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Targeting the enhanced ER stress response in Marinesco-Sjögren syndrome

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ABSTRACT

Background and objective: Marinesco-Sjögren syndrome (MSS) is an autosomal recessive infantile-onset disorder characterized by cataracts, cerebellar ataxia, and progressive myopathy caused by mutation of *SIL1*. In mice, a defect in SIL1 causes endoplasmic reticulum (ER) chaperone dysfunction, leading to unfolded protein accumulation and increased ER stress. However, ER stress and the unfolded protein response (UPR) have not been investigated in MSS patient-derived cells.

Methods: Lymphoblastoid cell lines (LCLs) were established from four MSS patients. Spontaneous and tunicamycin-induced ER stress and the UPR were investigated in MSS-LCLs. Expression of UPR markers was analyzed by western blotting. ER stress-induced apoptosis was analyzed by flow cytometry. The cytoprotective effects of ER stress modulators were also examined.

Results: MSS-LCLs exhibited increased spontaneous ER stress and were highly susceptible to ER stress-induced apoptosis. The inositol-requiring protein 1α (IRE1α)-X-box-binding protein 1 (XBP1) pathway was mainly upregulated in MSS-LCLs. Tauroursodeoxycholic acid (TUDCA) attenuated ER stress-induced apoptosis.

Conclusion: MSS patient-derived cells exhibit increased ER stress, an activated UPR, and susceptibility to ER stress-induced death. TUDCA reduces ER stress-induced death of MSS patient-derived cells. The potential of TUDCA as a therapeutic agent for MSS could be explored further in preclinical studies.

1. Introduction

Marinesco-Sjögren syndrome (MSS; OMIM 248800) is a rare autosomal recessive infantile-onset multisystem disorder characterized by bilateral cataracts, cerebellar ataxia, intellectual disability, and progressive muscle weakness due to myopathy [1]. Intellectual disability is highly variable in MSS, and there are also few patients with normal cognition. Other clinical features include short stature, hypergonadotropic hypogonadism [2], and strabismus [3]. Homozygous or compound heterozygous mutations of the *SIL1* gene on chromosome 5q31 are reported to cause MSS [1].

SIL1 is a co-chaperone of the HSP70 molecular chaperone BIP (also referred to as GRP78 or HSPA5) [4]. BIP is located in the lumen of the endoplasmic reticulum (ER) and binds to newly synthesized proteins to maintain proper protein folding and translocation in the ER. ADP-bound BIP binds tightly to its substrates, whereas ATP induces a conformational change that opens the substrate-binding pocket. SIL1 modulates BIP activation via nucleotide exchange during the ATP/ADP cycle of BIP. SIL1 protein releases ADP from BIP so that it can bind to ATP and re-start the protein-folding process [5,6]. Therefore, SIL1 deficiency causes BIP dysfunction, leading to accumulation of misfolded proteins in the ER and increased ER stress. The unfolded protein response (UPR) is a cellular adaptive response to ER stress and restores protein-folding homeostasis.

In this study, we aimed to evaluate ER stress and the UPR at the cellular level in MSS using patient-derived lymphoblastoid cell lines

Abbreviations: 4-PBA, sodium phenylbutyrate; ATF6α, activating transcription factor 6α; CASP3, caspase-3; DEXA, dexamethasone; EBV, Epstein-Barr virus; eIF2α, eukaryotic translation initiation factor 2α; ER, endoplasmic reticulum; IRE1α, inositol-requiring protein 1α; LCL, lymphoblastoid cell line; MSS, Marinesco-Sjögren syndrome; PDI, protein disulphide isomerase; PERK, PRKR-like ER kinase; TUDCA, tauroursodeoxycholic acid; UPR, unfolded protein response; VPA, sodium valproate; XBP1, X-box-binding protein 1

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(LCLs) and to explore a therapeutic approach. MSS patient-derived cells exhibited spontaneous ER stress and an activated UPR and were highly susceptible to ER stress-induced apoptosis. Tauroursodeoxycholic acid (TUDCA) alleviated excessive ER stress-induced apoptosis in these cells. The potential use of TUDCA as a therapeutic agent for MSS should be investigated further.

2. Materials and methods

2.1. Patient samples

All patients included in this study were clinically and genetically diagnosed with MSS. Blood samples and medical reports were obtained with written informed consent of the patients or their legal guardians. Research protocols were approved by the ethical committee of Tokyo Medical and Dental University (approval nos. 103 and 196).

Four Japanese patients were enrolled in this study. These patients carried the homozygous c.936dupG (p.Leu313fs) mutation in exon 9 of the *SIL1* gene, which is highly common in Japanese MSS patients [7]. Their ages at the time point for cell line establishment ranged from 14 months to 49 years (mean = 16.8 ± 18.9 years). All patients had a low average IQ or moderate intellectual disability and severe muscle weakness. The patients' phenotypes are described in Table 1.

2.2. Cell lines and cell culture

Epstein-Barr virus (EBV)-immortalized lymphoblastoid cell lines (EBV-LCLs) from control subjects (control LCLs) and patients with MSS (MSS-LCLs) were established according to standard protocols. LCLs were maintained in RPMI 1640 supplemented with 15% fetal bovine serum and 1% penicillin/streptomycin.

2.3. Western blotting

Cells were lysed in ice-cold RIPA buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1.0% NP-40, 0.5% DOC, 0.1% SDS, 50 mM NaF, 25 mM β-glycerophosphate, 1 mM PMSF, and a protease inhibitor cocktail (Roche, Basel, Switzerland). Lysates were resolved on SDS-polyacrylamide gels. The gels were transferred to nitrocellulose membranes (EMD Millipore, Billerica, MA, USA) and blocked with 5% non-fat milk prepared in TBST. The membranes were incubated with the following primary antibodies: anti-SIL1 (OriGene Technologies, Rockville, MD, USA); anti-spliced X-box-binding protein 1 (XBP1s), anti-BIP, anti-caspase-3 (CASP3), anti-PARP, anti-phospho-ASK1 (Thr645), anti-ASK1, anti-phospho-JNK (Thr183/Tyr185) (G9), and anti-JNK (Cell Signaling Technology, Danvers, MA, USA); and anti-β-actin (Sigma-Aldrich, St. Louis, MO, USA). Primary antibodies were detected by binding of a horseradish peroxidase-conjugated anti-rabbit or anti-mouse secondary antibody with an ECL kit (GE Healthcare, Little Chalfont, UK).

Table 1
Clinical features of patients.

	Patient 1	Patient 2	Patient 3	Patient 4
Sex	M	F	F	M
Present age	10 y	52 y	24 y	9 y
Age at cell line establishment	7 y5 m	49 y	21 y	1 y2 m
Ocular involvements				
Cataract	+	+	+	+
Cerebellar signs				
Cerebellar atrophy	+	+	+	+
Nystagmus	+	+	+	+
Dysarthria	5y	N/A	2y	3y
Ataxia	23 m	5 y	1 y	3 y
Psychomotor delay and Muscle symptom				
Intellectual disability (intelligence quotient)	+ (35–50)	− (81)	+ (40)	− (85)
Head control	6 m	6 m	8 m	2y6 m
Standing	3 y	5 y	−	4 y
Walk	−	−	−	−
Activity daily living	Wheel chair	Wheel chair	Wheel chair	Wheel chair
Serum CK (IU/L)	276	27	N/A	261
Myopathy	+	+	+	+
Other				
Hypogonadism	N/A	+	+	N/A

M: male, F: female, y: year, m: month, N/A: not applicable, CK: creatin kinase.

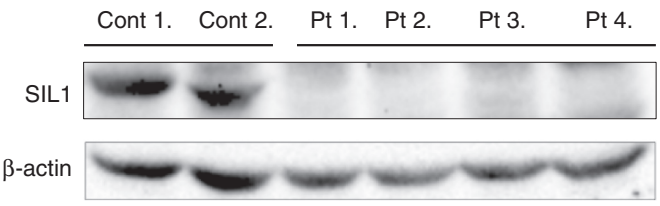

Fig. 1. Expression of SIL1 is defective in MSS-LCLs.
Western blot analysis of SIL1 expression. β-actin was used as a loading control. Cont: An EBV-transformed LCL derived from a healthy volunteer. Pt: An EBV-transformed LCL derived from a patient with MSS.

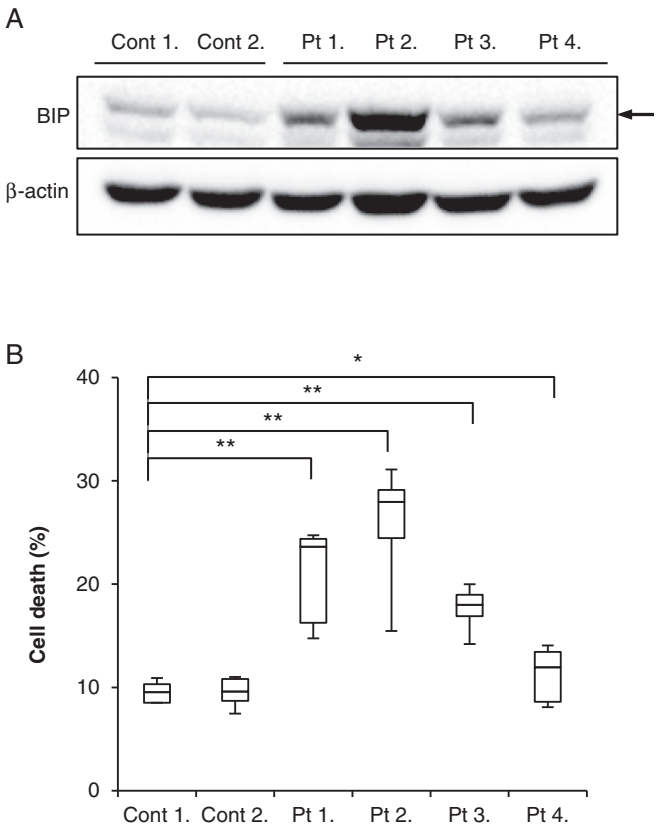

Fig. 2. Expression of BIP and the dead cell fraction in non-stressed culture conditions.
(A) Western blot images of BIP expression. β-actin was used as a loading control. (B) Percentage of dead cells (n = 5). Data are presented as mean ± standard deviation (SD) from multiple independent experiments. *p < 0.05, **p < 0.005.

2.4. Apoptosis assay, cell survival assay, and measurement of mitochondrial membrane potential

LCLs were plated at a density of 1×10^6 cells/mL and treated with 2–20 μg/mL tunicamycin (Sigma-Aldrich) for 24 h. LCLs (2.5×10^5 cells) were washed with PBS and resuspended in 100 μL of annexin