PERSPECTIVE

Neuroprotective modulation of the unfolded protein response in Marinesco-Sjögren syndrome: PERK signaling inhibition and beyond

Individuals with Marinesco-Sjögren syndrome (MSS; OMIM 248800), a genetic disease of infancy, suffer various disabilities, including loss of motor coordination due to cerebellar degeneration, and skeletal muscle weakness. After a progressive phase, symptoms stabilize and patients live to old age. Therefore, any pharmacological treatment that delays or attenuates cerebellar degeneration and/or muscle pathology can significantly improve their quality of life. We recently found that inhibiting the protein kinase RNA-like endoplasmic reticulum kinase (PERK) delays cerebellar degeneration, and ameliorates motor function and muscle pathology in a MSS mouse model (Grande et al., 2018). This is the first preclinical study of a pharmacological treatment for MSS. Here we summarize our findings, and discuss how this therapeutic strategy might be improved for effective treatment.

MSS is a rare autosomal recessive disorder that causes cerebellar ataxia, myopathy and cataracts. Most patients also have mild to moderate intellectual disability, skeletal abnormalities, strabismus, nystagmus and dysarthria, and some present hypergonadotropic hypogonadism (Anttonen and Lehesjoki, 2006). There is no pharmacological treatment, apart from hormone replacement for primary gonadal failure, and medical care involves mainly educational and rehabilitative programs to improve walking, cognition and speaking.

Approximately 60% of MSS patients carry homozygous or compound heterozygous mutations in the *SIL1* gene. A spontaneous *Sil1* mutation is also responsible for the ataxic behavior of woozy mice, which develop cerebellar degeneration with loss of Purkinje cells (PCs) and myopathic changes highly reminiscent of MSS (Buchkremer et al., 2016). The SIL1 protein is an ATP-exchange factor for the endoplasmic reticulum (ER) chaperone binding immunoglobulin protein (BiP; also known as GRP78), which plays a central role in protein folding.

BiP's ability to bind unfolded proteins and release the folded substrate is tightly regulated by a cycle of ATP binding, hydrolysis, and nucleotide exchange. SIL1 binds to ADP-bound BiP to catalyze the release of ADP and rebinding of ATP. If this nucleotide exchange is defective, as in the case of *SIL1* mutations, BiP remains associated with its client protein, ultimately leading to accumulation of unfolded proteins, ER stress and activation of the unfolded protein response (UPR). The SIL1 homologous 150-kDa oxygen-regulated protein (ORP150; also known as GRP170) can substitute for the SIL1 nucleotide exchange function, and its transgenic overexpression in woozy mice prevents UPR activation and PC death (Zhao et al., 2010).

The UPR is a complex signaling pathway whose purpose is to restore ER proteostasis by increasing the ER protein-folding capacity, degrade unfolded proteins, and inhibit protein synthesis as a mechanism to reduce the load of unfolded proteins entering the ER (**Figure 1**). This is achieved through activation of three distinct ER transmembrane protein sensors: inositol-requiring enzyme 1 (IRE1), activating transcription factor 6 (ATF6), and PERK. The IRE1 and ATF6 signaling pathways increase expression of chaperones and components of the protein degradation machinery, while activated PERK phosphorylates the α subunit of eukaryotic translation initiation factor 2 (eIF2α). This inhibits mRNA translation, reducing global protein synthesis. The mRNA of transcription factor ATF4, however, escapes eIF2α-P-mediated translational attenuation. ATF4 upregulates growth arrest and DNA-damage-inducible protein-34 (GADD34), which dephosphorylates eIF2α-P, providing a negative feedback mechanism that contributes to rapidly restoring protein synthesis upon resolution of ER stress. If cells are unable to handle the unfolded protein load, protracted ATF4 synthesis induces the expression of C/EBP homologous protein (CHOP), which triggers apoptosis. Thus persistent ER stress eventually leads to cell death.

Prolonged activation of the PERK branch of the UPR is detrimental to neurons, which are exquisitely dependent on new protein synthesis for synaptic maintenance and survival. Thus pharmacological inhibition of this pathway to restore protein synthesis rescues synaptic failure and behavioral deficits in prion-infected mice and slows disease progression in mouse models of frontotemporal dementia and Parkinson's disease (Moreno et al., 2013; Halliday et al., 2015; Radford et al., 2015; Mercado et al., 2018).

In woozy mice, motor deficits are first detectable in the accelerating rotarod task at 9–10 weeks of age, concomitantly with incipient degeneration of PCs (Grande et al., 2018). Rotarod performance worsens progressively up to 16 weeks of age, after which there is no further significant decline (Grande et al., 2018). At this stage, histological analysis finds marked cerebellar atrophy with almost complete loss of PCs (Grande et al., 2018).

Previous studies have found increased BiP and CHOP levels in degenerating PCs of woozy mice, supporting a role of the UPR in neurodegeneration (Zhao et al., 2005, 2010). We found that activation of the UPR – particularly the PERK/eIF2α branch – prefigured PC degeneration and ataxia in woozy mice (Grande et al., 2018). Presymptomatic treatment with GSK2606414, a potent inhibitor of PERK, delayed neurodegeneration and the onset of

motor deficits, prolonging the asymptomatic phase of the disease by ~3 weeks, and improved muscle pathology and motor performance in the symptomatic phase (Grande et al., 2018). Rescued PCs had higher ORP150 protein levels, suggesting that translational recovery promoted the synthesis of this alternative BiP co-factor, restoring BiP-assisted protein folding and synaptic protein transport (**Figure 2**) (Grande et al., 2018). Supporting this, we found that GSK2606414 attenuated ER chaperone aggregation and improved secretory protein trafficking in SIL1-deficient cells (Capone et al., 2018).

We found reduced numbers of CHOP-positive PCs in GSK2606414-treated woozy mice, suggesting that PERK inhibition may prevent CHOP-mediated apoptosis. However, genetic ablation of CHOP is not neuroprotective in woozy mice (Zhao et al., 2010), indicating that CHOP is not the final executor of cell death, and consistent with the idea that PERK pathway inhibition protects neurons by restoring synaptic protein synthesis rather than by blocking downstream pro-apoptotic signals.

Complete PERK inhibition by GSK2606414 is toxic to the pancreas, where a degree of eIF2α-P-mediated translational repression is essential to cope with the high secretory load of this tissue. Trazodone, a licensed antidepressant, and dibenzoylmethane (DBM), a licorice derivative with anti-cancer properties, partially inhibit PERK signaling downstream of eIF2α-P at the level of the translation initiation ternary complex, and are neuroprotective without pancreatic toxicity (Halliday et al., 2017). Therefore, these drugs could eventually be repurposed for treatment of MSS, and it may be worth testing them in the woozy mouse. However, PERK inhibition delayed but did not stop PC degeneration in woozy mice (Grande et al., 2018), so inhibiting PERK signaling alone may not be sufficient for long-term neuroprotection in MSS.

The ATF6 and IRE1 branches of the UPR were poorly activated in woozy PCs, and PERK inhibition by GSK2606414 did not lead to their over-activation (Grande et al., 2018). Thus targeting ATF6 and/or IRE1 may not provide any further neuroprotection.

Perhaps the increase in ORP150 induced by GSK2606414 is too small to fully sustain BiP's folding activity, and unfolded proteins continue to accumulate in the secretory pathway, eventually leading to neuronal dysfunction and death. It may therefore be necessary to further boost protein folding for long-term neuroprotection, and chemical chaperones might be useful. Tauroursodeoxycholic acid (TUDCA) in particular was recently found to attenuate ER stress-induced apoptosis in MSS cells (Kashimada et al., 2018). This compound crosses the blood brain barrier and is already used in patients for the treatment of primary biliary cirrhosis. It may be worth testing whether TUDCA has beneficial effects in the woozy model, and if combining TUDCA with trazodone or DBM provides better neuroprotection than PERK inhibitors alone (**Figure 2**). Evidence of a synergic effect of these drugs may lead to their rapid repurposing for MSS.

This work was supported by a grant from Fondazione Telethon, No. GGP12220 (to MS and RC).

Elena Restelli*, Antonio Masone, Michele Sallese, Roberto Chiesa*
Department of Neuroscience, IRCCS – Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy (Restelli E, Masone A, Chiesa R)
Department of Medical, Oral and Biotechnological Sciences, Università "G. d'Annunzio", Chieti, Italy; CeSI-MeT, Center for Research on Ageing and Translational Medicine, Università "G. d'Annunzio", Chieti, Italy (Sallese M)
***Correspondence to:** Elena Restelli, PhD, elena.restelli@marionegri.it; Roberto Chiesa, PhD, roberto.chiesa@marionegri.it.*
***orcid:** 0000-0002-6081-5178 (Elena Restelli)*
0000-0002-3842-3733 (Roberto Chiesa)
***Received:** July 12, 2018*
***Accepted:** September 12, 2018*

***doi:** 10.4103/1673-5374.243708*

***Plagiarism check:** Checked twice by iThenticate.*
***Peer review:** Externally peer reviewed.*

***Open peer reviewers:** Clévio Nobrega, Universidade do Algarve, Portugal; Marija Cvetanovic, University of Minnesota, USA.*
***Additional file:** Open peer review reports 1 and 2.*

References

Anttonen AK, Lehesjoki AE (1993) Marinesco-Sjogren Syndrome. In: GeneReviews® (Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, Amemiya A, eds). Seattle, WA, USA: University of Washington, Seattle.

Buchkremer S, González Coraspe JA, Weis J, Roos A (2016) Sil1-mutant mice elucidate chaperone function in neurological disorders. J Neuromuscul Dis 3:169-181.

Capone V, Clemente E, Restelli E, Di Clampi A, Sperduti S, Ornaghi F, Pietrangelo L, Protasi F, Chiesa R, Sallese M (2018) PERK inhibition attenuates the abnormalities of the secretory pathway and the increased apoptotic rate induced by SIL1 knockdown in HeLa cells. Biochim Biophys Acta Mol Basis Dis 1864:3164-3180.

Grande V, Ornaghi F, Comerio L, Restelli E, Masone A, Corbelli A, Tolomeo D, Capone V, Axten JM, Laping NJ, Fiordaliso F, Sallese M, Chiesa R (2018) PERK inhibition delays neurodegeneration and improves motor function in a mouse model of Marinesco-Sjögren syndrome. Hum Mol Genet 27:2477-2489.

Halliday M, Radford H, Sekine Y, Moreno J, Verity N, le Quesne J, Ortori CA, Barrett DA, Fromont C, Fischer PM, Harding HP, Ron D, Mallucci GR (2015) Partial restoration of protein synthesis rates by the small molecule ISRIB prevents neurodegeneration without pancreatic toxicity. Cell Death Dis 6:e1672.

Halliday M, Radford H, Zents KAM, Molloy C, Moreno JA, Verity NC, Smith E, Ortori CA, Barrett DA, Bushell M, Mallucci GR (2017) Repurposed drugs targeting eIF2α-P-mediated translational repression prevent neurodegeneration in mice. Brain J Neurol 140:1768-1783.

Kashimada A, Hasegawa S, Isagai T, Uchiyama T, Matsuo M, Kawai M, Goto M, Morio T, Hayashi YK, Takagi M (2018) Targeting the enhanced ER stress response in Marinesco-Sjögren syndrome. J Neurol Sci 385:49-56.

Mercado G, Castillo V, Soto P, López N, Axten JM, Sardi SP, Hoozemans JJM, Hetz C (2018) Targeting PERK signaling with the small molecule GSK2606414 prevents neurodegeneration in a model of Parkinson's disease. Neurobiol Dis 112:136-148.

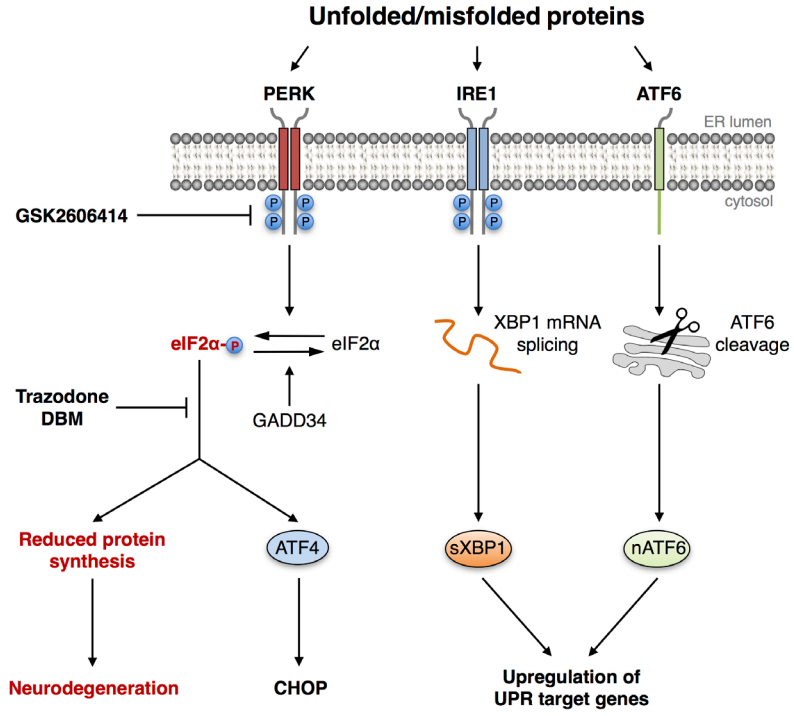

Figure 1 Scheme of the unfolded protein response (UPR), showing the sites of action of compounds that inhibit protein kinase RNA-like endoplasmic reticulum kinase (PERK) signaling.
The UPR is mediated by three endoplasmic reticulum (ER)-resident transmembrane proteins that sense ER stress. Once activated, the kinase PERK phosphorylates eukaryotic translation initiation factor 2 (eIF2α) in the cytoplasm. This inhibits protein translation, reducing the overload of misfolded proteins. Growth arrest and DNA-damage-inducible protein-34 (GADD34), a protein phosphatase upregulated by the PERK pathway, dephosphorylates eIF2α, providing a negative feedback mechanism that contributes to rapidly restoring protein synthesis upon resolution of ER stress. Sustained activation of PERK/eIF2α-P signaling causes chronic translational attenuation leading to synapse loss and neurodegeneration, and selectively enhances translation of ATF4 that induces the expression of C/EBP homologous protein (CHOP), eventually triggering apoptosis. Activated inositol-requiring enzyme 1 (IRE1) initiates the unconventional splicing of the mRNA encoding the transcriptional factor X-box-binding protein 1 (XBP1) to produce sXBP1, a more stable form of XBP1 with a potent transactivator domain that enhances transcription of genes involved in protein folding, secretion and ER-associated degradation. Activating transcription factor 6 (ATF6) is transported to the Golgi where it is processed within the transmembrane domain to release the cytosolic domain, which translocates to the nucleus (nATF6) and induces the expression of ER chaperones. GSK2606414 is a potent inhibitor of PERK. Trazodone and dibenzoylmethane (DBM) reverse translational attenuation, acting downstream of eIF2α-P.

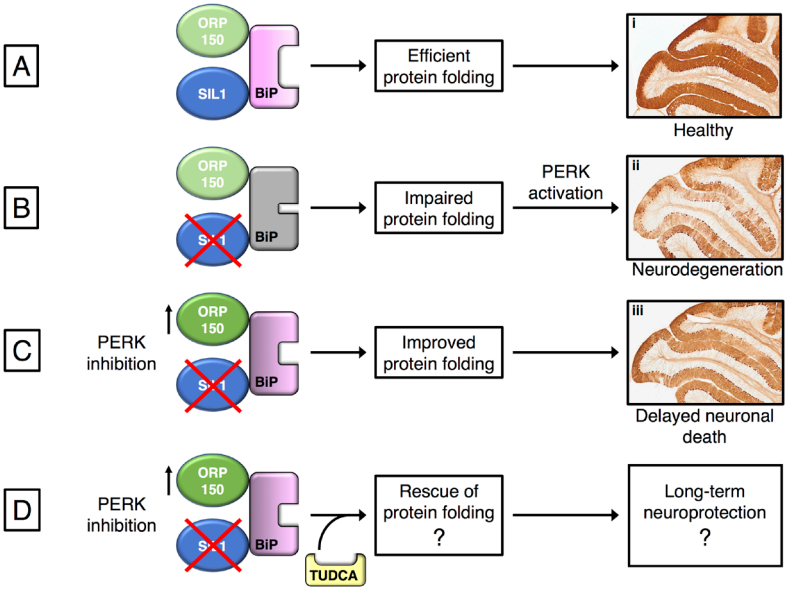

Figure 2 Theoretical model describing how a combined pharmacological treatment with PERK signaling inhibitors and chemical chaperones could protect SIL1-deficient Purkinje cells.
(A) In normal conditions SIL1 and 150-kDa oxygen-regulated protein (ORP150) work in parallel providing optimal nucleotide exchange for binding immunoglobulin protein (BiP)-assisted folding. Purkinje neurons, identified by calbindin immunostaining, are normal (panel i). (B) In the absence of SIL1, ORP150 nucleotide exchange function is insufficient and BiP activity is impaired. Unfolded proteins accumulate, activating the UPR. PERK-mediated translational attenuation reduces synaptic protein synthesis, leading to degeneration of Purkinje neurons (panel ii). (C) PERK signaling inhibitors increase ORP150 synthesis, improving BiP-assisted protein folding and prolonging neuronal survival (panel iii). However, BiP folding activity is still suboptimal, and unfolded proteins continue to accumulate ultimately leading to neuronal dysfunction and death. (D) Chemical chaperones, such as taurodeoxycholic acid (TUDCA), may boost protein folding in PERK inhibitor-treated cells, providing full neuroprotection. Panel i, vehicle-treated wild-type mouse; panel ii, vehicle-treated woozy mouse; panel iii, GSK2606414-treated woozy mouse, analyzed after 5 weeks of treatment (Grande et al., 2018).

Moreno JA, Halliday M, Molloy C, Radford H, Verity N, Axten JM, Ortori CA, Willis AE, Fischer PM, Barrett DA, Mallucci GR (2013) Oral treatment targeting the unfolded protein response prevents neurodegeneration and clinical disease in prion-infected mice. Sci Transl Med 5:206ra138.

Radford H, Moreno JA, Verity N, Halliday M, Mallucci GR (2015) PERK inhibition prevents tau-mediated neurodegeneration in a mouse model of frontotemporal dementia. Acta Neuropathol 130:633-642.

Zhao L, Longo-Guess C, Harris BS, Lee JW, Ackerman SL (2005) Protein accumulation and neurodegeneration in the woozy mutant mouse is caused by disruption of SIL1, a cochaperone of BiP. Nat Genet 37:974-979.

Zhao L, Rosales C, Seburn K, Ron D, Ackerman SL (2010) Alteration of the unfolded protein response modifies neurodegeneration in a mouse model of Marinesco-Sjogren syndrome. Hum Mol Genet 19:25-35.

P-Reviewers: Nobrega C, Cvetanovic M; C-Editors: Zhao M, Yu J; T-Editor: Liu XL