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EDITORIAL

(off)Targeting UPR signaling: the race toward intervening ER proteostasis

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1. ER proteostasis and disease

The secretory and folding capacity of the cell is constantly challenged by physiological demands and pathological perturbations that disturb the process of protein synthesis and maturation. To adjust the protein folding capacity of the endoplasmic reticulum (ER) according to the need, cells engage a dynamic intracellular signaling pathway known as the unfolded protein response (UPR). Homeostatic activation of the UPR enforces adaptive programs that improve key aspects of the secretory pathway, whereas chronic ER stress results in apoptosis. The generation of genetically modified mice for specific UPR components has uncovered the relevance of the pathway to the physiology of different organs and cell types. Due to the fundamental role of the UPR in controlling protein folding, abnormal levels of ER stress have been associated with a variety of pathologies involving specialized secretory cells, in addition to diseases linked to protein misfolding and aggregation, highlighting cancer, autoimmunity, diabetes, obesity, and neurodegeneration [1].

The UPR has evolved toward the establishment of a complex network of interconnected signaling pathways initiated by the activation of three main type of signal transducers located in the ER known as IRE1α, ATF6, and PERK. Abnormal levels of ER stress have been proposed as a major pathogenic mechanism underlaying in several neurodegenerative diseases, where sustained PERK signaling contributes to neuronal dysfunction and death [2]. Here we discuss recent advances and controversies in the generation of small molecules that inhibit or enhance PERK signaling and their applicability for the treatment of neurodegenerative diseases.

1.1. Therapeutic potential of PERK modulation

PERK is a type-I transmembrane protein located in the ER membrane that dimerizes and autophosphorylates in response to ER stress. The activation of PERK leads to the direct phosphorylation of the alpha subunit of the eIF2 complex at serine 51. This event inhibits protein synthesis at the level of translation, thus protecting cells against ER stress. The phosphorylation of eIF2α also allows the selective translation of ATF4, a transcription factor involved in the regulation of genes that encode proteins involved in redox control, amino acid metabolism, autophagy, and protein folding and synthesis. In addition, ATF4 participates on a feedback loop by inducing the expression of the eIF2α phosphatase PPP1R15A (also known as GADD34), the regulatory subunit of protein phosphatase 1 (PP1). ATF4 can also upregulate the transcription factor GADD153 (also known as CHOP), which inhibits the expression of the antiapoptotic BCL-2 gene to accelerate cell death, in addition to upregulating the expression of proapoptotic BCL-2 members, and to burst protein synthesis and oxidative stress [3]. eIF2α phosphorylation is also regulated by other stress kinases, a pathway known as the integrated stress response (ISR) [4].

Increased levels of phosphorylated PERK and eIF2α have been found in postmortem brain tissue of patients affected with several neurodegenerative diseases including Alzheimer's and Parkinson's disease, in addition to ALS [2,5]. Besides the known consequences of eIF2α phosphorylation in stress mitigation and apoptosis control, recent studies suggest that in the context of central nervous system pathology the sustained activation of PERK causes chronic translational attenuation, leading to the repression of the expression of synaptic proteins. This pathogenic mechanism contributes to neurodegeneration and cognitive decline in prion-infected mice, in addition to the AD and frontotemporal dementia (FTD) models [2]. Remarkably, the oral administration of the PERK inhibitor GSK2606414 recovered the expression of synaptic proteins, preventing neurodegeneration in prion-infected mice [6] and in a mouse model of FTD [7]. Furthermore, GSK2606414 has also demonstrated to successfully inhibit tumor growth *in vivo*, and its optimized analog, GSK2656157, was selected for preclinical development [8]. However, in the context of other neurodegenerative diseases, enhancement of eIF2α phosphorylation may be beneficial [2]. Thus, the modulation of PERK signaling represents an interesting avenue for drug discovery with potential for the treatment of numerous diseases.

During the past 5 years, several small molecules to modulate PERK signaling have been identified that target the pathway at different levels (Figure 1). GSK2606414 and GSK2656157, as well as AMG'44, have been described as selective and potent inhibitors of the kinase domain of PERK, in addition to the PERK activator CCT020312 [9]. Salubrinal was

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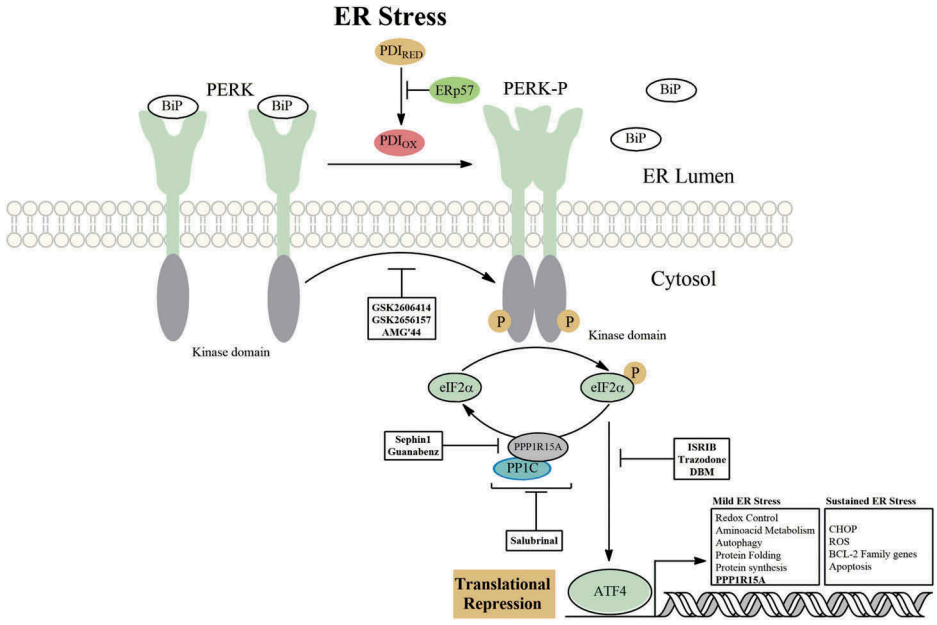

Figure 1. PERK signaling modulators. PERK activation leads to phosphorylation of eIF2α and consequent inhibition of protein translation and expression of the transcription factor ATF4. ATF4 translocates to the nucleus and induces expression of pro-survival genes. ATF4 also controls genes related to apoptosis, including CHOP. CHOP in turn can induce the expression of GADD34, promoting the dephosphorylation of eIF2α under prolonged ER stress. Upon ER stress Several small molecule modulators of the PERK and eIF2α function have been described, acting at different levels of the pathway. The most studied PERK modulators are shown, including GSK2606414, GSK2656157, and AMG'44 have been described as inhibitors of the kinase domain of PERK. Guanabenz and Sephin 1 bind directly to PPP1R15A, preventing eIF2α-P dephosphorylation. Salubrinal also prevents eIF2α dephosphorylation by inhibition of the inducible and constitutive phosphatase. ISRIB, trazodone, and dibenzoylmethane (DBM) prevent the consequences of eIF2α phosphorylation.

identified as a general eIF2α phosphatase inhibitor that also targets the constitutive phosphatase complex [4]. Guanabenz and its derivative, Sephin 1, directly and reversibly bind to PPP1R15A, inhibiting the dephosphorylation of eIF2α, thus prolonging the adaptative response elicited by the pathway [10]. Another compound known as ISRIB is also effective in blocking the consequences of eIF2α phosphorylation [11]. More information on PERK modulators, their mechanisms of action, and specificity and safety issues are indicated in Table 1. Several studies have used these compounds to assess their efficacy in different models of neurodegeneration, generating very conflicting results where the therapeutic potential may be observed only in certain diseases (see specific references in [2]). For example, Sephin 1 was able to prevent the motor, morphological, and molecular defects on mouse models of Charcot-Marie-Tooth 1B disease and ALS. Similarly, Guanabenz and Salubrinal administration were reported to protect ALS models, whereas one study indicated the opposite in the context of prion disease, where treatment of animals with salubrinal enhanced the severity of the disease possibly due to enhanced translational repression of synaptic proteins. In agreement with this, ISRIB delays the occurrence of experimental FTD and prion disease. However, one study in AD models indicated that ISRIB is ineffective in improving cognitive function. In contrast, another study suggested that PERK activation may be beneficial in FTD. In models of multiple sclerosis and spinal cord injury, PERK/eIF2α signaling is proposed to attenuate symptoms and improve oligodendrocyte function. Overall, accumulating studies suggests that pharmacological targeting of PERK signaling and the ISR may have contrasting and even opposite effects depending on the specific disease analyzed.

2. Expert opinion

Although great advances have been made using PERK modulators to assess their biomedical potential, several specificity, safety, and physicochemical issues are under discussion. Administration of GSK2606414 results in body weight loss and mild hyperglycemia, possibly due to pancreatic islet cell death [6]. This led to the development of GSK2656157, which reported no toxicity on animals due to lower lipophilicity and improved pharmacokinetic properties [12]. However, it was recently reported that both small molecules are potent RIPK1 inhibitors, a kinase implicated in the promotion of inflammation and necroptosis, highlighting the risk of misinterpretation of results obtained with these drug [12]. However, most data provided in that study relies on *in vitro* characterization. The small molecule ISRIB has been shown to be neuroprotective *in vivo* without the occurrence of pancreatic toxicity; however, it has solubility issues [10]. Guanabenz is well known as a centrally active hypotensive drug acting on the α2-adrenergic receptor that can provoke drowsiness and coma at higher doses, but its derivative, Sephin 1, has no activity over the α2-adrenergic receptor, making it a suitable candidate for further preclinical development [13]. However, a recent study questioned the mechanism of action of the compound based on *in vitro* phosphatase assays [14]. As a response to this matter, Carrara et al. demonstrated, by using a recombinant system containing the PPP1R15A regulatory subunit, the PP1 catalytic subunit, and the eIF2α substrate, that the beneficial effects of Guanabenz and Sephin 1 are due to allosteric inhibition of PPP1R15A, as they selectively bind to the amino-terminal region. This phenomenon induces a conformational change, comprising its function, and inhibiting eIF2α dephosphorylation [10]. Although controversy was generated, Crespillo-Casado and Carrara studies are not

Table 1. Compounds targeting PERK and eIF2α phosphatases.

Compound	Mechanism of action	Specificity	Side effects	Physicochemical properties
GSK2606414	Inhibitor of the kinase domain of PERK	Displayed at least a 100-fold selectivity over other eIF2α kinases, but also inhibits RIPK1 at low concentrations	Pancreatic β-cell toxicity. Broadly inhibits CYP450 due to its lipophilicity	Orally available, valuable PK, high lipophilicity
GSK2656157	PERK kinase domain inhibitor	Also one of the most potent inhibitors of RIPK	No toxicity reported on animals	Optimized analog of GSK2606414. Less lipophilic, with improved PK parameters
Salubrinal	eIF2α phosphatase inhibitor	Block selectively dephosphorylation of eIF2α PP1-mediated	Nontoxic in *in vitro* assays, promote apoptosis in rat model of brain ischemic model. It accelerates experimental prion disease.	–
Guanabenz	eIF2α phosphatase inhibitor – Directly and reversibly binds to PPP1R15A, modulating the PPP1R15-PPA complex affinity for eIF2α	It is a centrally active hypotensive drug with nanomolar affinity for the a2 adrenergic receptor	Drowsiness and coma at high doses. Can accelerate disease in a mutant SOD1 model of ALS	Crosses the BBB
Sephin 1	eIF2α phosphatase inhibitor – Directly and reversibly binds to PPP1R15A, modulating the PPP1R15-PPA complex affinity for eIF2α	Guanabenz derivative with no a2 adrenergic blocking activity	Lacks the adverse effects of guanabenz on mice and has no measurable adverse effect on general health or memory in diverse experimental paradigms	Orally available crosses the BBB and reaches concentration in the brain known to inhibit R15A
ISRIB	Acts downstream of eIF2α-P allowing ternary eIF2β complex to be formed	Target the interaction between eIF2α and eIF2β	Safe toxicological profile, with no pancreatic toxicity	Crosses the BBB but has poor solubility
Trazodone	Prevents the reduction on ternary complex levels acting downstream of eIF2α-P	Did not act on other UPR branches	Safe in Humans (FDA approved)	Orally available, crosses the BBB
Dibenzoylmethane	Prevents the reduction on ternary complex levels acting downstream of eIF2α-P	Did not act on other UPR branches	Safe in Humans (FDA approved)	Crosses the BBB
AMG'44	Inhibitor of the kinase domain of PERK	1000-fold selectivity of PERK over GCN2	–	Orally available, good PK properties in mouse
CCT020312	Activation of PERK – eIF2α phosphorylation	Selectively activates PERK signaling	–	–

The table summarizes the mechanism of action, specificity, side effects, and physicochemical properties of indicated compounds.
BBB: blood–brain barrier; PK: pharmacokinetic; FDA: Food and Drug Administration.

comparable, mainly due to differences in the systems used: both express recombinant proteins, but one of them is chaperonin assisted (GroEL/GroES chaperonin system) [15], while the other used actin as a cofactor for the enzyme (which is not completely required for R15 holoenzyme activities) [14]. Also, the duration of the *in vitro* assays differs between both studies. Altogether, these differences could affect the properties of recombinant proteins regarding selectivity and specificity for inhibitors and regulatory subunits.

Because of all these issues related to safety, specificity, and mechanisms of action, alternative strategies need to be developed in order to discover new molecules that are more suitable for human use. In this context, a recent screening used a library of FDA-approved drugs to identify small molecules that mimic the cellular effects of ISRIB, uncovering Trazodone and Dibenzoylmethane as drug candidates that can prevent translational repression acting downstream of eIF2α phosphorylation [10]. These compounds were effective in providing neuroprotection to models of FTD and prion disease after oral administration with clinically relevant doses. Thus, repurposing existing drugs opens new potentials for drug discovery. However, clinical trials that tested the effects of trazodone administration iti Alzheimer's disease patients showed no beneficial effect or even adverse consequences in cognition. Since the pharmacological modulation of protein translation is becoming an important target for disease intervention, it is essential to better define possible side effects of the long-term administration of small molecules specially because the pathway has essential physiological roles in different specialized secretory cells, memory storage and bioenergetics as reported in many studies.

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Declaration of interest

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