BBA - Molecular Basis of Disease xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadis

PERK inhibition attenuates the abnormalities of the secretory pathway and the increased apoptotic rate induced by *SIL1* knockdown in HeLa cells

Vanessa Capone[a,b], Emanuela Clemente[a,b], Elena Restelli[c], Antonella Di Campli[d], Samantha Sperduti[a,b], Francesca Ornaghi[c], Laura Pietrangelo[b,e], Feliciano Protasi[b,f], Roberto Chiesa[c], Michele Sallese[a,b,*]

[a] *Department of Medical, Oral and Biotechnological Sciences, University G. d'Annunzio, Chieti 66100, Italy*
[b] *CeSI-MeT, Centre for Research on Ageing and Translational Medicine, University G. d'Annunzio, Chieti 66100, Italy*
[c] *Department of Neuroscience, IRCCS - Mario Negri Institute for Pharmacological Research, Via G. La Masa 19, 20156 Milan, Italy*
[d] *Institute of Protein Biochemistry, National Research Council, Naples, Italy*
[e] *DNISC, Dept. of Neuroscience, Imaging, and Clinical Sciences, University G. d'Annunzio, Chieti, 66100, Italy*
[f] *DMSI, Dept. of Medicine and Aging Science, University G. d'Annunzio, Chieti, 66100, Italy*

ARTICLE INFO

Keywords:
Marinesco-Sjögren syndrome
SIL1
Membrane trafficking
Cell survival

ABSTRACT

Loss-of-function mutations in the *SIL1* gene are linked to Marinesco-Sjögren syndrome (MSS), a rare multisystem disease of infancy characterized by cerebellar and skeletal muscle degeneration. SIL1 is a ubiquitous adenine nucleotide exchange factor for the endoplasmic reticulum (ER) chaperone BiP. The complexity of mechanisms by which loss of SIL1 causes MSS is not yet fully understood. We used HeLa cells to test the hypothesis that impaired protein folding in the ER due to loss of SIL1 could affect secretory trafficking, impairing the transport of cargoes essential for the function of MSS vulnerable cells. Immunofluorescence and ultrastructural analysis of SIL1-knocked-down cells detected ER chaperone aggregation, enlargement of the Golgi complex, increased autophagic vacuoles, and mitochondrial swelling. SIL1-interefered cells also had delayed ER-to-plasma membrane transport with retention of Na^+/K^+-ATPase and procollagen-I in the ER and Golgi, and increased apoptosis. The PERK pathway of the unfolded protein response was activated in SIL1-interfered cells, and the PERK inhibitor GSK2606414 attenuated the morphological and functional alterations of the secretory pathway, and significantly reduced cell death. These results indicate that loss of SIL1 is associated with alterations of secretory transport, and suggest that inhibiting PERK signalling may alleviate the cellular pathology of *SIL1*-related MSS.

1. Introduction

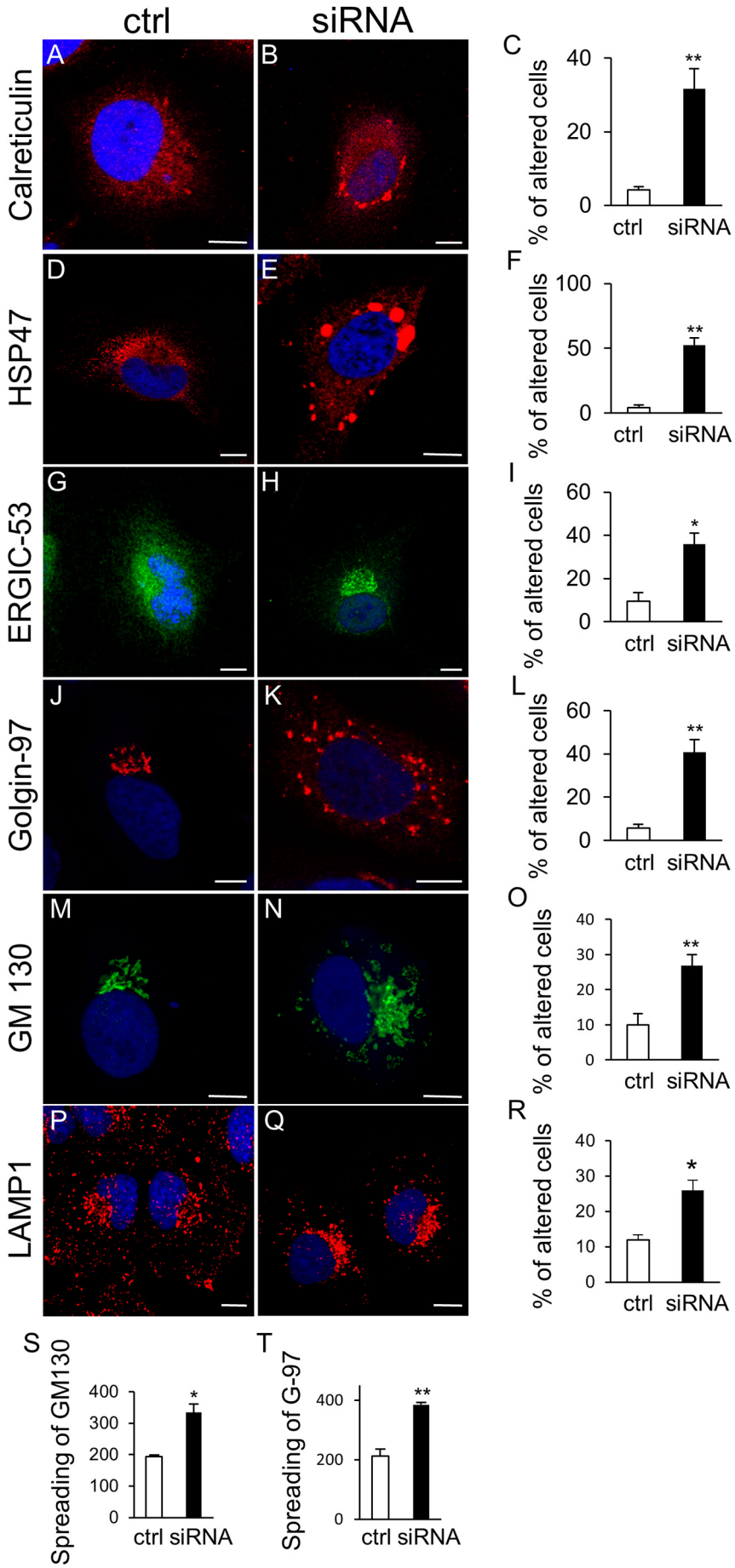
Marinesco-Sjögren syndrome (MSS) is a rare, early-onset, autosomal recessive genetic disease (MIM 248800) causing cataracts, cerebellar ataxia, hypotonia, dysarthria, short stature, and mental retardation ranging from mild to severe [1–4]. Clinical signs appear in early infancy, and progress for a number of years; then they stabilise and patients live to old age. There is no therapy for MSS, and medical care is mainly symptomatic, with educational and rehabilitative programs to improve walking, cognition and speaking.

Approximately 60% of MSS cases are linked to loss-of-function mutations in the *SIL1* gene [3,4]. The SIL1 protein is an endoplasmic reticulum (ER) ATPase exchange factor that assists in the release of ADP from the essential chaperone BiP, also known as GRP78 [5]. BiP plays vital roles in the translocation of newly synthesized proteins into the ER lumen, in the ion gating of the aqueous pore translocon, in protein folding, and in the retrotranslocation of misfolded proteins targeted to ER-associated degradation (ERAD) [6–8]. BiP also participates in activating the unfolded protein response (UPR), a complex signalling pathway triggered by the accumulation of unfolded/misfolded proteins in the ER, whose purposes are to restore proteostasis by boosting the ER protein-folding capacity, reduce the load of newly synthesized proteins entering the ER, and degrade the unfolded proteins [9].

The UPR signals through three distinct ER transmembrane protein sensors: inositol-requiring enzyme 1 (IRE1), protein kinase RNA (PKR)-like ER kinase (PERK), and activating transcription factor 6 (ATF6). Under normal conditions these sensors are inactive due to BiP's association with their luminal domains [10–13]. When the level of unfolded proteins in the ER rises, BiP dissociates from the ER stress sensors to bind to exposed hydrophobic regions of unfolded proteins, enabling

* Corresponding author at: Department of Medical, Oral and Biotechnological Sciences, University G. d'Annunzio, Chieti 66100, Italy.
E-mail address: michele.sallese@unich.it (M. Sallese).

https://doi.org/10.1016/j.bbadis.2018.07.003
Received 16 January 2018; Received in revised form 5 June 2018; Accepted 3 July 2018



(caption on next page)