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UPR activation and CHOP mediated induction of *GBA1* transcription in Gaucher disease

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ABSTRACT

Chronic presence of mutant, misfolded proteins in the endoplasmic reticulum (ER) initiates ER stress and induces the Unfolded Protein Response (UPR).
In Gaucher disease (GD), resulting from mutations in the *GBA1* gene, encoding lysosomal acid β-glucocerebrosidase (GCase), a certain fraction of the mutant variants is retained in the ER and activates the UPR. We have previously shown UPR activation in GD derived fibroblasts, in fibroblasts that derived from carriers of GD mutations and in *Drosophila* models of carriers of GD mutations.
In the present work we extended our studies to include a large collection of fibroblasts, EBV-transformed B-cells and white blood cells (WBCs) that derived from GD patients.
The results showed UPR activation in all tested cells. They also indicated that transcription of the *GBA1* gene is upregulated through activation of the UPR-induced CHOP transcription factor. Transcription of the *MAN2B* gene, encoding alpha-mannosidase and of the *ACP* gene, encoding acid phosphatase was also elevated presumably through CHOP activation.
Our results highlight the existence of chronic stress in GD derived cells due to the presence of ER-retained mutant GCase, which leads to upregulation of *GBA1* expression.

1. Introduction

Gaucher disease (GD), an autosomal recessive genetic disorder, results mainly from mutations in the *GBA1* gene (OMIM #606463), encoding acid β-glucocerebrosidase (GCase; EC 3.2.1.45) [1]. Mutations in the saposin C domain of the *prosaposin* gene (*PSAP*) also result in GD [2–4]. Presence of mutant enzyme leads to decreased lysosomal activity and accumulation of the substrate glucosylceramide (glucocerebroside) [1]. More so, a certain fraction of the mutant enzyme is retained in the ER and undergoes ER associated degradation (ERAD) [5]. The chronic presence of mutant enzyme in the ER leads to ER stress and upregulates the Unfolded Protein Response (UPR), a cascade of signaling pathways aiming at reaching homeostasis in the cells. If not achieved, the cells undergo apoptotic death. The ER membrane harbors three ER stress sensors: the type 1 transmembrane protein kinase endoribonuclease (IRE1), the type 1 protein kinase (PERK), and the activating transcription factor 6 (ATF6). These three UPR transducers are constitutively expressed in metazoan cells, and are maintained in an inactive state through interaction with the ER chaperone BiP (Immunoglobulin heavy chain-binding protein) [6,7]. ER-accumulated unfolded proteins bind and sequester BiP, thus promoting its dissociation from PERK, IRE1 and ATF6. Dissociation of BiP from the three stress sensors allows their modification and activation, which results in a response to the accumulation of misfolded proteins [7–9]. Thus, IRE1 undergoes dimerization and phosphorylation, and participates in a cytoplasmic complex, which splices the transcription factor X-box binding protein 1 (Xbp1). Upon its splicing the *Xbp1* mRNA (*Xbp1s*) is translated into a protein that translocates into the nucleus and activates UPR related genes [10–13]. PERK is a kinase that undergoes dimerization and autophosphorylation, and mediates phosphorylation of the eukaryotic translation initiation factor 2α (eIF2α) [8]. Phosphorylated eIF2α attenuates general protein translation in the cells [7,8,12,14,15]. Modified PERK also initiates translation of ATF4, which activates transcription of UPR related genes, like the CAAT/Enhancer binding protein (C/EBP) homologous protein (CHOP), which is a proapoptotic bZIP transcription factor [16,17]. CHOP is essential for cell cycle arrest as part of the apoptotic response to chronic ER stress [6,14,18]. ATF6 shuttles to the Golgi, where it is sequentially cleaved by proteases. Its cleaved N-terminal cytosolic

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fragment serves as a transcription factor of UPR upregulated genes [7,11,12,14,19].

Activation of UPR in GD derived cells has already been noted in fibroblast lines that originated from GD patients, homozygous for the N370S or the L444P mutations [20,21]. We documented it in several fibroblast lines that derived from GD patients and carriers of GD mutations by assaying the increase in *BiP* and *CHOP* mRNAs and proteins, the increase in *Xbp1* splicing and phosphorylation of eIF2α [22]. Accumulation of glucosylceramide per se, induced by conduritol-β-epoxide (CBE), did not result in UPR [22]. Likewise, in the absence of mutant GCase there was no UPR [23], underscoring the importance of mutant GCase in the activation of UPR.

GD patients and carriers of GD mutations have a higher propensity to develop Parkinson disease (PD) than the non-GD population [24]. We and others have shown that the presence of mutant GCase activates UPR and leads to development of parkinsonian signs or neurodegeneration in *Drosophila melanogaster* [22,25,26].

It has already been documented that *GBA1* mRNA levels are increased in GD derived cells, the nature of which was unknown [27]. Previous studies indicated that a 630 bp promoter fragment of the human *GBA1* gene was sufficient to confer the same tissue specificity as the entire gene. This fragment contains 365 bp upstream the transcription start site and 265 bp of the first exon [27,28]. Using Electrophoretic Mobility Shift Assays (EMSAs) and Chloramphenicol Acetyl Transferase (CAT) assays our lab showed that OCTA binding protein (OBP), activator protein 1 (AP-1), polyoma enhancer activator 3 (PEA3) and a CAAT enhancer binding protein (C/EBP) participate in regulating the *GBA1* gene [29].

In the present study we document activation of UPR in GD derived cultured fibroblasts, in cultured lymphoblasts and in white blood cells (WBCs) from GD patients. We also show that the increase in *GBA1* transcription in GD-derived cells is mediated by the UPR-activated CHOP protein.

2. Materials and methods

2.1. Cell lines

Human primary skin fibroblasts and EBV-transformed B-cells derived from GD patients and carriers were from the "Cell Line and DNA Biobank from Patients Affected by Genetic Diseases" (G. Gaslini Institute). Skin fibroblasts (cultured fibroblasts) were grown in DMEM supplemented with 20% FBS (Biological Industries, Beit Haemek, Israel). Human EBV transformed B-cells (cultured lymphoblasts) were grown in RPMI supplemented with 10–20% FBS (Biological Industries, Beit Haemek, Israel). HEK293 (Human epithelial embryonic kidney) cells were grown in DMEM supplemented with 10% FBS. All cells were grown at 37 °C in the presence of 5% CO_2.

2.2. Antibodies

The following primary antibodies were used in this study: rabbit polyclonal anti-phospho-eIF2α (Ser51) antibodies, rabbit polyclonal anti-eIF2α antibodies (from cell signaling Technology, Beverly, MA, USA), Rabbit polyclonal anti-ERK antibodies (Santa Cruz Biotechnology, CA, USA) and mouse monoclonal anti-myc antibody (Cell Signaling Technology, Beverly, MA, USA).

Secondary antibodies used were: horseradish peroxidase-conjugated goat anti-mouse antibodies and horseradish peroxidase-conjugated goat anti-rabbit antibodies (both from Jackson Immuno Research Laboratories, West Grove, PA, USA).

2.3. Chemicals

Leupeptin, phenylmethylsulfonyl fluoride (PMSF), aprotinin and phosphatase inhibitor cocktails 2 and 3 were from Sigma-Aldrich (Rehovot, Israel). KAPA SYBR FAST UNIVERSAL qPCR kit was from KAPA Biosystems Inc. (Wilmington, MA, USA). CBE and thapsigargin were from Sigma-Aldrich (Rehovot, Israel).

2.4. Plasmids

pGL3-N: A 630 bp normal human *GBA1* promoter fragment, prepared by cleavage of an existing clone [24] with the restriction enzyme *Sac*I, was introduced into the *Sac*I restriction site of the mammalian vector pGL3 (Promega Corporation, Madison, WI, USA), harboring the luciferase gene.

pGL3-M: The above mentioned vector, harboring a 630 bp human *GBA1* promoter fragment, mutated at its CHOP binding site (CCAAT → CTGGC).

pGL2: A mammalian vector expressing the renilla gene coupled to the CMV promoter (Promega Corporation, Madison, WI, USA).

pcDNA4-myc-His-CHOP: CHOP sequence was amplified from a human cDNA library, prepared from a normal fibroblast line (F0908, kindly provided by Prof. Eli Sprecher, Department of Dermatology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel), using the primers: CHOP cDNA-F: TTAGCG**GAATTC**ATGGCAACT and CHOP cDNA-R: TCCAAT**CTCGAG**ATTGCTTGG. The 1028 bp amplified CHOP cDNA fragment, which contained *Eco*RI and *Xho*I restriction sites on its 5′ and 3′ ends, respectively (appears in bold), was cloned between the *Eco*RI and *Xho*I restriction sites of pcDNA4-myc-His-plasmid (Invitrogen Life Technologies Co., Carlsbad, CA, USA).

2.5. Separation of WBCs from whole blood

Pellets of WBCs were isolated by applying whole blood samples on polysucrose sodium metrizoate in UNI-SEP lymphocyte separation tubes (Novamed, Jerusalem, Israel) and processed according to the manufacturer's instructions.

2.6. Patients' genotypes

Molecular characterization of the samples used in the present study was performed at the Gaslini Institute (Genoa, Italy) on fibroblasts and EBV transformed B-cells and at Shaare Tzedek Hospital (Jerusalem, Israel) on patients' derived blood samples.

Since the traditional amino-acid residue numbering, which excludes the first 39 amino acids of the leader sequence (GenBank accession *GBA1* no. NP_000148.2) is regularly used in GD literature and not the

Table 1
Primers used in this study. The table contains the sequence of all the primers used in this work. RT = real time, R = reverse, F = forward.

Primer	Primer sequence
Human-GAPDH-RT-F	5′-CTCCTCCTGTTCGACAGTCA-3′
Human-GAPDH-RT-R	5′-GTTGACTCCGACCTTCACCT-3′
Human-CHOP-RT-F	5′-AGCGACAGAGCCAAAATCAG-3′
Human-CHOP-RT-R	5′-TCTGCTTTCAGGTGTGGTGA-3′
Human-BiP-RT-F	5′-CATCAAGTTCTTGCCGTTCA-3′
Human-BiP-RT-R	5′-ATGTCTTTGTTTGCCCACCT-3′
Human-ATF4-RT-F	5′-GTTCTCCAGCGACAAGGCTA-3′
Human-ATF4-RT-R	5′-ATCCTGCTTGCTGTTGTTGG-3′
Human-GBA1-RT-F	5′-AGGCAGTGTCGTGGGCAT-3′
Human-GBA1-RT-R	5′-ACCAAGGGCAGGAAAGGT-3′
Human-MAN2B-RT-F	5′-GATCATTGGACAAGCCAGAC-3′
Human-MAN2B-RT-R	5′-CGTCTGCCCTATTTACCCAT-3′
Human-ACP-RT-F	5′-AACCTAAACCAGCAGCCATC-3′
Human-ACP-RT-R	5′-AGCACATCAAGATCATGGGA-3′
Human-GAPDH-F	5′-CCATCAATGACCCCTTCATTGACC-3′
Human-GAPDH-R	5′-CTCAYGGYYCACACCCATGAC-3′
Human-Xbp1s-F	5′-TCTGCTGAGTCCGCAGCAG-3′
Human-Xbp1s-R	5′-GAAAAGGGAGGCTGGTAAGGAAC-3′