dependent regulation of lipogenesis during mouse mammary gland development and adipocyte differentiation. Proc Natl Acad Sci U S A. 2008; 105:16314–16319. [PubMed: 18852460]

119. Desvergne B, Michalik L, Wahl W. Transcriptional regulation of metabolism. Physiol Rev. 2006; 86:465–514. [PubMed: 16601267]

120. Espenshade PJ, Hughes AL. Regulation of sterol synthesis in eukaryotes. Annu Rev Genet. 2007; 41:401–427. [PubMed: 17666007]

121. Kim YL, Park K, Kim JY, Seo HS, Shin KO, Lee YM, et al. An Endoplasmic Reticulum Stress-Initiated Sphingolipid Metabolite, Ceramide-1-Phosphate, Regulates Epithelial Innate Immunity by Stimulating beta-Defensin Production. Mol Cell Biol. 2014; 34:4368–4378. [PubMed: 25312644]

122. Volmer R, van der Ploeg K, Ron D. Membrane lipid saturation activates endoplasmic reticulum unfolded protein response transducers through their transmembrane domains. Proc Natl Acad Sci U S A. 2013; 110:4628–4633. [PubMed: 23487760]

123. Shiozaki K, Takahashi K, Hosono M, Yamaguchi K, Hata K, Shiozaki M, et al. Phosphatidic acid-mediated activation and translocation to the cell surface of sialidase NEU3, promoting signaling for cell migration. Faseb J. 2015.

124. Gupta S, Read DE, Deepti A, Cawley K, Gupta A, Oommen D, et al. Perk-dependent repression of miR-106b-25 cluster is required for ER stress-induced apoptosis. Cell Death Dis. 2012; 3:e333. [PubMed: 22739985]

125. Chitnis NS, Pytel D, Bobrovnikova-Marjon E, Pant D, Zheng H, Maas NL, et al. miR-211 Is a Prosurvival MicroRNA that Regulates chop Expression in a PERK-Dependent Manner. Mol Cell. 2012; 48:353–364. [PubMed: 23022383]

126. Jiang HY, Wek SA, McGrath D, Scheuner D, Kaufman RJ, Cavener DR, et al. Phosphorylation of the alpha subunit of eukaryotic initiation factor 2 is required for activation of NF-kappaB in response to diverse cellular stresses. Mol Cell Biol. 2003; 23:5651–5663. [PubMed: 12897138]

127. Deng J, Lu PD, Zhang Y, Scheuner D, Kaufman RJ, Sonenberg N, et al. Translational repression mediates activation of nuclear factor kappa B by phosphorylated translation initiation factor 2. Mol Cell Biol. 2004; 24:10161–10168. [PubMed: 15542827]

128. Byrd AE, Aragon IV, Brewer JW. MicroRNA-30c-2* limits expression of proadaptive factor XBPl in the unfolded protein response. J Cell Biol. 2012; 196:689–698. [PubMed: 22431749]

129. Chitnis N, Pytel D, Diehl JA. UPR-inducible miRNAs contribute to stressful situations. Trends Biochem Sci. 2013; 38:447–452. [PubMed: 23906563]

130. Coelho DS, Domingos PM. Physiological roles of regulated Ire1 dependent decay. Front Genet. 2014; 5:76. [PubMed: 24795742]

Oncogene. Author manuscript; available in PMC 2016 May 18.
131. Suzuki HI, Arase M, Matsuyama H, Choi YL, Ueno T, Mano H, et al. MCPIP1 ribonuclease antagonizes dicer and terminates microRNA biogenesis through precursor microRNA degradation. Mol Cell. 2011; 44:424–436. [PubMed: 22055188]

132. Balkwill FR, Capasso M, Hagemann T. The tumor microenvironment at a glance. J Cell Sci. 2012; 125:5591–5596. [PubMed: 23420197]

133. Ackerman D, Simon MC. Hypoxia, lipids, and cancer: surviving the harsh tumor microenvironment. Trends Cell Biol. 2014; 24:472–478. [PubMed: 24985940]

134. 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]

135. 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]

136. 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]

137. 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]

138. Gao Y, Sartori DJ, Li C, Yu QC, Kushner JA, Simon MC, et al. PERK Is Required in the Adult Pancreas and is Essential for Maintenance of Glucose Homeostasis. Mol Cell Biol. 2012; 32:5129–5139. [PubMed: 23071091]

139. Kusio-Kobialka M, Podszywalow-Bartnicka P, Peidis P, Glodkowska-Mrowka E, Wolanin K, Leszak G, et al. The PERK-eIF2alpha phosphorylation arm is a pro-survival pathway of BCR-ABL signaling and confers resistance to imatinib treatment in chronic myeloid leukemia cells. Cell Cycle. 2012; 11:4069–4078. [PubMed: 23095523]

140. Zhang K, Shen X, Wu J, Sakaki K, Saunders T, Rutkowski DT, et al. Endoplasmic reticulum stress activates cleavage of CREBH to induce a systemic inflammatory response. Cell. 2006; 124:587–599. [PubMed: 16469704]

141. 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 U S A. 2011; 108:6561–6566. [PubMed: 21464300]

142. Garg AD, Kaczmarek A, Krysko O, Vandenabeele P, Krysko DV, Agostinis P. ER stress-induced inflammation: does it aid or impede disease progression? Trends Mol Med. 2012; 18:589–598. [PubMed: 22883813]

143. Mahadevan NR, Anufreichik V, Rodvold JJ, Chiu KT, Sepulveda H, Zanetti M. Cell-extrinsic effects of tumor ER stress imprint myeloid dendritic cells and impair CD8(+) T cell priming. PLoS One. 2012; 7:e51845. [PubMed: 23272178]

144. Meares GP, Liu Y, Rajbhandari R, Qin H, Nozell SE, Moley JA, et al. PERK-dependent activation of JAK1 and STAT3 contributes to endoplasmic reticulum stress-induced inflammation. Mol Cell Biol. 2014; 34:3911–3925. [PubMed: 25113558]

145. Wang M, Kaufman RJ. The impact of the endoplasmic reticulum protein-folding environment on cancer development. Nat Rev Cancer. 2014; 14:581–597. [PubMed: 25145482]

146. Yan Y, Gao YY, Liu BQ, Niu XF, Zhuang Y, Wang HQ. Resveratrol-induced cytotoxicity in human Burkitt’s lymphoma cells is coupled to the unfolded protein response. BMC Cancer. 2010; 10:445. [PubMed: 20723265]

147. Oda T, Kosuge Y, Arakawa M, Ishige K, Ito Y. Distinct mechanism of cell death is responsible for tunicamycin-induced ER stress in SK-N-SH and SH-SY5Y cells. Neurosci Res. 2008; 60:29–39. [PubMed: 18029041]

148. Mohammad MK, Avila D, Zhang J, Barve S, Arteel G, McClain C, et al. Acrolein cytotoxicity in hepatocytes involves endoplasmic reticulum stress, mitochondrial dysfunction and oxidative stress. Toxicol Appl Pharmacol. 2012; 265:73–82. [PubMed: 23026831]
149. Wang S, Kaufman RJ. The impact of the unfolded protein response on human disease. J Cell Biol. 2012; 197:857–867. [PubMed: 22733998]

150. Bhattacharya S, Huang Fu WC, Dong G, Qian J, Baker DP, Karar J, et al. Anti-tumorigenic effects of Type 1 interferon are subdued by integrated stress responses. Oncogene. 2013; 32:4214–4221. [PubMed: 23045272]

151. Hami LS, Green C, Leshinsky N, Markham E, Miller K, Craig S. GMP production and testing of Xcellerated T Cells for the treatment of patients with CLL. Cytotherapy. 2004; 6:554–562. [PubMed: 15764021]

152. Bhattacharya S, Zheng H, Tzimas C, Carroll M, Baker DP, Fuchs SY. Bcr-abl signals to desensitize chronic myeloid leukemia cells to IFNalpha via accelerating the degradation of its receptor. Blood. 2011; 118:4179–4187. [PubMed: 21821707]

153. Rosalia RA, Silva AL, Camps M, Allam A, Jiskoot W, van der Burg SH, et al. Efficient ex vivo induction of T cells with potent anti-tumor activity by protein antigen encapsulated in nanoparticles. Cancer Immunol Immunother. 2013; 62:1161–1173. [PubMed: 23613147]

154. Lin W, Bailey SL, Ho H, Harding HP, Ron D, Miller SD, et al. The integrated stress response prevents demyelination by protecting oligodendrocytes against immune-mediated damage. J Clin Invest. 2007; 117:448–456. [PubMed: 17273557]

155. Lees JR, Cross AH. A little stress is good: IFN-γ, demyelination, and multiple sclerosis. J Clin Invest. 2007; 117:297–299. [PubMed: 17273549]

156. Ryu EJ, Harding HP, Angelastro JM, Vitolo OV, Ron D, Greene LA. Endoplasmic reticulum stress and the unfolded protein response in cellular models of Parkinson’s disease. J Neurosci. 2002; 22:10690–10698. [PubMed: 12486162]

157. Hoozemans JJ, van Haastert ES, Eikelenboom P, de Vos RA, Rozemuller JM, Schepers W. Activation of the unfolded protein response in Parkinson’s disease. Biochem Biophys Res Commun. 2007; 354:707–711. [PubMed: 17254549]

158. Nagata T, Iiwea H, Murakami T, Shiote M, Narai H, Ohta Y, et al. Increased ER stress during motor neuron degeneration in a transgenic mouse model of amyotrophic lateral sclerosis. Neurol Res. 2007; 29:767–771. [PubMed: 17672929]

159. Wilhelmus MM, Verhaar R, Andringa G, Bol JG, Cras P, Shank L, et al. Presence of tissue transglutaminase in granular endoplasmic reticulum is characteristic of melanized neurons in Parkinson’s disease brain. Brain Pathol. 2011; 21:130–139. [PubMed: 20731657]

160. Wang L, Popko B, Roos RP. The unfolded protein response in familial amyotrophic lateral sclerosis. Hum Mol Genet. 2011; 20:1008–1015. [PubMed: 21159797]

161. Wang L, Popko B, Tixier E, Roos RP. Guanabenz, which enhances the unfolded protein response, ameliorates mutant SOD1-induced amyotrophic lateral sclerosis. Neurobiol Dis. 2014; 71:317–324. [PubMed: 25134731]

162. Moreno JA, Radford H, Peretti D, Steinert JR, Verity N, Martin MG, et al. Sustained translational repression by eIF2alpha-P mediates prion neurodegeneration. Nature. 2012; 485:507–511. [PubMed: 22622579]

163. Finnie JW, Manavis J, Blumbergs PC, Kuchel TR. Axonal and neuronal amyloid precursor protein immunoreactivity in the brains of guinea pigs given tunicamycin. Vet Pathol. 2000; 37:677–680. [PubMed: 11105962]

164. Moreno JA, Halliday M, Molloy C, Radford H, Verity N, Axten JM, et al. Oral treatment targeting the unfolded protein response prevents neurodegeneration and clinical disease in prion-infected mice. Sci Transl Med. 2013; 5:206ra138.

165. Obeng EA, Carlson LM, Gutman DM, Harrington WJ Jr, Lee KP, Boise LH. Proteasome inhibitors induce a terminal unfolded protein response in multiple myeloma cells. Blood. 2006; 107:4907–4916. [PubMed: 16507771]
Figure 1.
PERK activation caused by a variety of cellular stresses. PERK can be activated by physiologically relevant stresses such as glucose deprivation, oxygen restriction (hypoxia), viral infection, proteotoxicity (increased load of misfolded/unfolded proteins in ER) and increased lipid biosynthesis.
Figure 2.
Direct PERK substrates. Activated PERK, in response to ER stress, phosphorylates downstream substrates such as: translation initiation factor 2α (eIF2α), transcription factors FOXO (Forkhead box O protein), nuclear factor erythroid-derived 2 transcription factor (Nrf2) and a lipid signaling second messenger diacylglycerol (DAG) and regulates cell homeostasis.
Figure 3.
PERK lipid kinase activity and regulation of downstream effectors. PERK possesses lipid kinase activity toward its substrate diacylglycerol (DAG), forming phosphatidic acid (PA) and activating AKT, mTOR and MAP kinase pathways.