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Drp1 guarding of the mitochondrial network is important for glucose-stimulated insulin secretion in pancreatic beta cells

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ABSTRACT

Mitochondria form a tubular network in mammalian cells, and the mitochondrial life cycle is determined by fission, fusion and autophagy. Dynamin-related protein 1 (Drp1) has a pivotal role in these processes because it alone is able to constrict mitochondria. However, the regulation and function of Drp1 have been shown to vary between cell types. Mitochondrial morphology affects mitochondrial metabolism and function. In pancreatic beta cells mitochondrial metabolism is a key component of the glucose-induced cascade of insulin secretion. The goal of the present study was to investigate the action of Drp1 in pancreatic beta cells. For this purpose Drp1 was down-regulated by means of shDrp1 in insulin-secreting INS1 cells and mouse pancreatic islets. In INS1 cells reduced Drp1 expression resulted in diminished expression of proteins regulating mitochondrial fusion, namely mitofusin 1 and 2, and optic atrophy protein 1. Diminished mitochondrial dynamics can therefore be assumed. After down-regulation of Drp1 in INS1 cells and spread mouse islets the initially homogenous mitochondrial network characterised by a moderate level of interconnections shifted towards high heterogeneity with elongated, clustered and looped mitochondria. These morphological changes were found to correlate directly with functional alterations. Mitochondrial membrane potential and ATP generation were significantly reduced in INS1 cells after Drp1down-regulation. Finally, a significant loss of glucose-stimulated insulin secretion was demonstrated in INS1 cells and mouse pancreatic islets. In conclusion, Drp1 expression is important in pancreatic beta cells to maintain the regulation of insulin secretion.

1. Introduction

Mitochondria form a dynamic tubular network in living cells. Continuous fusion and fission events maintain this network morphology in terms of quantity, distribution, interconnectivity and shape, and allow fast adaptation to cellular requirements [1–5]. Processes interfering with the mitochondrial life cycle have been shown to increase mitochondrial heterogeneity and to contribute to metabolic disorders, such as the development of type 2 diabetes [6–9].

Different proteins act in concert to maintain the mitochondrial life cycle. The homologous proteins mitofusin 1(Mfn1) and mitofusin 2 (Mfn2) regulate fusion of the outer mitochondrial membrane, while optic atrophy protein 1 (Opa1) is essential for inner mitochondrial membrane fusion [10,11]. Fission is initiated by translocation of fission protein 1 (Fis1), mitochondrial fission factor (Mff) [12] and the mitochondrial dynamics proteins to mitochondria [13,14]. Attachment of these proteins to mitochondria paves the way for increased binding of dynamin-related protein 1 (Drp1) which alone has the capacity eventually to constrict mitochondria [13,15–17].

In this process Drp1 polymerises into dimeric spirals around the outer mitochondrial membrane [18,19]. Drp1 consists of an N-terminal GTPase domain, a centrally localized dynamin-like domain and a C-terminal GTPase effector domain. Conformational changes of Drp1 polymers, which are essential for mitochondrial fission, are dependent on GTP hydrolysis [20]. Several post-translational modifications – for example, phosphorylation, S-nitrosylation, SUMOylation, ubiquitination and interactions with linking effector proteins such as Mff – govern the activity of Drp1 in a cell-type-specific manner [17,18]. There is therefore a growing body of

Abbreviations: Drp1, dynamin-related protein 1; ER, endoplasmic reticulum; Fis1, fission protein 1; Mff, mitochondrial fission factor; Mfn1, mitofusin 1; Mfn2, mitofusin 2; Opa1, optic atrophy protein 1.

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evidence to suggest that Drp1 is a key regulator of the mitochondrial life cycle [18].

Glucose-stimulated insulin secretion in pancreatic beta cells is a function of mitochondrial metabolism and the generation of ATP [21]. The mitochondrial life cycle in pancreatic beta cells shows specific adaptation to this vital signalling cascade for regulation of blood glucose homoeostasis [5,22,23]. Recently, mitochondrial dysfunction in terms of mitochondrial heterogeneity has been linked to insulin secretion deficits in the development of type 2 diabetes [24–26].

Glucose and free fatty acids have been reported to affect the mitochondrial life cycle in pancreatic beta cells and therefore probably contribute to the pathogenesis of type 2 diabetes [24–26]. Compared with a dominant negative mutant (K38A), overexpression of wild-type Drp1 in insulin-secreting INS1 cells has been found to increase Drp1 expression under high nutrient stress, thus mediating endoplasmic reticulum (ER) stress and apoptosis [24,27,28]. In line with these findings, Drp1 has been identified as an ER-resident protein that regulates ER morphology in stressed beta cells [29]. It has recently been postulated that PGC-1α and rhein (an anthraquinone compound isolated from rhubarb) confer protection against Drp1 up-regulation in stressed beta cells [30,31].

However, while the importance of Drp1 for mitochondrial metabolism has been clearly demonstrated, this effect varies depending on cell type and has to date not been investigated in pancreatic beta cells [15,32,33]. Therefore, in the present study, we have down-regulated Drp1 in insulin-secreting INS1 cells and mouse pancreatic islets and have characterised the mitochondrial network and glucose-stimulated insulin secretion.

2. Materials and methods

2.1. Lentivirus generation

Lentivirus was generated using the Trans Lentiviral Packaging Kit (Fisher Scientific, Pittsburgh, PA, USA) and the HEK293T producer cell line according to the manufacturer's instructions. shRNA for Drp1 in the pGIPZ vector (Fisher Scientific) was used for down-regulation. Empty vector served as control during viral transduction.

2.2. Cell culture and transduction

Clonal rat glucose-responsive INS1-832/13 (INS1 cells) was generated as described [34]. INS1 cells were transduced with lentiviruses for three days and cell clones were selected through resistance against puromycin (500 μg/ml) and green fluorescence of the pGIPZ vector. INS1 cells were cultured in RPMI 1640 media containing 10 mmol/l glucose and supplemented with 10% foetal bovine serum and 5% penicillin/streptomycin, 1 mmol/l sodium pyruvate, 10 mmol/l HEPES, 50 μM 2-mercaptoethanol and 200 mmol/l glutamine in a humidified atmosphere at 37 °C and 5% CO_2. Mouse pancreatic islets were isolated from male 12-week-old C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME, USA) by collagenase P digestion (Roche Diagnostics, Mannheim, Germany) and Ficoll gradient purification (Ficoll PM 400; Sigma, Seelze, Germany). Islets were dissociated using calcium-free medium, seeded on collagen-coated well plates or PCA-coated Lab-Tek chamber slides (Nunc, Naperville, USA) for microscopy and transduced with lentiviruses for three days.

2.3. Real-time PCR

Total RNA samples were prepared using the RNeasy total RNA isolation kit (Qiagen, Courtaboeuf, France), quantified using a spectrophotometer (ND-2000, PeqLab, Erlangen, Germany) and reverse transcribed into cDNA using the Maxima® First Strand cDNA Synthesis Kit (Fermentas, St. Leon-Rot, Germany). Gene expression was quantified using TaqMan Universal PCR Master Mix and the following assays: Rn_01405085 for Drp1, Rn_01480914_m1 for Fis1, Rn_01404701_m1 for OPA1, Rn_01462175_m1 for Mfn1 and Rn_01639201_m1 for Mfn2. PCR reactions were performed in triplicates using the 7900HT Real-Time PCR System (Applied Biosystems). Relative mRNA expression was calculated with the comparative ($2^{-\Delta\Delta Ct}$) method and normalised to GAPDH (4352338E-0908012).

2.4. Western blot analyses

40 μg of cellular proteins were separated by SDS-PAGE and blotted onto Roti®Fluoro PVDF membrane (Roth, Karlsruhe, Germany). Membranes were probed for 1 h at room temperature with anti-Drp1 (1:1000) and anti-beta-tubulin (1:1000) (Santa-Cruz Biotechnology, Santa Cruz, CA, USA) antibodies. Immunoreactive bands were visualised using IRDye 680, IRDye 800 CW fluorescence-labelled secondary antibodies and analysed via the Odyssey imaging system. Densitometry measurements of bands were performed using the Odyssey infrared imaging system (LI-COR, Lincoln, NE, USA) and normalised to beta-tubulin expression.

2.5. Immunohistochemistry

Cells were fixed with 4% formaldehyde for 15 min and permeabilised with 0.2% Tween 20 for 5 min in phosphate-buffered saline. Cells were stained for 1 h with anti-Drp1 (1:100) (Santa-Cruz Biotechnology) antibody. Positive signals were visualised using Cy5 (1:250) (Molecular Probes Invitrogen, Darmstadt, Germany). The samples were counterstained with DAPI and mounted using VECTASHIELD® mounting medium (Vector Laboratories, Peterborough, UK). Cells were analysed using a Fluoview FV10i confocal microscope (405 and 635 LD laser) and the Fluoview-SW software (Olympus, Hamburg, Germany).

2.6. Glucose-stimulated insulin secretion

INS1 cells and mouse islets were incubated for 1 h in bicarbonate-buffered Krebs-Ringer solution without glucose, supplemented with 0.1% albumin. Subsequently cells were incubated for 1 h in Krebs-Ringer solution containing 3 or 25 mmol/l glucose. The secreted insulin in the supernatant and the insulin content in the homogenised scraped cells were measured by ELISA (Mercodia, Uppsala, Sweden) and protein content by Bradford protein assay.

2.7. Cellular ATP content

Analysis of ATP was performed with the ATPlite Detection Assay System (PerkinElmer Life Sciences, Zaventem, Belgium) according to the manufacturer's instructions.

2.8. Measurement of mitochondrial membrane potential

5×10^5 INS1 cells grown on glass-bottom dishes (MatTak Corporation, Ashland, MA, USA) were loaded with 6.2 μmol/l tetramethylrhodamine ethylester perchlorate (TMRE, Molecular Probes Invitrogen Detection Technologies, Eugene, OR, USA) for 20 min at 37 °C, as described [35]. Cells were analysed using a Fluoview FV10i confocal microscope (559 LD laser) and the Fluoview-SW software (Olympus).