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Research Article

ATF6β regulates the Wfs1 gene and has a cell survival role in the ER stress response in pancreatic β-cells

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ABSTRACT

Endoplasmic reticulum (ER) stress is implicated in pancreatic β-cell dysfunction and death resulting in type 2 diabetes. Activating transcription factor 6 (ATF6) is an essential component of the Unfolded Protein Response (UPR) and consists of two isoforms, ATF6α and ATF6β. Here we investigated the role of ATF6β. ATF6β mRNA was detected in pancreatic β-cell lines and rodent and human islets. We also detected ATF6β protein and production of the active form (ATF6βp60) in response to ER stress. Knock-down of ATF6β in INS-1 832/13 insulinoma cells did not affect mRNA induction of several major UPR genes in response to ER stress, suggesting ATF6β is not essential for the basic UPR. Expressing active ATF6βp60 or ATF6αp50 followed by microarray analysis showed that they regulate similar UPR genes, although some genes such as *Wfs1* are ATF6β-specific. ATF6β, but not ATF6α, is able to bind the *Wfs1* promoter and induce *Wfs1* gene and protein expression. Knock-down of ATF6β increased the susceptibility of β-cells to ER stress-induced apoptosis, while overexpression of active ATF6βp60 reduced apoptosis. Thus, ATF6β is not essential for induction of most UPR genes, but is required to maintain cell survival in β-cells undergoing chronic ER stress, which in part relates to its ability to induce *Wfs1*, a pro-survival gene.

Introduction

The endoplasmic reticulum (ER) of eukaryotic cells has an essential role in the production and folding of membrane and secretory proteins [1]. ER stress occurs when the protein folding capacity of the ER is not sufficient to meet demand or if there is a significant alteration in the ER luminal environment. Accumulation of unfolded, misfolded and aggregated proteins can be detrimental to cell survival. Thus, eukaryotic cells have evolved an adaptive mechanism called the Unfolded Protein Response (UPR) to deal with such situations [1]. In mammalian cells, sensing of ER stress and subsequent signal transduction is mediated by three transmembrane proteins, PERK (double stranded RNA-activated protein kinase (PKR)-like endoplasmic reticulum kinase), IRE1 (inositol requiring enzyme 1), and ATF6 (activating transcription factor 6). In general, the UPR involves four distinct processes: (i) transient translational attenuation, (ii) an increase in the protein folding capacity through transcriptional

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transduction of ER chaperones, (iii) enhanced ER-Associated Degradation (ERAD) of terminally misfolded proteins, and (iv) apoptosis, when the adaptive UPR fails to restore ER homeostasis [2–4]. Thus, proper functioning of the ER and UPR is crucial to cell survival, particularly in specialized secretory cells such as pancreatic β-cells where protein folding capacity needs to respond to acute production of large amounts of insulin [5,6].

In mammalian cells, ATF6 consists of two isoforms, ATF6α [7,8] and ATF6β [9–11]. Both are ubiquitously expressed in mammalian cells and are activated in response to ER stress [9]. Under basal conditions, ATF6 is retained in an inactive form in the ER membrane by association with the chaperone protein glucose-regulated protein 78 kDa (GRP78) [12]. Upon ER stress, GRP78 dissociates from ATF6 to assist in protein folding, and this causes ATF6 to translocate to the Golgi, where it is cleaved by site 1 and site 2 proteases to release the cytosolic N-terminal portion [9,12]. The cleaved active form of ATF6 acts as a transcription factor in the nucleus, where it binds to ER stress elements (ERSE) of target genes to increase gene transcription [13].

While ATF6α has been extensively studied in a number of cell types including pancreatic β-cells [13–15], comparatively little is known about the ATF6β isoform. In this study we demonstrate that ATF6β is expressed in pancreatic β-cell lines and rodent and human islets and identify ATF6β-regulated genes. Interestingly, while ATF6β is dispensable for regulating the expression of major UPR genes that are also induced by ATF6α, depletion of ATF6β increased the susceptibility of β-cells to ER stress-induced apoptosis, while overexpression had the opposite effect. These results identify a pro-survival role for ATF6β in pancreatic β-cells.

Materials and methods

Cell culture

Rat INS-1 (obtained from Dr. Claus Wollheim, University of Geneva, Switzerland [16]) and INS-1 832/13 (obtained from Dr. Chris Newgard, Duke University, Durham, NC, [17]) insulinoma cell lines were maintained as described [14]. INS-1 832/13 TetR cells stably transfected with doxycycline-inducible FLAG-WFS1 were obtained from Dr. Fumihiko Urano, Washington University, St. Louis, MI [18]. Mouse MIN6 insulinoma cells (obtained from Dr. Michael Wheeler, University of Toronto, Toronto, ON, Canada) were cultured in DMEM (25 mM glucose, 2 mM L-glutamine, and 1 mM sodium pyruvate) supplemented with 10% FBS and 55 μM β-mercaptoethanol at 37 °C and 5% CO_2. Human islet samples were obtained from Dr. Patrick MacDonald (Alberta Diabetes Institute, University of Alberta). Ethics approval for obtaining human islets for basic research studies was obtained from the University Health Network. Mouse and rat pancreatic islets were isolated as described previously [19,20]. All animal procedures were approved and performed in accordance with the Animal Use Protocols at the Toronto Centre for Phenogenomics and the University Health Network.

Cell lysis and western blot analysis

Cells were placed on ice, washed once with ice-cold phosphate-buffered saline (PBS) then incubated in lysis buffer (1% Triton X-100, 100 mM KCl, 20 mM HEPES, 2 mM EDTA, pH 7.3) containing 0.5 mM PMSF and protease inhibitors (Roche) on ice for 20 min, with occasional shaking. Lysates were collected, centrifuged at 13,200 rpm for 10 min at 4 °C and the protein concentration in the supernatant was determined using the bicinchoninic acid (BCA) protein assay (Pierce Inc.). Cell lysate preparation for blotting with anti-ATF6β antibody involved scraping the cells in 500 μl of ice-cold PBS (containing protease inhibitor cocktail and 10 μM MG132) and centrifugation at 5, 000 rpm for 2 min. Cell pellets were then lysed in 100 μl SDS-sample buffer (50 mM Tris/HCl, pH 6.8, containing 2% SDS, 10% glycerol, protease inhibitor cocktail and 10 μM MG132). The lysates were boiled for 5 min then centrifuged at 13,200 rpm for 2 min and the supernatant was collected and protein concentration measured. Equal amounts of protein were boiled in 2X SDS sample buffer supplemented with 10% β-mercaptoethanol. Proteins were resolved on either 10% SDS-PAGE gels or 4–12% NuPAGE gels (Invitrogen) and transferred to Hybond-ECL nitrocellulose membranes (GE Healthcare). Western blotting and enhanced chemiluminescence detection was performed as described previously [21]. The following primary antibodies were used: ATF6β (obtained from Dr. Kazutoshi Mori, Kyoto University, Kyoto, Japan); WFS1 (1:500; 11558-1-AP, Proteintech Group); FLAG tag (1:1,000; F3165, Sigma); PARP (1:500; 9542P, Cell Signaling); cleaved caspase 3 (1:500; 9661S, Cell Signaling); γ-tubulin (1:1000; T6557, Sigma). Band densitometry was performed by Image J software on images from scanned films within a linear range of exposure.

Short-interfering RNA-mediated knock-down

Briefly, 24 pmol siRNA was diluted in 400 μl OptiMEM directly into each well of a 6-well plate, mixed gently and incubated at room temperature for 5 min. After incubation, 4 μl of Lipofectamine RNAiMAX reagent was added to each well containing the diluted siRNAs, mixed gently and incubated at room temperature for 30 min. INS-1 832/13 cells were trypsinized and resuspended in RPMI growth media at 300,000 cells/ml. Two milliliters of cell-containing media were added to each well (i.e. 600,000 cells/well) on top of the siRNA complexes to obtain a final siRNA concentration of 10 nM. The cells were mixed gently and incubated at 37 °C for 48 h or 72 h. GFP siRNA was used as control. Short-interfering RNAs targeted to ATF6β and GFP were obtained from Invitrogen.

RNA isolation and reverse transcription PCR

After cell treatments total RNA was isolated using TRIzol reagent (Invitrogen). Isolated RNA was then extracted using the RNeasy RNA Isolation Kit (Qiagen) as per the manufacturer's instructions. RNA concentration and integrity were measured using a Nano-drop machine. RT-PCR (Qiagen OneStep RT-PCR kit) was used to amplify ATF6β cDNA using primers specific for ATF6β: Rat/mouse; Forward: 5'-atg gcg gag ctg atg ctc ctct-3'; Reverse: 5'-tcc tgt ttc cag acc cca gct-3' Human; Forward: 5'-atg gcg gag ctg atg ctg ctc-3'; Reverse: 5'-cag acc cta act tga gct cgc-3'. The following experimental conditions were used for the RT-PCR: 50 °C (30 min); 95 °C (15 min); 30 cycles of 94 °C (30 s), 53 °C (30 s), 72 °C (1.5 min); 72 °C (10 min). RT-PCR products were resolved in a 1% agarose gel and visualized using ethidium bromide. The RNA concentration used for cell line samples was 1 μg and 500 ng for islet samples.