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Tumor progression and the Different Faces of the PERK kinase

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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 diacyglycerol (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

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Conflict of Interest

The authors declare no conflict of interest.

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.[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.[5,6] Ire1 regulates expression of numerous ER chaperones through activation of the X-box binding protein 1 (Xbp1) transcription factor.[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.[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.[10] Xbp1 is also a transcriptional target of ATF6, an ER bound transcription factor induced by ER stress.[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.[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[14,15] and the transcription factor Nrf2.[16] Recent studies have identified new PERK substrates that include protein substrates such as FOXO[17] and a lipid signaling second messenger diacylglycerol (DAG).[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,[19–24] viral infection,[25–27] proteotoxicity[28,29] and alterations in membrane lipid composition[30–34] (Figure 1). Since such stresses are prevalent in human diseases such

as cancer, obesity and neurodegenerative disorders[35–39] 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[40–43] and Ire1.[44–47] 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*).[48–52] 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[53–55] 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.[56] ER stress-induced growth arrest occurs as a result of reduced translation of the critical G1/S-specific cyclin-D1.[54] 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.[57,58]

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.[53,59] 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).[60,61] The Cip/Kip family of CKIs (including $p21^{Cip1}$, $p27^{Kip1}$, and $p57^{Kip2}$) 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.[53,62,63] In proliferating fibroblasts, most of the $p21^{Cip1}$ and $p27^{Kip1}$ molecules are found as components of active cyclin D-dependent holoenzymes.[53,62,64] 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.[65] 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

synthesis rather than any acceleration in protein degradation.[53] 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.[53,54,65] 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[14,15] (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.[14]

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).[66] The noted increase in translation efficiency reflects the presence of a short uORF (upstream open reading frame) located in 5′ untranslated region (UTR).[67–69] ATF4 translation is increased in response to variety of stresses including hypoxia, nutritional deprivation (amino acid limitation and glucose deprivation) and viral infection.[19,27,50,70] 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 gens (*p62*, *Nbr1*, *Atg3, Atg5, Atg7, Atg10, Atg12, Atg16l1, Becn1, Map1lc3b*, *Gabarap*, *Gabarapl2*. These genes are involved in the formation, elongation and function of the autophagosome.[71] 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).[72]

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.[73–75] 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.[76–79] 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,[80–82] constitutive Nrf2 activity is also associated with fibrosis and Nrf2 activating mutations have been identified in a variety of human cancers.[77,83,84]

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.[16] PERK phosphorylates Nrf2 on threonine 80 located within the Neh2 domain of Nrf2.[85] 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.[86–89] 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),[86,88,90–92] rendering Nrf2−/− mice susceptible to oxidative stress.[87,93–95] Heterodimeric Nrf2 partners include small Maf proteins[88,90,96–98] and ATF4, whose accumulation is under PERK-dependent translational control.[67,99] 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.[100]

Forkhead/FOXO transcription factors

The Forkhead or FOXO transcription factor family regulates a diverse set of genes that contribute to organismal homeostasis.[101,102] 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.[17] PERK was identified in an RNAi screen for modifiers of reduced FOXO activity in *Drosphila*.[17] Additional work demonstrated that

PERK phosphorylates FOXO3 at serines 261, 298, 301, 303 and 311 and increase FOXO activity.[17] 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,[18,66,103] 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 dysgerulated in variety of cancers) therefore FOXO may play role in controlling proliferation and apoptosis of tumor cells.[104_107]

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[66,103] activity resulted in the identification of diacyglycerol (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.[108_111] 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[112,113] and thereby contributing to MAPK signaling.[18,114] PA also triggers mTOR activation through direct binding and activation of Akt[18,115_117] (Figure 3). PERK directly phosphorylates multiple DAG species[18] 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;[118] master regulators of fatty acid and cholesterol biosynthesis.[119,120] ER stress is associated with the generation of sphingolipids and ceramides[121] through unknown mechanisms. Finally, PERK activity is also sensitive to membrane fluidity, which is a feature of membrane lipid composition.[4,122] 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.[123]

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.[124] 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.[125] MiR-211 is embedded within an intron of *trpm1* while miR-204 is located within intronic sequences of *trpm3*.[125] 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.[126,127] The relevant miR-30c-2-3p target is XbpI.[128] 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.[10,129] Among the key targets of miR-17, miR-34a, miR-96, and miR-125b is caspase 2.[10,130] 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.[10] 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.[10,131] 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.[14,99] 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.[21,23,132,133] 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.[134] However, upon transplantation of transformed PERK−/− fibroblasts into immune compromised mice, a significant inhibition of tumor growth was noted.[19,134] 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.[85] 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.[125,129] 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.[125]

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.[135_137] 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 in PERK excision was delayed until late embryogenesis were essentially normal.[135_137] 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.[138] 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.[139] 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.[140_145] 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.*[146_149] In addition, ER stress and PERK have been implicated in dampening the effects of type 1 interferon.[150] IFN has robust anti-tumor activity in vitro, but limited impact clinically.[150_153] 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.[150,154,155]

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).[38,156_162] 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.[39] 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).[163] 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.[40–43,164] 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).[41] 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.[43]

Based on GSK2606414, a second compound, GSK2656157, was developed for preclinical studies.[40,42] GSK2656157 exhibited promising results in multiple human tumor xenograft models. Consistent with murine genetically engineered mouse models of PERK deficiency,[135,138] mice receiving GSK2656157 exhibited significant pancreatic toxicity.[40] 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.[165] 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.

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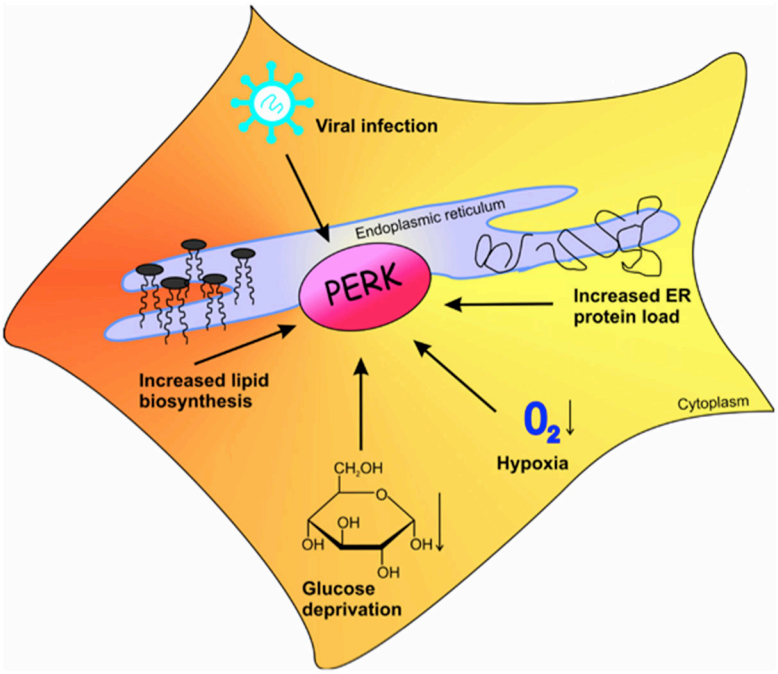

Figure 1.

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.

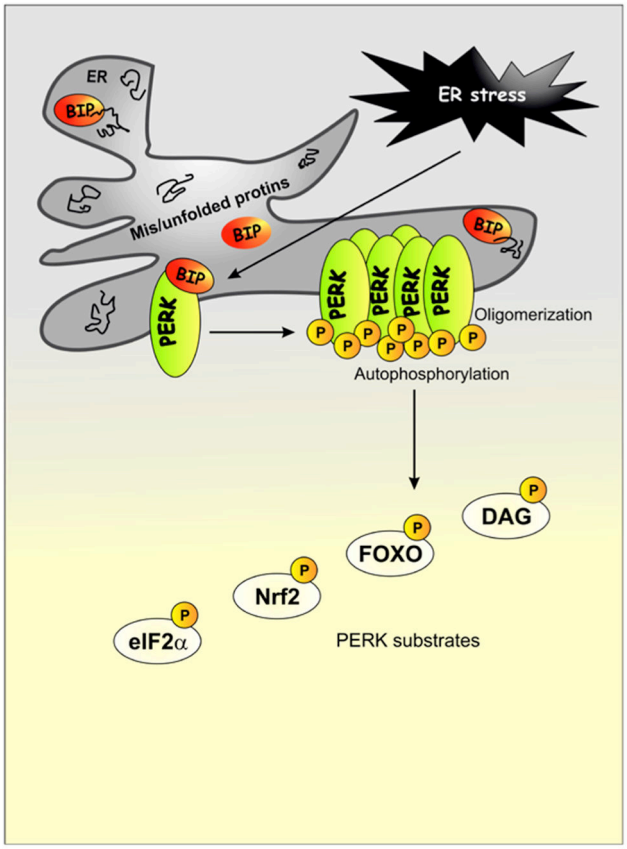

Figure 2.

Direct PERK substrates. Activated PERK, in response to ER stress, phophorylates 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 diacyglycerol (DAG) and regulates cell homeostasis.

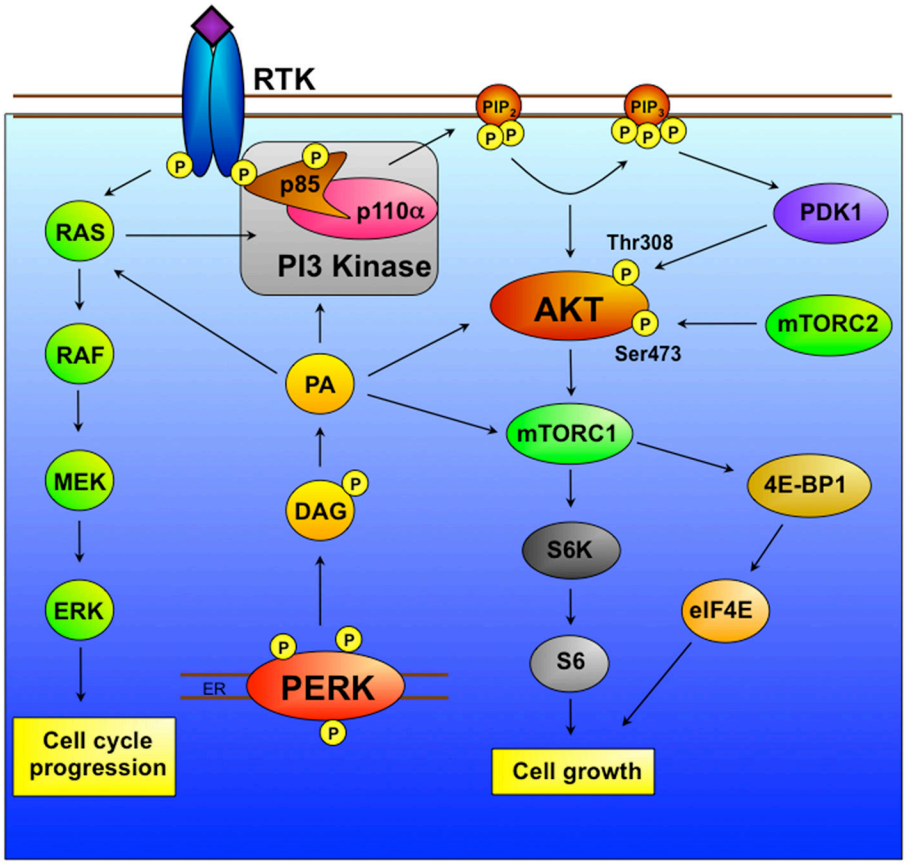

Figure 3.

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.