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The PERKs of mitochondria protection during stress: insights for PERK modulation in neurodegenerative and metabolic diseases

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Abstract

Protein kinase RNA-like ER kinase (PERK) is an endoplasmic reticulum (ER) stress sensor that responds to the accumulation of misfolded proteins. Once activated, PERK initiates signaling pathways that halt general protein production, increase the efficiency of ER quality control, and maintain redox homeostasis. PERK activation also protects mitochondrial homeostasis during stress. The location of PERK at the contact sites between the ER and the mitochondria creates a PERK-mitochondria axis that allows PERK to detect stress in both organelles, adapt their functions and prevent apoptosis. During ER stress, PERK activation triggers mitochondrial hyperfusion, preventing premature apoptotic fragmentation of the mitochondria. PERK activation also increases the formation of mitochondrial cristae and the assembly of respiratory supercomplexes, enhancing the cellular ATP generating capacity. PERK strengthens mitochondrial quality control during stress by promoting the expression of mitochondrial chaperones and proteases and by increasing mitochondrial biogenesis and mitophagy, which results in a renewal of the mitochondrial network. But how does PERK mediate all these changes in mitochondrial homeostasis? In addition to the classic PERK-eIF2α -ATF4 pathway, PERK can activate other protective pathways - PERK-OGT, PERK-TFEB, and PERK-NRF2 - contributing to a broader regulation of mitochondrial dynamics, metabolism, and quality control. The pharmacological activation of PERK was protective in models of neurodegenerative and metabolic diseases, such as Huntington's disease, progressive supranuclear palsy and obesity, while the inhibition of PERK was protective in models of Parkinson's and prion diseases and diabetes. We have here reviewed the molecular mechanisms by which PERK regulates mitochondrial dynamics, metabolism and quality control, and discusses the therapeutic potential of targeting PERK in neurodegenerative and metabolic diseases.

Abbreviations List

6–OHDA	6–hydroxydopamine
AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
ATF4	Activating transcription factor 4
ATF5	Activating transcription factor 5
ATFS-1	Transcription factor associated with stress-1
ATP	Adenosine triphosphate
Ca^{2+}	Calcium ion
CHOP	C/EBP-homologous protein
eIF2α	Eukaryotic translation initiation factor 2α
ER	Endoplasmic reticulum
ER-UPR	Endoplasmic reticulum unfolded protein response
FGF21	Fibroblast growth factor 21
FTD	Fronto–Temporal Dementia
GRP75	Mitochondrial Hsp70

HD	Huntington's disease
HO-1	Heme oxygenase–1
Htt	Huntingtin
ISR	Integrated stress response
KEAP1	Kelch–like ECH–associated protein 1
MAM	Mitochondrial-associated ER membranes
Mfn	Mitofusin
MSS	Marinesco–Sjögren syndrome
mtDNA	Mitochondrial DNA
NADH	Nicotinamide adenine dinucleotide
NRF1	Nuclear respiratory factor 1
NRF2	Nuclear factor erythroid 2–related factor 2
OGT	O-linked N-acetyl-glucosamine transferase
p- eIF2α	Phosphorylated eukaryotic translation initiation factor 2α
PD	Parkinson's disease
PERK	Protein kinase RNA-like ER kinase

PGC1α	Proliferator–activated receptor gamma coactivator 1–alpha
Proteostasis	Protein homeostasis
PSP	Progressive Supranuclear Palsy
ROS	Reactive oxygen species
SCAF1	Supercomplex assembly factor 1
SIMH	Stress-induced mitochondrial hyperfusion
SOD1	Superoxide Dismutase 1
TCA	Tricarboxylic acid cycle
TFAM	Mitochondrial transcription factor A
TFEB	Transcription factor EB
TIM	Translocase of the inner membrane
UPR	Unfolded protein response
UPR^{mt}	Mitochondrial unfolded protein response

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Keywords

Endoplasmic Reticulum	Mitochondria	Dynamics
PERK	Unfolded Protein Response	Metabolic Diseases
Stress	Metabolism	Neurodegeneration

I. Introduction

Mitochondria - the cellular powerhouses - arrange in a highly dynamic network where mitochondrial fusion, fission, transport, and renewal processes occur (Sharma et al. 2019, Giacomello et al. 2020). In addition to contacts within the mitochondrial network, mitochondria also contact with other organelles, such as the endoplasmic reticulum (ER) (Prinz, Toulmay and Balla 2020). The ER is the organelle of translation and assembly of proteins destined for the ER, lysosomes, plasma membrane, the extracellular space and a subset of cytosolic proteins (Reid and Nicchitta, 2015). Contacts between the ER and the mitochondria occur in microdomains called mitochondria–associated ER membranes (MAM) (Sharma et al. 2019), which mediate essential functions, such as: mitochondrial dynamics, autophagy, and the exchange of calcium (Ca^{2+}), lipids, and reactive oxygen species (ROS) between the two organelles (Prinz et al. 2020).

Different functional disruptions can instigate stress in the ER or mitochondria, and both organelles possess signalling pathways that restore homeostasis. In the ER, alterations to Ca^{2+} homeostasis, redox balance, availability of nutrients, and disruption of the ER folding capacity promote the accumulation of unfolded or misfolded proteins that activate the *ER unfolded protein response* (ER–UPR) (Hetz et al. 2020). The ER–UPR is an ensemble of signalling pathways that restore the protein homeostasis (proteostasis) by promoting a dual response: a decrease in general protein synthesis that reduces ER protein load, and a selective increase in the expression of proteins that control cellular processes such as redox homeostasis, protein folding and clearance, autophagy and apoptosis (Hetz and Papa 2018). In the mitochondria, loss of redox homeostasis, impairment of mitochondrial protein import, and dissipation of the mitochondrial membrane potential activate the *mitochondrial UPR* (UPR^{mt}) (Shpilka and Haynes 2018). The UPR^{mt} is a transcriptional program that increases the expression of

mitochondrial chaperones and proteases, restoring mitochondrial homeostasis (Anderson and Haynes 2020, Nargund et al. 2012).

The UPR^{mt} and the ER-UPR integrate a global stress response pathway, the integrated stress response (ISR) (Pakos–Zebrucka et al. 2016, Anderson and Haynes 2020). The ISR responds to a variety of stressful stimuli, through the activation of a group of stress-sensing kinases (Pakos–Zebrucka et al. 2016). Under stress, these kinases phosphorylate eukaryotic translation initiation factor–2α (eIF2α), halting the general translation of proteins and boosting the synthesis of specific transcription factors such as ATF4, ATF5 and C/EBP homologous protein (CHOP) (Zhou et al. 2008, Palam, Baird and Wek 2011, Vattem and Wek 2004, Anderson and Haynes 2020). The source and duration of the stress determine the outcome of the ISR activation (Pakos–Zebrucka et al. 2016). Acute activation of the ISR relieves stress - by increasing the expression of ER and mitochondrial chaperones and proteases, of components of the autophagic pathway, and of the amino acid metabolism pathways, which are under the control of ATF4, ATF5 and CHOP (B'Chir et al., 2013, Hetz et al. 2020, Harding et al. 2003, Quiros et al. 2017, Fiorese et al. 2016, Anderson and Haynes 2020). Chronic activation of the ISR triggers a maladaptive response - where CHOP restores protein synthesis, overloading the already affected proteostasis network (Ma and Hendershot 2003, Marciniak et al. 2004), and promotes the expression of pro-apoptotic proteins, directing the cell to apoptosis (Urra et al. 2013).

PERK is one of the ISR stress-sensing kinases. In presence of ER stress, PERK auto-phosphorylates and phosphorylates the eIF2α, activating the PERK-ISR pathway of the ER-UPR (Pakos–Zebrucka et al. 2016, Hetz et al. 2020). Subcellular fractionation studies indicated that PERK is located at the MAM, where it functions as a structural component since the knockout of PERK compromised the integrity of ER–mitochondria contacts (Verfaillie et al. 2012). At the MAM, PERK physically interacts with mitofusin 2 (Mfn2) and controls

mitochondrial morphology, Ca^{2+} homeostasis, and ROS production (Munoz et al. 2013). Recent data suggests that early signalling via the PERK-mitochondria axis can produce protective adaptations on mitochondrial function (Lebeau et al. 2018, Balsa et al. 2019, Kim et al. 2018a). A deeper knowledge of the mechanisms behind PERK-mediated effects on mitochondria may reveal new therapeutic targets for diseases where ER stress and mitochondrial dysfunction are present. This review examines the mechanisms behind PERK-mediated protective adaptations in mitochondrial dynamics, metabolism and UPR^{mt} and discusses the therapeutic potential of targeting PERK in neurodegenerative and metabolic diseases.

II. PERK unites mitochondria against fragmentation

Mitochondria can respond to stress by altering its fission and fusion dynamics (Eisner, Picard and Hajnoczky 2018). Mitochondrial fission regulates the selective autophagy of damaged mitochondria (mitophagy) and the re–distribution of mitochondria within the cell (Eisner et al. 2018). Mitochondrial fusion dilutes the existing damage and allows sharing of functional machinery between mitochondria, ensuring respiration, maintenance of membrane potential, and mitochondrial DNA (mtDNA) integrity (Eisner et al. 2018). ER stress triggers adaptations in mitochondrial dynamics via the activation of the stress-sensor PERK (Lebeau et al. 2018). In this section, we discuss how PERK adapts mitochondrial dynamics and we review the gaps in knowledge on the mechanisms driving such adaptations.

In models of ER stress, alterations to mitochondrial morphology split into 3 stages (Hom et al. 2007, Lebeau et al. 2018): *Stage 1*, 30 minutes of ER stress – initiation of a reversible mitochondrial fragmentation associated with the approximation between ER and mitochondrial membranes and dependent on an increase in mitochondrial Ca^{2+} content; *Stage 2*, 6 hours of ER stress – mitochondria undergo stress-induced mitochondrial hyperfusion (SIMH), which is

a process where mitochondria become more interconnected, elongated and efficient in ATP production; *Stage 3*, 24 hours of ER stress – new round of mitochondrial fragmentation that irreversibly triggers apoptosis (Hom et al. 2007, Lebeau et al. 2018, Bravo et al. 2011, Tondera et al. 2009). Inhibition of PERK during ER stress blocked mitochondrial hyperfusion occurring at stage 2, indicating that PERK is responsible for ER stress-induced mitochondrial hyperfusion (Lebeau et al. 2018) (Fig. 1). Consistently, in ER-stressed cells, blocking the effects of p-eFI2□ - a downstream effector of PERK - with the small molecule ISRIB induced premature mitochondrial fragmentation (Lebeau et al. 2018). Thus, PERK-induced mitochondrial hyperfusion is a potential pro-survival adaptation that protects cells from apoptotic mitochondrial fragmentation during stress (Lebeau et al. 2018).

The mechanism behind mitochondrial hyperfusion typically depends on three proteins: the outer membrane GTPase mitofusin 1 (Mfn1), the inner membrane GTPase OPA1 and the inner membrane scaffold protein SLP-2 (Tondera et al. 2009). SLP-2 binds to the OMA1 protease, preventing it from processing OPA1 and starting mitochondrial fission (Eisner et al. 2018, Wai et al. 2016), while Mfn1 closely tethers mitochondria, allowing the lipid merge that is required for mitochondrial fusion (Gao & Hu, 2021). However, a study in cells treated with ER-stress inducers found that OPA1 processing and Mfn1 levels remained unaltered (Lebeau et al. 2018), suggesting that PERK-induced mitochondrial hyperfusion occurs by a different mechanism from OPA1 processing or Mfn1 accumulation. Although the mechanism involved is not yet clarified, the same study showed that PERK-induced mitochondrial hyperfusion depends on the phosphorylation of eIF2α and on its ability to interrupt general protein translation, and on the presence of the mitochondrial scaffold SLP-2 and the protease YME1L (Fig. 1) (Lebeau et al. 2018).

In conclusion, a chronic activation of the ER-UPR induces mitochondrial permeabilization, cytochrome c release and consequent apoptosis; however, during the early activation of the

ER-UPR, PERK favors mitochondrial hyperfusion through a mechanism dependent on SLP-2, YME1L and p- eIF2α (Lebeau et al. 2018). The control that PERK exerts over the mitochondrial dynamics facilitates the recovery of homeostasis after acute ER stress, preventing premature apoptosis. This protective effect of PERK may be particularly helpful in early disease stages where ER stress has not yet reached the chronic maladaptive phase, where it directs cells for apoptosis.

III. PERKing up mitochondrial ATP production

When cells are under ER-stress, besides mitochondrial hyperfusion, there is an increase in the efficiency of ATP production, sustaining cell viability (Tondera et al. 2009, Lebeau et al. 2018, Balsa et al. 2019). This suggests that PERK activation might also modulate mitochondrial metabolism. In this section, we discuss the putative mechanisms behind PERK–induced adaptations to mitochondrial metabolism under stress.

In ER-stressed cells, PERK promotes the formation of mitochondrial cristae, the assembly of respiratory supercomplexes and the efficiency of oxidative phosphorylation (Balsa et al. 2019). In human cells with a missense mutation in mitochondrial complex I, the pharmacological activation of PERK partially recovered the assembly of respiratory complex I into supercomplexes, boosted oxygen consumption and conferred resistance to glucose deprivation (Balsa et al. 2019). These results suggest that PERK activation may potentially confer some protection against mitochondrial bioenergetic impairment.

Mechanistically, PERK activates the eIF2α-ATF4 pathway and drives the expression of the supercomplex assembly factor 1 (SCAF1), a protein that is crucial for supercomplex assembly, oxidative phosphorylation and ATP production (Fig. 1) (Balsa et al. 2019, Cogliati et al. 2016). Concerning formation of cristae, however, the mechanisms likely involve an alternative pathway, since mitochondrial cristae morphology remained unchanged upon SCAF1 deletion

and blockade of the eIF2α pathway (Balsa et al. 2019). Recent data indicate that the PERK-OGT-TOM70 is a potential pathway by which PERK activation regulates mitochondrial cristae formation (Latorre-Muro et al. 2021): PERK phosphorylates and activates the enzyme O-linked N-acetyl-glucosamine transferase (OGT), which catalyses the O-GlcNAcylation of specific substrates such as the translocase of the outer mitochondrial membrane 70 (TOM70). O-GlcNAcylated TOM70 increases the mitochondrial import of MIC19, a protein involved in the maintenance of the inner mitochondrial membrane architecture. Translocated MIC19 is assembled into the MICOS (mitochondrial contact site and cristae-organizing system) complex, a master regulator of cristae morphology, that enhances mitochondrial cristae formation (Latorre-Muro et al. 2021) (Fig. 1).

Mitochondrial cristae are the site of ATP production through oxidative phosphorylation (Cogliati, Enriquez & Scorrano 2016, Gilkerson, Selker & Capaldi 2003). Under normal conditions, the tricarboxylic acid cycle (TCA) cycle provides electron donors such as NADH, which participate in ATP production through oxidative phosphorylation (Martinez - Reyes and Chandel 2020). Under ER stress, the translation of TCA genes decreases, which can limit the activity of the TCA cycle and reduce the production of NADH (Rendleman et al. 2018). In turn, PERK reprograms the mitochondrial metabolism to one-carbon metabolism (Rendleman et al. 2018), which encompasses both the folate and methionine cycles (Newman & Maddocks, 2017), ensuring the production of NADH for ATP synthesis and glutathione for redox buffering (Rendleman et al. 2018, Celardo et al. 2016). During one-carbon metabolism, serine donates one-carbon units to the folate cycle while producing glycine. The folate cycle generates NADH that can be used in oxidative phosphorylation and glycine participates in the biogenesis of glutathione, along with other glutathione precursors that are synthesized by the methionine cycle (Yang & Vousden, 2016).

In summary, ER stress activated-PERK can adapt mitochondrial metabolism by increasing respiratory supercomplex assembly, boosting cristae formation, and reprogramming NADH production. These PERK–driven adaptations sustain an increase in the production of ATP (Balsa et al. 2019) and antioxidant defences (Rendleman et al. 2018) that fuel cellular recovery and prevent the rise of oxidative stress during ER stress.

IV. Mitochondrial network renewal: a result of PERK's versatility

PERK promotes the renewal of the mitochondrial network, ensuring a healthy pool of mitochondria during stress (Martina et al. 2016, Kim et al. 2018a). In this section, we discuss how PERK regulates mitochondrial network renewal via the activation of the transcription factor EB (TFEB).

TFEB is a transcription factor that responds to alterations in nutrient availability (Pastore et al., 2019). PERK activation also activates TFEB and increases its translocation to the nucleus, where TFEB promotes the expression of autophagic and lysosomal genes (Martina et al. 2016, Kim et al. 2018a). Mechanistically, activated PERK interacts with calcineurin, which in turn increases the activity of ryanodine receptors in the ER, triggering the outflow of Ca^{2+} from the ER to the cytosol (Kim et al. 2018a, Bollo et al., 2010, Liu et al., 2014). Increased cytosolic Ca^{2+} promotes the activity of calcineurin, which is a Ca^{2+}-dependent phosphatase that dephosphorylates TFEB and directs it to the nucleus (Fig. 2) (Creamer 2020, Kim et al. 2018a).

In the nucleus, TFEB conducts a multivalent transcriptional response that acts on three axes (Fig. 2): i) increases the expression of ER-UPR genes (namely CHOP and ATF4) and ATF4 target genes, suggesting a boost in ISR induction (Martina et al. 2016); ii) increases the expression of lysosomal and autophagic genes, as well as the recruitment of proteins that participate in mitophagy (such as parkin) to the mitochondria (Kim et al. 2018a); and iii) increases the expression of transcription factors, such as peroxisome proliferator-activated

receptor 1-alpha gamma coactivator (PGC1α), mitochondrial transcription factor A (TFAM) and nuclear respiratory factor 1 (NRF1), involved in mitochondrial biogenesis (Martina et al 2016, Kim et al. 2018a).

Renewing the mitochondrial network through increased mitophagy and mitochondrial biogenesis via the PERK-TFEB pathway was an important survival response in an *in vivo* model of hepatitis (Kim et al. 2018a). Carbon monoxide, which was shown to activate PERK in cells, increased the expression of markers of autophagy, lysosomal biogenesis and mitochondrial biogenesis in mitochondrial extracts, attenuated mitochondrial dysfunction and decreased liver damage in an *in vivo* model of hepatitis (Kim et al. 2018a).

Thus, PERK activates TFEB, that boosts ER–UPR activation, mitophagy and mitochondrial biogenesis (Martina et al. 2016, Kim et al. 2018a), renewing the mitochondrial network and limiting cell damage during stress.

V. Integrating the PERK–NRF2 axis in the mitochondrial adaptations to stress

The activation of PERK during ER stress can prevent the generation of oxidative stress as a result of the interaction between PERK and nuclear factor erythroid 2–related factor 2 (NRF2), the master regulator of antioxidant responses (Cullinan et al. 2003). NRF2 is normally complexed with Kelch–like ECH–associated protein 1 (KEAP1), which directs NRF2 to degradation by the ubiquitin–proteasome system (Dinkova–Kostova and Abramov 2015). During ER stress, PERK phosphorylates NRF2, separating it from KEAP1 (Cullinan et al. 2003, Zhang et al. 2019). NRF2 is no longer degraded and moves to the nucleus where it induces the expression of antioxidant enzymes to prevent oxidative stress (Zhang et al. 2019) (Fig. 2). In this section we discuss how the PERK–NRF2 pathway controls mitochondrial homeostasis in addition to redox homeostasis.

PERK increases the levels of ATF4, which can dimerize with NRF2 and increase the expression of heme oxygenase-1 (HO–1) (He et al. 2001), an antioxidant enzyme that protects cells against oxidative stress and programmed cell death (Gozzelino, Jeney and Soares 2010, Alcaraz and Ferrandiz 2020) (Fig. 2). Specifically in the mitochondria, the activation of the PERK-NRF2 axis can control biogenesis and metabolism (Zheng et al. 2012, Mohamed et al. 2020). Both PERK and HO–1 seem essential for the expression of transcription factors NRF1 and TFAM and for the increase in mtDNA content required for mitochondrial biogenesis (Zheng et al. 2012) (Fig. 2). PERK silencing abrogated signaling by NRF2, resulting in mitochondrial dysfunction that was characterized by increased ROS production, mtDNA release to the cytosol, decreased mitochondrial mass, and impaired mitochondrial bioenergetics, with decreased membrane potential and oxidative phosphorylation (Mohamed et al. 2020). The expression of a degradation-resistant form of NRF2 restored oxidative phosphorylation and decreased ROS production, which highlights the role of NRF2 activation in the control of mitochondrial metabolism and protection of mitochondrial function (Mohamed et al. 2020).

Thus, during ER stress, the PERK-NRF2 pathway increases the expression of antioxidant defences that prevent oxidative stress. Specifically in mitochondria, the PERK-NRF2 pathway protects against damage to oxidative phosphorylation processes and increases the expression of genes involved in mitochondrial biogenesis (NRF1 and TFAM), preventing apoptotic damage (Zheng et al. 2012, Mohamed et al. 2020).

VI. PERK: an ER stress sensor that paves the way for UPR^{mt}

Perturbations to mitochondrial oxidative phosphorylation, and to protein synthesis, folding, degradation and trafficking can activate the UPR^{mt}, a stress response that increases the expression of mitochondrial chaperones and proteases, restoring mitochondrial homeostasis

(Shpilka and Haynes 2018). In this section, we discuss how PERK, an ER-stress sensor and initiator of the ISR, can also participate in the activation of the UPR^{mt}.

The UPR^{mt} mechanisms have been mainly characterized in *Caenorhabditis elegans*, (Melber and Haynes 2018), where the mediator of UPR^{mt} is the transcription factor ATFS–1 (Nargund et al. 2012). ATFS–1 has a mitochondrial targeting sequence that drives its import to healthy mitochondria, and a nuclear localization sequence that directs it to the nucleus when stress impairs mitochondrial import (Nargund et al. 2012). Once in the nucleus, ATFS–1 initiates UPR^{mt} by increasing the expression of mitochondrial chaperones and proteases (Nargund et al. 2012). Comparison between the mechanisms of UPR^{mt} in nematodes and in mammals identified ATF5 as the main mediator of UPR^{mt} in mammals (Fiorese et al. 2016). Like ATFS-1, ATF5 possesses both a mitochondrial targeting sequence and a nuclear localization sequence (Fiorese et al. 2016), and promotes the expression of mitochondrial chaperones and proteases in a situation of mitochondrial stress (Fiorese et al. 2016, Shpilka and Haynes 2018).

In ER-stressed cells, eIF2α phosphorylation induces CHOP and ATF4 expression, which increases ATF5 levels (Teske et al. 2013). PERK is essential for ATF5 expression since PERK genetic deletion prevented the increase of ATF5 levels in ER stressed cells (Teske et al. 2013, Zhou et al. 2008). In fact, levels of Lon protease and mitochondrial Hsp70 (GRP75), which are typical UPR^{mt} transcripts, increased in a PERK– eIF2α –ATF4-dependent way during ER stress (Hori et al. 2002, Lebeau et al. 2018). These data suggest that upon ER stress the PERK- eIF2α -ATF4 pathway can favour the cooperation between ATF4 and CHOP, increasing ATF5 levels and driving the expression of mitochondrial chaperones and proteases (Fig. 3).

Activated PERK also impairs the mitochondrial import (Rainbolt et al. 2013), one of the triggers of UPR^{mt} that putatively promotes nuclear translocation of ATF5 (Fiorese et al. 2016, Melber and Haynes 2018). Cells treated with activators of the ISR, ER-UPR and PERK

presented decreased levels of TIM17A, a subunit of the mitochondrial protein import complex called translocase of the inner membrane 23 (TIM23) (Rainbolt et al. 2013). The decreased levels of TIM17A during stress may be due to: i) decreased production of TIM17A, since the phosphorylation of eIF2α by PERK decreases general mRNA translation; and ii) increased TIM17A degradation by the YME1L protease (Rainbolt et al. 2013, Lebeau et al. 2018). TIM17A depletion impaired mitochondrial protein import, modestly increased mRNA levels of the mitochondrial chaperone Hsp60 and the protease YME1L, and protected cells and nematodes against paraquat–induced toxicity (Rainbolt et al. 2013). Such results indicate that PERK activation triggers the decrease in TIM17A levels and the putative activation of UPR^{mt}, conferring resistance against toxic stimuli both *in vitro* and *in vivo.*

In summary, PERK activation may promote the UPR^{mt} during ER stress: PERK activates the eIF2α -ATF4-CHOP pathway that increases the expression of ATF5 - the main mediator of UPR^{mt} in mammals - increasing the expression of chaperones and mitochondrial proteases (Teske et al. 2013, Zhou et al. 2008). In addition, PERK activation can decrease TIM17A levels impairing mitochondrial protein import (Rainbolt et al. 2013), possibly favoring ATF5 translocation to the nucleus (Fig. 3).

VII. PERK as a drug target in neurodegenerative and metabolic diseases

Mitochondrial dysfunction and ER stress are hallmarks of neurodegenerative and metabolic diseases (Hetz and Saxena 2017, Rocha et al. 2020). Levels of phosphorylated PERK or effectors of the PERK/ISR pathway - p- eIF2α and CHOP - are increased in post-mortem brain samples from patients with progressive supranuclear palsy (PSP), amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD) or Huntington's disease (HD), suggesting a chronic activation of PERK or ISR pathways in advanced stages of such diseases (Hetz and Saxena 2017, Ito et al. 2009, Hoozemans et al. 2009, Stutzbach et al. 2013,

Hoozemans et al. 2007, Carnemolla et al. 2009). The same occurs in metabolic diseases, where levels of p- eIF2α or CHOP were increased in pancreas or liver biopsy samples from patients with obesity, diabetes or non-alcoholic fatty liver disease (Kumashiro et al., 2011; Laybutt et al., 2007; Sharma et al., 2008). These data suggest that dysregulated signaling via the PERK or ISR pathways may be a common pathogenic mechanism between neurodegenerative and metabolic diseases (Hughes & Mallucci, 2019), which prompted the study of PERK as a pharmacological target in these disorders (Hetz, Axten & Patterson, 2019). In this section we describe the effects of pharmacological PERK modulation (inhibition or activation) in models of neurodegenerative and metabolic diseases.

While PERK inhibition was found protective in models of disorders such as PD, prion disease or diabetes (Bilekova, Sachs and Lickert 2021, Mercado et al. 2018, Moreno et al. 2013), PERK activation was protective in models of other disorders such as HD, PSP and obesity (Ganz et al. 2020, Bruch et al. 2017, Joe et al. 2018) (Table 1). The explanation for why PERK pharmacological activation may hold promise in diseases where the protein already seems activated may reside on the levels of eIF2α and the extent of its phosphorylation. The balance between phosphorylation and dephosphorylation of eIF2α can determine the balance of pathways between cell survival or death (Costa-Mattioli & Walter, 2020): high levels of p-eIF2α may block general protein synthesis, directing cells for apoptosis; while low levels of p-eIF2α may be insufficient to reach the protective response associated to the ISR, hindering the recovery of cellular homeostasis. Thus, the pharmacological inhibition of PERK could be protective in conditions where high p- eIF2α levels chronically block protein synthesis, contributing to the pathophysiology of the disease, such as in PD and diabetes (Bilekova, Sachs and Lickert 2021, Mercado et al. 2018, Moreno et al. 2012, Moreno et al. 2013) (Table 1). Activation of PERK appears to provide protection in models of neurodegenerative diseases with lower levels of p- eIF2α, such as HD and PSP (Ganz et al. 2020, Bruch et al. 2017) (Table

1) or metabolic diseases where energy expenditure is dysregulated, such as obesity (Joe et al. 2018). Finally, we suggest that the strategy of PERK modulation requires adjustment to each disease, taking into account the extent of activation of the PERK-p- eIF2α pathway and the stage of disease progression.

(1) PERK inhibition

Studies in models of PD, prion disease or diabetes found an association between PERK activation and a persistent block in protein synthesis. This block is thought to hinder synaptic neurotransmission in PD and prion disease, or the synthesis of insulin in diabetes (Mercado et al. 2018, Moreno et al. 2012, Kim et al., 2018b, Kim et al., 2019, Bilekova, Sachs and Lickert 2021), thus prompting the therapeutic strategy of PERK inhibition to counteract its hyper-activation. Consistently, the inhibition of PERK with GSK2606414 in *in vivo* models of PD and prion disease unblocked protein synthesis, and restored synaptic protein levels and dopamine synthesis (Moreno et al. 2013, Mercado et al. 2018) (Table 1).

In a mouse model of diabetes, PERK inhibition with GSK2606414 was protective when it was administered in the low nanomolar range (Kim et al., 2018b, Kim et al., 2019, Bilekova, Sachs and Lickert 2021). Physiologically, insulin production in pancreatic cells requires a large amount of ER activity, which chronically activates the ER-UPR (Bilekova, Sachs and Lickert 2021). However, double knockout mice for PERK presented a decrease in insulin production, which provoked hyperglycaemia and aggravated the diabetic phenotype (Harding et al., 2001, Bilekova, Sachs and Lickert 2021). PERK inhibition with GSK2606414 in the low nanomolar range decreased blood glucose in hyperglycemic mice by allowing the partial phosphorylation of eIF2α and activation of ISR, which increases the expression of the ER chaperone BiP that supports insulin secretion (Kim et al., 2018b, Kim et al., 2019, Bilekova, Sachs and Lickert 2021, Gao et al., 2012).

(2) PERK activation

PERK activation is predominantly reported as the protective strategy in models of neurodegenerative diseases such as HD and PSP (see Table 1), and of the metabolic disorder obesity (Ganz et al. 2020, Bruch et al. 2017, Joe et al. 2018).

The increased levels of CHOP in post-mortem samples from the brains of HD patients (Carnemolla et al. 2009) suggest that the PERK-ISR pathway may be chronically activated. Regarding to HD models, the comparison between different murine cell lines and different murine brain regions suggest that the striatum, the brain region more vulnerable in HD, presents low levels of p- eIF2α: a wild-type striatal cell line ($STHdh^{Q7/7}$) presented lower levels of p-eIF2α than a fibroblast (NIH 3T3) and a neuroblastoma-derived (N2a) cell lines; also, the striatum presented lower levels of p- eIF2α than the cortex in both wild-type and HD mice (Leitman et al, 2014). The pharmacological activation of PERK with MK-28 or CCT020312 protected a striatal cell line expressing mutant huntingtin ($STHdh^{Q111/111}$) against death and attenuated motor deficits in HD mice (Ganz et al. 2020). These results raise the hypothesis that the HD models may be mimicking an early stage of HD where the extent of PERK-ISR activation is insufficient to restore homeostasis, and thus benefit from PERK activation strategies. Still, this hypothesis awaits confirmation as the available literature in human samples still lacks a description of PERK-ISR activation at different stages of HD.

In brains of PSP patients, levels of phosphorylated PERK were increased, but levels of eIF2α and p- eIF2α were decreased (Bruch et al. 2017). Such results indicate that, although PERK is activated, the amount of p- eIF2α may be insufficient to trigger the protective effects of the ISR pathway (Bruch et al. 2017). The pharmacological activation of PERK with CCT020312 attenuated mortality in cell models of PSP, and recovered memory and locomotion skills in an animal model of PSP (Bruch et al. 2017). PERK activation in cell models of PSP favoured the

activation of NRF2, normalized the production of ATP and decreased the phosphorylation of tau, attenuating cell death (Bruch et al. 2017).

In mouse models of obesity (high fat diet), PERK activation with carbon monoxide decreased blood glucose levels, increased insulin sensitivity and protected against weight gain (Joe et al. 2018). Mechanistically, the activation of the PERK- eIF2α -ATF4 pathway increased the expression of proteins involved in mitochondrial biogenesis such as PGC1α, NRF1, and TFAM, increased mitochondrial DNA content, and normalized oxidative phosphorylation (Joe et al. 2018, Figueiredo–Pereira et al. 2020). In addition, the activation of the PERK- eIF2α -ATF4 pathway increased the expression of the fibroblast growth factor 21 (FGF21) protein, a hormone known to decrease fat mass (Geng, Lam & Xu, 2020, Joe et al. 2018). The increases in FGF21 expression, mitochondrial biogenesis, and oxidative phosphorylation contribute to increase energy expenditure, decrease the size of adipocytes, and prevent weight gain in animal models of obesity (Joe et al. 2018, Figueiredo–Pereira et al. 2020).

VIII. Conclusions

(1) PERK is classically defined as an ER-stress sensor that activates the ISR (eIF2α –ATF4) pathway of the ER-UPR (Pakos–Zebrucka et al. 2016, Hetz et al. 2020). During ER stress, PERK can also activate complementary pathways such as PERK-OGT, PERK-NRF2 or PERK-TFEB that contribute to the protection of mitochondrial homeostasis (Latorre-Muro et al. 2021, Martina et al. 2016, Kim et al. 2018a, Zheng et al. 2012). This allows PERK to control various aspects of mitochondrial function (dynamics, metabolism, biogenesis, mitophagy and the UPR^{mt}), preventing its impairment and delaying apoptosis in stressful situations.

(2) PERK activation during ER stress promotes mitochondrial hyperfusion (Lebeau et al. 2018), boosts the assembly of respiratory supercomplexes, and increases the formation

of mitochondrial cristae (Balsa et al. 2019). Both mitochondrial hyperfusion and the assembly of respiratory supercomplexes depend on the activation of the PERK- eIF2α - ATF4 pathway (Balsa et al. 2019). The formation of mitochondrial cristae is mediated by the PERK-OGT pathway (Latorre-Muro et al. 2021). Such alterations to mitochondrial dynamics and metabolism result in a pro-survival response that prevents premature mitochondrial fragmentation and optimizes energy production during stress.

(3) Activated PERK reprogrammes NADH production from TCA to one–carbon metabolism, favouring also the production of glutathione (Rendleman et al. 2018). NADH feeds oxidative phosphorylation–dependent ATP synthesis and glutathione prevents a rise in oxidative stress.

(4) Both PERK-NRF2 and PERK-TFEB pathways contribute to increase the expression of genes that are involved in mitochondrial biogenesis (Kim et al. 2018a, Zheng et al. 2012). Additionally, PERK-TFEB boosts mitophagy, promoting the renewal of the mitochondrial network, and PERK-NRF2 protects oxidative phosphorylation, preserving mitochondrial function (Mohamed et al. 2020, Martina et al. 2016, Kim et al. 2018a).

(5) PERK also regulates mitochondrial quality control pathways such as UPR^{mt}. Current studies suggest that mammalian UPR^{mt} activation is mediated by ATF5 expression and impaired mitochondrial import (Anderson and Haynes 2020, Melber and Haynes 2018, Fiorese et al. 2016). During ER stress, activated PERK can drive both triggers of UPR^{mt}: increased ATF5 expression (Zhou et al. 2008) and decreased mitochondrial protein import (Rainbolt et al. 2013).

(6) Dysregulation of PERK signaling is a common trait between neurodegenerative and metabolic diseases, and its pharmacological modulation prevented disease manifestations in several disease models (Hughes & Mallucci, 2019). The specific

strategy for PERK/ISR modulation, however, requires fine-tuning to each disease (Costa-Mattioli & Walter, 2020), possibly being conditioned by the levels of eIF2α and the extent of its phosphorylation. PERK inhibition conferred protection in models of PD, prion disease and diabetes (Bilekova, Sachs and Lickert 2021, Mercado et al. 2018, Moreno et al. 2013) by attenuating p- eIF2α levels, thus preventing a chronic blockage of protein synthesis. PERK activation conferred protection in models of other neurodegenerative diseases with lower levels of p- eIF2α, such as HD and PSP, or in diseases with dysregulated energy expenditure, such as obesity (Ganz et al. 2020, Bruch et al. 2017, Joe et al. 2018).

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Figure legends

Figure 1. Proposed mechanism for PERK–driven adaptations to mitochondrial morphology and metabolism. (A) Under ER stress, PERK phosphorylates eIF2α, which elicits a translational arrest that, along with mitochondrial scaffold SLP–2 and protease YME1L, mediates mitochondrial hyperfusion (Lebeau et al. 2018). (B) Phosphorylated eIF2α increases ATF4 levels that promotes the expression of supercomplex assembly factor 1 (SCAF1) and drives the assembly of respiratory supercomplexes (Balsa et al. 2019). (C) Activated PERK phosphorylates O-linked N-acetyl-glucosamine transferase (OGT), catalysing the O-GlcNAcylation of TOM70. O-GlcNAcylated TOM70 increases the translocation of MIC19 into the mitochondria. MIC19 is a protein involved in the maintenance of inner mitochondrial membrane architecture that, once translocated to the mitochondria, increases mitochondrial cristae assembly (Latorre-Muro et al. 2021). (D) The increase in mitochondrial cristae and supercomplex assembly together with mitochondrial hyperfusion result in an optimization of oxidative phosphorylation activity and increased ATP production (Balsa et al. 2019, Lebeau et al. 2018). This figure was created with modified images from Servier Medical Art templates, licensed under a Creative Commons Attribution 3.0 Unported License; https://smart.servier.com/.

Figure 2. PERK regulation of mitochondrial turnover. Under ER stress, activated PERK interacts with calcineurin and increases cytosolic Ca^{2+} concentration, triggering TFEB

translocation to the nucleus (Bollo et al., 2010, Martina et al. 2016, Kim et al. 2018). TFEB nuclear targeting increases the expression of ATF4 and ATF4-target genes such as CHOP (Martina et al. 2016). TFEB also increases the expression of lysosomal and autophagic genes and of NRF1, TFAM and PGC1α (Kim et al. 2018). This transcriptional regulation increases mitochondrial biogenesis and mitophagy, which results in the renewal of the mitochondrial network (Kim et al. 2018). PERK also directly phosphorylates NRF2, preventing its targeting for degradation by KEAP1 (Cullinan et al. 2003). NRF2 dimerizes with ATF4, increasing heme oxygenase–1 (HO–1) expression (He et al. 2001). HO–1 induces the expression of NRF1 and TFAM, which are transcription factors that promote mitochondrial biogenesis (Zheng et al. 2012). This figure was created with modified images from Servier Medical Art templates, licensed under a Creative Commons Attribution 3.0 Unported License; https://smart.servier.com/.

Figure 3. Proposed mechanism for PERK–induced UPR^{mt} upon ER stress. Under ER stress, PERK phosphorylates eIF2α, which arrests general translation and increases the expression of the transcription factors ATF4, CHOP and ATF5 - the main regulator of UPR^{mt} (Hetz et al. 2020, Teske et al. 2013, Zhou et al. 2008). The decrease in general translation decreases TIM17A levels, impairing the mitochondrial import efficiency, which may attenuate the import of ATF5 to the mitochondria (Rainbolt et al. 2013, Nargund et al. 2012). In the nucleus, ATF5 promotes the UPR^{mt} by increasing the expression of mitochondrial chaperones and proteases (Fiorese et al. 2016, Shpilka and Haynes 2018). YME1L is a mitochondrial protease that increases the degradation of TIM17A even further (Rainbolt et al. 2013, Nargund et al. 2012). This figure was created with modified images from Servier Medical Art templates, licensed under a Creative Commons Attribution 3.0 Unported License; https://smart.servier.com/.

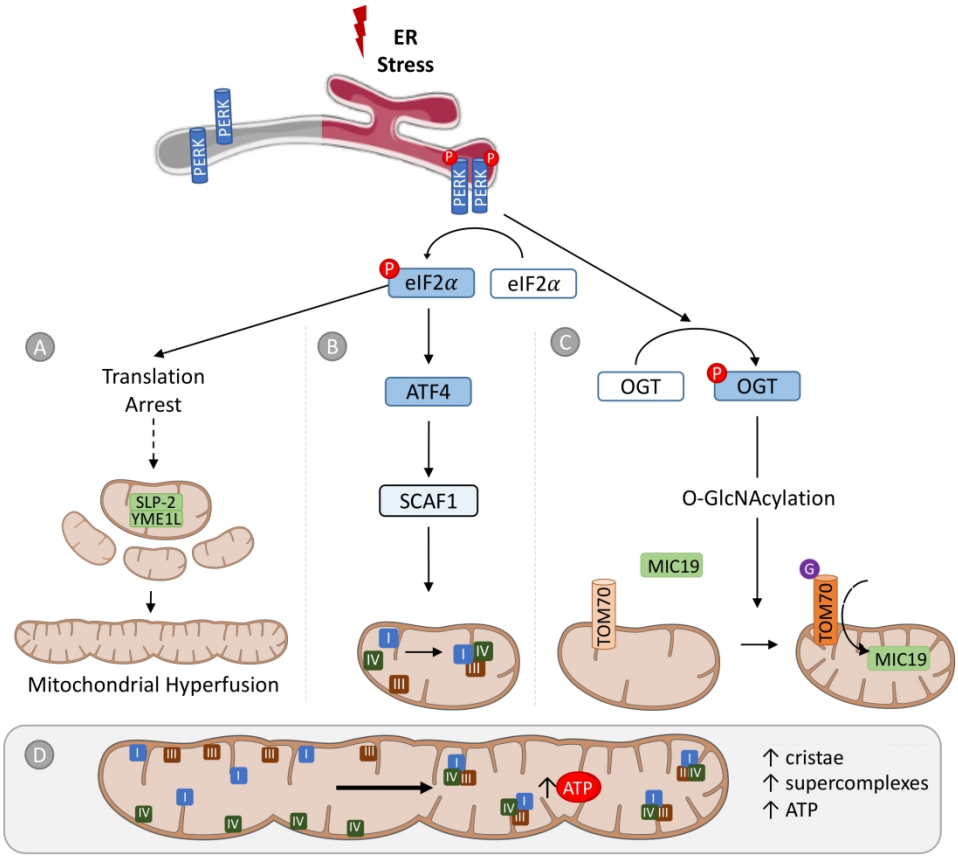

Figure 1. Proposed mechanism for PERK–driven adaptations to mitochondrial morphology and metabolism.

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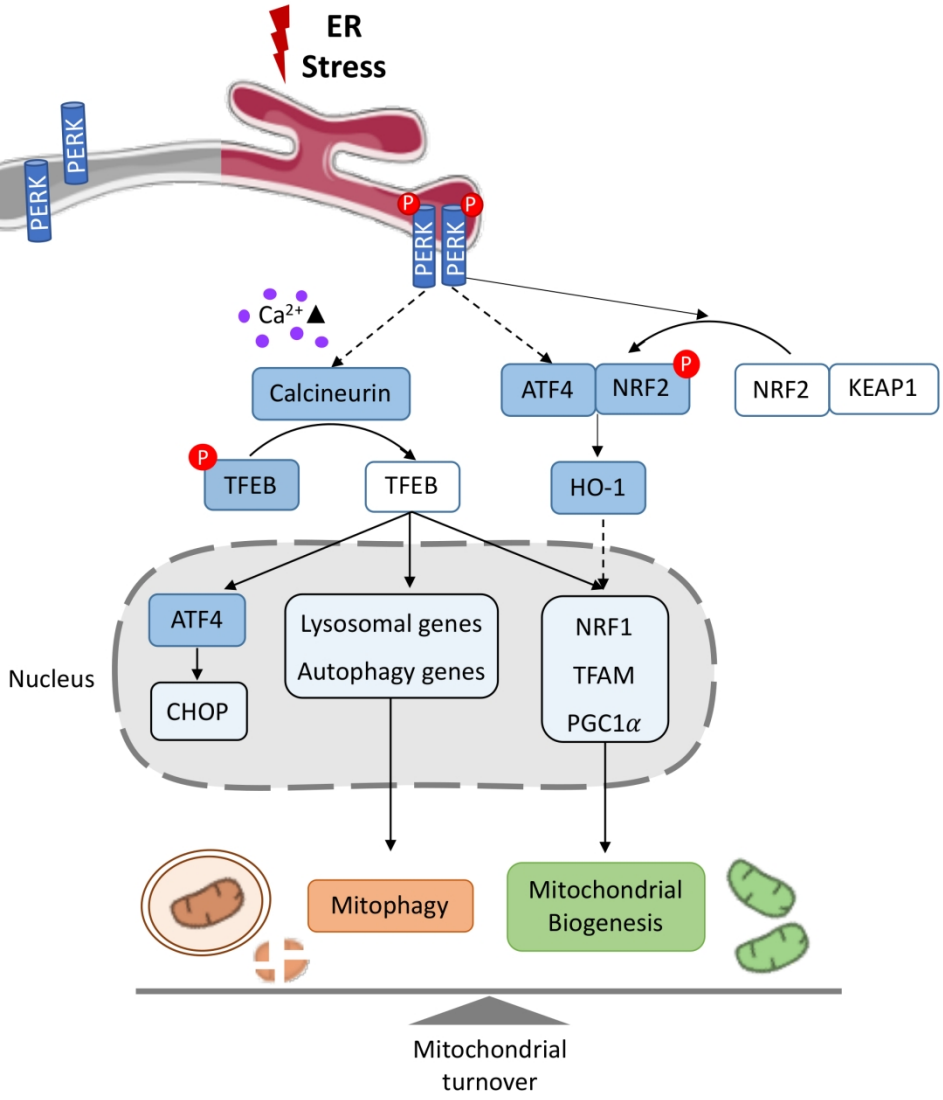

Figure 2. PERK regulation of mitochondrial turnover.

170x189mm (300 x 300 DPI)

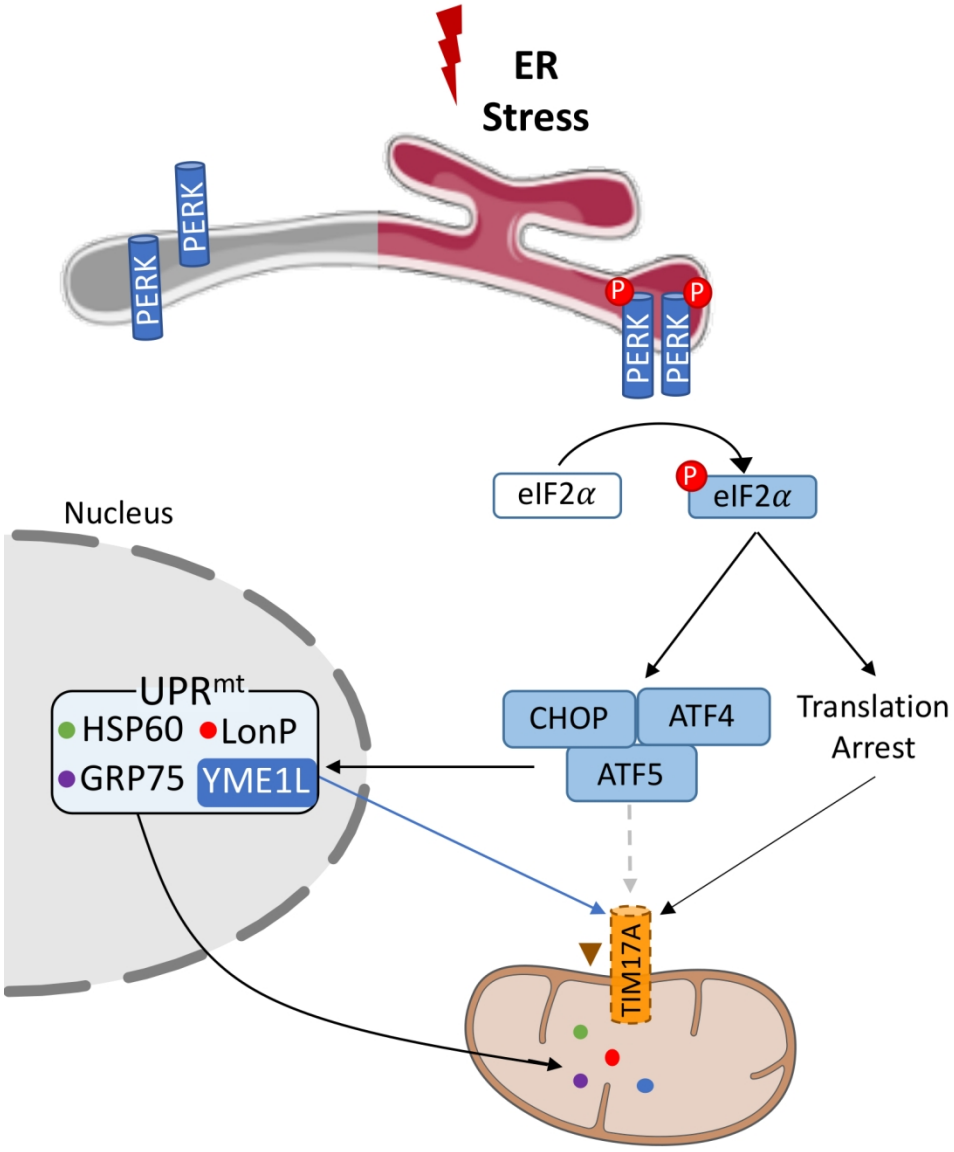

Figure 3. Proposed mechanism for PERK–induced UPRmt upon ER stress.

143x173mm (300 x 300 DPI)

Table 1. Pharmacological modulations of PERK signaling in neurodegenerative diseases. Grey lines represent inhibition strategies. White lines represent activation strategies.

Disease	Drug	Modulation	Models	Protection	Reference
ALS	GSK2606414	Inhibition	UAS–TDP–43 flies	Yes	Kim et al. 2014
			TDP–43 transfected primary neurons	Yes	Kim et al. 2014
			G93–SOD1 transfected primary neurons	No	Bugallo et al. 2020
			TDP43 transfected HEK293 cells	No	Hans et al. 2020
FTD	GSK2606414	Inhibition	Tau P301L mice	Yes	Radford et al. 2015
GM2	GSK2606414	Inhibition	GM2 treated primary neurons	Yes	Virgolini et al. 2019
HD	CCT020312	Activation	$STHdh^{Q111/111}$ cells	Yes	Ganz et al. 2020
	MK–28	Activation	R6/2 mice	Yes	Ganz et al. 2020
	A4	Inhibition	$STHdh^{Q111/111}$ cells	Yes	Leitman et al. 2014
MSS	GSK2606414	Inhibition	Woozy mice (CXB5/By–Sil1wz/J)	Yes	Grande et al. 2018
PD	GSK2606414	Inhibition	6–OHDA injected mice	Yes	Mercado et al. 2018
			Pink1/parkin mutant flies	Yes	Celardo et al. 2016
Prion	GSK2606414	Inhibition	Prion infected mice	Yes	Moreno et al. 2013
PSP	CCT020312	Activation	Annonacin treated LUHMES neurons	Yes	Bruch et al. 2017
			Tau P301S mice	Yes	Bruch et al. 2017

Abbreviations: Amyotrophic Lateral Sclerosis (ALS), Fronto–Temporal Dementia (FTD), Progressive Supranuclear Palsy (PSP), Parkinson's Disease (PD), GM2 gangliosidoses, Huntington's Disease (HD), Marinesco–Sjögren syndrome (MSS), huntingtin (Htt), Superoxide Dismutase (SOD1), 6–hydroxydopamine (6–OHDA).