EBioMedicine 2 (2015) 378–385

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Original Article

Pancreatic α Cells are Resistant to Metabolic Stress-induced Apoptosis in Type 2 Diabetes

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ARTICLE INFO

Article history:
Received 17 December 2014
Received in revised form 12 March 2015
Accepted 13 March 2015
Available online 17 March 2015

Keywords:
Pancreatic α cells
Type 2 diabetes
Apoptosis
Metabolic stress
Palmitate
Bcl2l1
ER stress

ABSTRACT

Pancreatic α cells are exposed to metabolic stress during the evolution of type 2 diabetes (T2D), but it remains unclear whether this affects their survival. We used electron microscopy to search for markers of apoptosis and endoplasmic reticulum (ER) stress in α and β cells in islets from T2D or non-diabetic individuals. There was a significant increase in apoptotic β cells (from 0.4% in control to 6.0% in T2D), but no α cell apoptosis. We observed, however, similar ER stress in α and β cells from T2D patients. Human islets or fluorescence-activated cell sorting (FACS)-purified rat β and α cells exposed in vitro to the saturated free fatty acid palmitate showed a similar response as the T2D islets, i.e. both cell types showed signs of ER stress but only β cells progressed to apoptosis. Mechanistic experiments indicate that this α cell resistance to palmitate-induced apoptosis is explained, at least in part, by abundant expression of the anti-apoptotic protein Bcl2l1 (also known as Bcl-xL).

1. Introduction

Type 2 diabetes (T2D) affects 380 million individuals worldwide, and its prevalence will further increase in the coming decades with the obesity epidemic (IDF Diabetes Atlas, 2013). T2D is characterized by progressive loss of pancreatic β cell function and insulinopenia, and by non-suppressed post-prandial glucagon secretion by pancreatic α cells (D'Alessio, 2011). β cell failure is associated with β cell apoptosis and a progressive decrease in β cell mass (Butler et al., 2003; Rahier et al., 2008). β cell death is probably secondary to metabolic stress mediated by high levels of saturated free fatty acids (FFAs) and glucose (Poitout and Robertson, 2008). Possible mechanisms of metabolic stress-induced β cell apoptosis in T2D include endoplasmic reticulum (ER) stress (Kharroubi et al., 2004), oxidative stress (Carlsson et al., 1999) and ceramide production (Shimabukuro et al., 1998), and this culminates in activation of the intrinsic or mitochondrial pathway of apoptosis (Cunha et al., 2012; Gurzov and Eizirik, 2011). A high-fat and high-sugar diet decreases β cell mass but increases α cell mass in nonhuman primates (Fiori et al., 2013). Recent evidence obtained in mice, monkeys and humans suggests that β cells may dedifferentiate and adopt α cell characteristics (Fiori et al., 2013; Gao et al., 2014; Talchai et al., 2012; White et al., 2013), putatively contributing to the decreased insulin production and increased glucagon secretion in T2D. α Cell dysfunction and hyperglucagonemia is induced by postprandial lipemia in healthy subjects and by treating mouse islets with triglyceride-rich lipoproteins (Niederwanger et al., 2014).

Pancreatic α and β cells have similar embryonic origins (Teitelman et al., 1993), and are equally exposed to metabolic stress during the evolution of T2D, but it remains unclear whether metabolic stress affects α cell survival. Here we examined the presence of apoptosis and ER stress in α and β cells of T2D individuals and in human islets exposed to palmitate. Both cell types show signs of ER stress, but only β cells progress to apoptosis. To clarify the mechanisms involved, we developed a method to fluorescence-activated cell sorting (FACS)-isolate pure (>90%) and viable (>90%) rat α cells. In keeping with the human islet data, palmitate induced ER stress in α and β cells, but apoptosis was only present in β cells. This α cell resistance to lipotoxicity is due to higher anti-apoptotic protein expression.

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http://dx.doi.org/10.1016/j.ebiom.2015.03.012

2. Materials and Methods

2.1. Human Islet EM

Organ donor pancreata were handled with the approval of the Ethics Committee of the University of Pisa, Italy. Samples from 8 non-diabetic and 9 T2D organ donors were studied, individual characteristics of age, gender, BMI, cause of death and anti-diabetic treatment are listed in Table S1. Samples were processed as previously described (Cnop et al., 2014; Marchetti et al., 2007). In brief, the tissue was fixed with 2.5% glutaraldehyde in 0.1 M of cacodylate buffer, pH 7.4 for 1 h at 4 °C. After rinsing in cacodylate buffer, the tissue was postfixed in 1% cacodylate buffered osmium tetroxide for 2 h at room temperature, then dehydrated in a graded series of ethanol, quickly transferred to propylene oxide and embedded in Epon-Araldite. Ultrathin sections (60–80 nm thick) were cut with a diamond knife, placed on formvar-carbon coated copper grids (200 mesh), and stained with uranyl acetate and lead citrate. β and α cells were identified by the ultrastructural characteristics of the insulin and glucagon granules, respectively (Orci, 1985) and in some cases confirmed by immunogold (see below). Morphometric analyses were performed as previously detailed (Cnop et al., 2014; Marchetti et al., 2007). Briefly, micrographs obtained at ×10,000, were analyzed by overlay with a graticule (11 × 11 cm) composed of 169 points. Volume densities were calculated according to the formula: volume density = Pi/Pt, where Pi is the number of points within the subcellular component and Pt is the total number of points; values are expressed as ml/100 ml tissue (ml%). By electron microscopy analysis, morphological evidence of marked chromatin condensation and/or the presence of blebs were considered as signs of apoptosis (Masini et al., 2009).

2.2. Immunoelectron Microscopy Methods

Immunogold experiments were accomplished according to the procedures detailed by Zuber et al. (Zuber et al., 2005). Ultrathin sections, from glutaraldehyde–osmium tetroxide fixed tissue mounted on nickel grids, were placed on droplets of freshly prepared 1% aqueous periodic acid for 6 min at room temperature. Sections were conditioned with PBS (0.01 M phosphate buffer, pH 7.2, 0.15 M NaCl) containing 1% BSA, 0.01% Triton X-100, and 0.01% Tween 20 and exposed to guinea pig anti-insulin antibody (Sigma-Aldrich, Saint Louis, MO, USA), diluted 1:200, for 2 h at room temperature, or to rabbit anti-glucagon antibody (Zymed-Invitrogen, Carlsbad, CA, USA), diluted 1:100, overnight at 4 °C. Afterwards, samples were rinsed with buffer and grids were incubated with 15 nm of protein A-gold complex (Agar Scientific, Stansted, UK), diluted 1:10, for 1 h at room temperature. Finally, sections were contrasted with uranyl acetate and lead citrate prior to examination with the 902 Zeiss electron microscope. Control incubations were performed by omission of the primary antibody.

2.3. Human Islet Isolation and Exposure to Palmitate

For the in vitro studies, islets were isolated from 3 non-diabetic donors (age, 61 ± 6 years; 2 males/1 female; body mass index, 25.4 ± 0.8 kg/m^2; cause of death: 2 cardiovascular diseases, 1 trauma; intensive care unit stay, 2.7 ± 0.6 days; pancreas cold ischemia time: 16.7 ± 2.3 h) by collagenase digestion and density gradient purification, as previously reported (Del Guerra et al., 2005; Cnop et al., 2014). Islets were cultured for 2–3 days in M199 medium (containing 5.5 mM of glucose) supplemented with 10% adult bovine serum. Then, the islets were cultured for 48 h in supplemented M199 medium containing 1% bovine serum albumin (BSA) and 0.5 mM of palmitate (Cnop et al., 2014; Cunha et al., 2008).

2.4. FACS Purification, Culture and Treatment of rat α and β Cells

Male Wistar rats (Charles River) were housed and used according to the guidelines of the Belgian Regulations for Animal Care, with the approval by the local Ethical Committee. Rat islets were isolated by collagenase digestion and handpicked. For β and α cell isolation, islets were dissociated into single cells by mechanical and enzymatic dispersion using trypsin (1 mg/ml) (Sigma) and DNaseI (1 mg/ml) (Roche) for 5 min at 31 °C under agitation. Dissociated cells were re-suspended in HEPES-buffered Earle's medium containing 2.8 mM of glucose. FACS sorting of β and α cells was done using an Aria I cell sorter (BD Biosciences) equipped with violet, blue and red lasers and a 70 μm nozzle. Forward scatter (FSC) and side scatter (SSC) indicated the relative differences in, respectively, size and granularity of the cells. Cells were selected with the blue argon laser at 488 nm while the FAD-like cellular autofluorescence was excited at 488 nm and selected after a 530/30 band-pass filter (525 nm). Cell doublets and cell fragments/death cells were excluded from FACS analysis and collection. Rat β cells have a threefold higher FAD fluorescence than α cells at low glucose concentration (2.8 mM). This property, coupled to the size and granularity difference between β and α cells (β cells are larger and more granulated than α cells), allows the separation of the β and α cell fractions, with a high purity, using an average side scatter-width intensity of 170,000 units for the β cells and 120,000 units for the non-β cells. The non-β cell fraction was gated under 50,000 units of fluorescence to avoid contamination by β cells. Additional used parameters were: voltage FSC: 105, SSC: 205, FITC: 730, laser window extension: 0.5, and FACS area scaling: 0.75. To obtain α cells from the non-β cell population, we narrow the gate on the fluorescence parameter and define a sorting window between ±20,000 and 40,000 units of fluorescence. This allows us to reach a very high purity in α cells (>90–95%) and very good viability (>90%) (protocol modified from (Pipeleers et al., 1985)). After sorting, purified β cells were cultured in Ham's F-10 medium containing 10 mM of glucose, 2 mM of GlutaMAX, 0.5% BSA, 50 μM of isobutylmethylxanthine, 50 units/ml of penicillin and 50 μg/ml of streptomycin and 5% heat-inactivated fetal bovine serum (FBS, Gibco Life Technologies). α cells were cultured in the same medium but with 6.1 mM of glucose and 10% FBS. In some experiments α and β cells were cultured in parallel at 6.1, 10 and 20 mM of glucose.

Palmitate (sodium salt, Sigma) was dissolved in 90% ethanol to a concentration of 50 mM and diluted in medium containing 0.75% BSA (fatty acid-free fraction V, Roche) and 2% FBS to a final concentration of 0.5 mM (modified from (Cnop et al., 2001)).

CPA and tunicamycin (Sigma) were dissolved in DMSO and used at a concentration of 12.5 μM and 5 μg/ml, respectively. BFA (Sigma) was dissolved in ethanol and used at 0.1 μg/ml. The treatments were performed in specific culture medium for β and α cells with 2% FBS.

2.5. RNA Interference

β and α cells were transfected with 30 nM of the previously validated siRNAs for Bcl2l1 (Invitrogen, Carlsbad, CA) (Miani et al., 2013), Bcl2 (Invitrogen, Carlsbad, CA) (Cunha et al., 2012) or Allstars Negative Control siRNA (siCTRL, Qiagen, used as a negative control) using the Lipofectamine RNAiMAX lipid reagent (Invitrogen). siCTRL does not affect β and α cell gene expression, function or viability ((Moore et al., 2012) and data not shown). Cells were cultured for 48 h and then exposed to palmitate.

2.6. Assessment of Cell Viability

The percentage of viable, apoptotic, and necrotic cells was determined after incubation with the DNA-binding dyes propidium iodide (5 μg/ml; Sigma) and Hoechst 33342 (5 μg/ml; Sigma) (Rasschaert et al., 2005). A minimum of 600 cells was counted in each experimental condition. Viability was evaluated by two independent observers, one of