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ABSTRACT

Metabolic endotoxemia has been implicated in the pathogenesis of type 2 diabetes. In addition to adipose tissue inflammation, inflammatory cell infiltration is also observed in islets, although its effect on islets is largely unknown. We hypothesized that macrophage infiltration into islets leads to impairment of α or β cell function, which ultimately act to exacerbate the pathophysiology of diabetes. Gene expression in a murine α cell line, αTC1, and β cell line, βTC6, was investigated by DNA microarray after co-culturing the cells with a murine macrophage cell line, RAW 264.7, in the presence or absence of bacterial endotoxin. Among the genes showing highly upregulated expression, genes specifically upregulated only in β cells were evaluated to determine the roles of the gene products on the cellular function of β cells. In both α and β cells, expression of type I interferon-responsive genes was highly upregulated upon endotoxin stimulation. Among these genes, expression of the X-linked inhibitor of apoptosis (Xiap)-associated factor 1 (Xaf1) gene, which is associated with the induction of apoptosis, was specifically enhanced in β cells by endotoxin stimulation. This upregulation appeared to be mediated by macrophage-derived interferon β (IFNβ), as endotoxin-stimulated macrophages produced higher amounts of IFNβ, and exogenous addition of IFNβ into βTC6 cultures resulted in increased Xaf1 protein production and cleaved caspase 3, which accelerated β-cell apoptosis. Macrophages activated by metabolic endotoxemia infiltrated into islets and produced IFNβ, which induced β-cell apoptosis by increasing the expression of Xaf1.

Introduction

Low-grade inflammation is thought to play an important role in the pathogenesis of type 2 diabetes. The primary target tissue of mild inflammation is adipose tissue, which is associated with an insulin-resistant state [1–3]. Moreover, recent studies indicated that high fat intake leads to changes in the gut microflora, which induces the so-called metabolic endotoxemia, and leads to the enhancement of systemic inflammatory responses [4, 5]. Persistent insulin resistance caused by adipose tissue inflammation forces islet β cells to produce higher amounts of insulin, a state known as hyperinsulinemia. Thus, sustained insulin resistance results in β-cell dysfunction and, ultimately, cell apoptosis, leading to an insufficient insulin secretary state [6, 7]. Although islet inflammation caused by endoplasmic reticulum stress, amyloid deposition, hyperglycemia, and impaired autophagy has been suggested to be associated with reduced numbers of β cells in a diabetic state, the precise molecular mechanism is largely unknown [8–11]. Under normal conditions, M2 macrophages reside in islets, function to enhance angiogenesis and have a protective role against exogenous stimuli [12]. In contrast, under obese and/or diabetic conditions, the number of immune cells increases in islets [13], and enhanced chemokine expression leading to the accumulation of inflammatory cells and β-cell dysfunction caused by infiltrated macrophages is observed [9, 14]. These previous studies suggested that low-grade inflammation affects not only insulin resistance, but also β-cell function. We hypothesized that metabolic endotoxemia stimulates and activates macrophages and that these macrophages may accumulate in islets, interact with resident cells such as α and/or β cells, and alter the functions of these cells.

Materials and Methods

Cells and cell culture

Alpha cell line αTC1-6 and beta cell line βTC6 [American Type Culture Collection (ATCC), Manassas, VA, USA] were maintained in Dulbecco's modified Eagles medium (DMEM) (Gibco, Massachusetts, USA) containing 15 mM HEPES, 3.0 g/l (+)-d-glucose, 3 mM BSA, 17 mM $NaHCO_3$, 1 % NEAA, 10 % fetal bovine serum (FBS) (Biowest, Nuaillé, France) in an atmosphere of 10 % CO_2 at 37 °C. Murine macrophage cell line RAW264.7 (ATCC) was maintained in DMEM containing 10 % FBS. Co-cultures of α cells and macrophages or β cells and macrophages were performed using a transwell system (Corning Inc., Acton, MA, USA) with a 0.4 µm porous membrane to separate the upper and lower chambers. An amount of 1×10^6 α cells or β cells were cultured in the lower chamber, while 1×10^5 RAW cells were cultured in the upper chamber [15]. The cells were treated with 1 ng/mL Escherichia coli (E. coli) lipopolysaccharide (LPS) (Sigma Aldrich, St. Louis, MO, USA) for the indicated times.

DNA microarray analysis

To identify differentially expressed genes in αTC1-6 and βTC6 co-cultured with RAW264.7 macrophages in response to LPS, total RNA was isolated from αTC1-6 and βTC6 at 0 (pre-treatment), 4, 8, and 16 h after the addition of LPS using RNeasy Mini kit (Qiagen, Hilden, Germany). The quantity and quality of the extracted RNA were checked with a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Double-stranded cDNA and biotinylated cRNA were synthesized using a Low Input Quick Amp Labeling Kit, One-Color (Agilent Technologies). Labeled RNA was then fragmented and hybridized to Agilent Expression Array (Agilent Technologies). The arrays were scanned using the SureScan Microarray Scanner G2600D (Agilent Technologies) and analyzed with Agilent Feature Extraction Software Version 11.5.1.1 (Agilent Technologies). To identify upregulated genes, we calculated intensity-based z-scores [16] and ratios (non-log-scaled fold change) from the average of normalized signal intensities of each probe for comparison between LPS-stimulated and unstimulated samples. Then we established criteria for upregulated genes: z-score > 2.0 and ratio > 1.5-fold. In order to investigate the functional implications of these genes, we used the Database for Annotation, Visualization and Integrated Discovery (DAVID) software (http://david.ncifcrf.gov). Among selected genes, a number of interferon (IFN)-related genes and chemokines were included. We then selected 8 upregulated genes in αTC1 and/or βTC-6 for further analysis.

Enzyme linked immunosorbent assay (ELISA)

Monocyte chemoattractant protein-1 (MCP-1) and IFNβ protein concentration in the culture media were determined by ELISA kits (R & D Systems, Minneapolis, MN, USA and Biolegend, San Diego, CA, USA) according to the manufacturer's instructions. Absorbances at 450 nm were determined using a microplate reader Thermo Scientific™ Multiskan™ FC (Thermo Fisher Scientific, Waltham, MA, USA).

Real-time polymerase chain reaction (PCR)

Total RNA was isolated from whole cells with TRIzol (Thermo Fisher Scientific) and reverse transcribed using Prime Script RT reagent Kits (Takara, Shiga, Japan). The protocol for the reverse transcription cycle was 42 °C for 30 min and 95 °C for 2 min. RT-PCR was performed using the KAPA SYBR® FAST qPCR Kits (Kapa Biosystems, MA, USA) and Step One Plus Real-Time PCR System (Applied Biosystems, CA, USA). PCR was carried out in two steps, the first at 95 °C for 5 s and the second at 60 °C for 30 s, which were then repeated 40 times. Gene expression levels were normalized to GAPDH. Primer sequences are shown in **Table 1S**.

Western blot analysis

The cells were solubilized with CytoBuster™ Protein Extraction Reagent (Merck, Darmstadt, Germany). Equal amounts of protein from whole cell lysates were resolved by SDS-PAGE. Then, the proteins were transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA) using the semi-dry system [Trans-Blot®SD Semi-Dry Transfer Cell (Bio-Rad Laboratories, Hercules, CA, USA)]. Antibodies were as follows: anti-β-actin (Cell Signaling Technology, Inc., MA, USA), anti-Xaf1 (Abcam, Cambridge, UK), anti-Cleaved Caspase-3 (Cell Signaling Technology, Inc.). After incubation with secondary antibodies (HRP-conjugated anti-rabbit and anti-mouse, Cell Signaling Technology, Inc.), immunoreactive proteins were visualized using enhanced chemiluminescence (Chemi-Lumi One Super, Nacalai Tesque), and signals were analyzed using Image Quant LAS4000 (GE Healthcare, Chalfont, UK).

Glucose-stimulated insulin assay

βTC6 Cells were plated at a density of 2×10^5 cells/well in 24 well plates. After overnight, cells were washed with PBS and pre-incubated with Krebs–Ringer bicarbonate HEPES buffer (KRBH; 115 mM NaCl, 24 mM $NaHCO_3$, 5 mM KCl, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, and 25 mM HEPES, pH 7.4) for 1 h. The medium was then removed and replaced with KRBH containing 0 or 2.8 mM glucose and incubation was performed for 2 h [17]. All incubations were performed at 37 °C under an atmosphere of 5 % CO_2. Following the stimulation period, the insulin levels in the medium were measured with an insulin ELISA kit (Morinaga Institute of Biological Science, Inc., Kanagawa, Japan).

siRNA Treatment of βTC6 cells

βTC6 cells were transfected with siRNAs against mouse Xaf1, Xiap, Ifnar1, and Ifnar2, using Metafectene Pro (Nippon genetics, Tokyo, Japan), according to the manufacturer's instructions. The siRNA duplexes were designed by Sigma-Aldrich as follows: Xaf1, 5′-GUAAAGAUGUUCGUCCAAATT-3′ (sense) and 5′-UUUGGACGAACAUCUUUACTT-3′ (antisense); Xiap, 5′-GCAAUAGAUAGAUGGCAGUTT-3′ (sense) and 5′-ACUGCCAUCUAUCUAUUGCTT-3′ (antisense); Ifnar1, 5′-GUUAUAACUGGAUUAGGUTT-3′ (sense) and 5′-ACCUAAUCCAGUUAUAAUCTT-3′ (antisense); Ifnar2, 5′-CAGAUGAACCUUGCACUAUTT-3′ (sense) and 5′-AUAGUGCAAGGUUCAUCUGTT-3′ (antisense). Stealth RNAi Negative Control Low GC (Invitrogen, Carlsbad, CA, USA) was used as a control. Forty-eight hours after siRNA transfection, cells were stimulated with IFNβ for 24 h.

TUNEL staining

βTC6 cells were stimulated with IFNβ and/or palmitate for 24 h. Evaluation of apoptosis was performed with ApopTag® Plus In Situ

Apoptosis Fluorescein Detection Kit (Merck, Darmstadt, Germany) to find DNA strand breaks using the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) reagent according to the manufacturer's protocol. Propidium iodide (PI) (Dojindo, Kumamoto, Japan) was used to visualize the nuclei. Cells that showed TUNEL positive in the nuclei were identified as apoptotic. One μM Thapsigargin (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was used as a positive control. Stained cells were photographed and analyzed using BZ-9000 BZ-X Analyzer (Keyence, Osaka, Japan). The ratio of cell apoptosis was expressed as the percentage of TUNEL positive cells versus PI positive cells. Five random fields from each sample were selected and quantitated.

Statistical analysis

Data are presented as mean ± SD. The significance of the difference between groups was evaluated with the Student's t-test. Data were statistically analyzed using one-way analysis of variance with Tukey's test when multiple groups were compared. The tests were performed using SPSS/w 17.0 software (Statistical Package for Social Sciences for Windows, Inc., Chicago, IL, USA). A p-value of < 0.05 was considered significant.

Results

Interferon-associated genes were upregulated, particularly in βTC6 co-cultured with endotoxin-stimulated macrophages

To determine the effects of metabolic endotoxemia-stimulated macrophages on islet cell function, changes in gene expression in both αTC1 and βTC6 cells co-cultured with RAW264.7 macrophages in the presence or absence of E. coli LPS (1 ng/ml) were investigated by microarray analysis; the results are shown in ▶ **Fig. 1a** (αTC1) and b (βTC6). Upon endotoxin stimulation, the expression of Mcp-1, Cxcl1, and IFN-associated genes were highly upregulated compared to that in cells co-cultured with macrophages without endotoxin stimulation. We conducted real-time PCR to confirm these results (▶ **Fig. 1c, d**). Interestingly, changes in the expression of most genes appeared to be larger in βTC6 than in αTC1 cells. Among these genes, Xaf1 gene expression was high and only upregulated in βTC6 cells, but not in αTC1 cells.

MCP-1 protein secretion into the culture medium was observed in both αTC1 and βTC6 cultures with macrophages upon endotoxin stimulation (▶ **Fig. 2a**). These results suggest that metabolic endotoxemia upregulates interferon-responsive genes in islets and that enhanced MCP-1 secretion by both α and β cells may cause further immune cell infiltration. Based on these results, soluble factors secreted from endotoxemia-stimulated macrophages appear to upregulate Xaf1 expression. Endotoxin-stimulation of RAW 264.7 macrophages resulted in higher expression of the Ifnβ gene (▶ **Fig. 2b**) and protein secretion (▶ **Fig. 2c**). Because interferon-responsive genes are highly upregulated, particularly in βTC6 cells, we compared the basal expression of both interferon-α/β receptor 1 (Ifnar1) and Ifnar2 gene, and confirmed that receptor expression was higher in βTC6 cells (▶ **Fig. 2d**). We also checked the glucose-stimulated insulin secretion in βTC6 (Supporting Information **Fig. 1S**).

Macrophage-derived IFNβ induced expression of Xaf1 in βTC6 cells

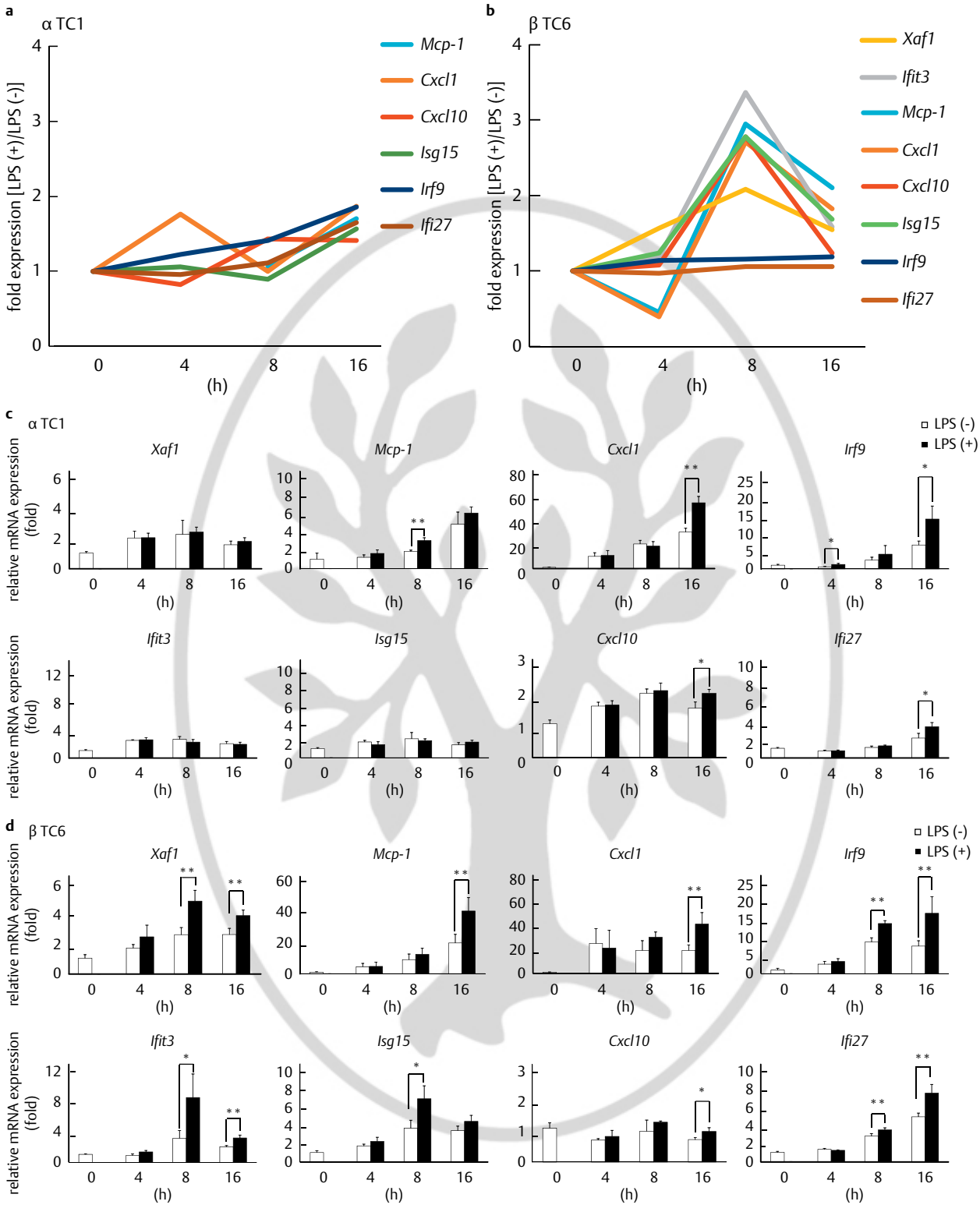
Particularly, in β cells, higher gene expression of interferon-responsive genes was induced and apoptosis-associated signals appeared to be activated. In fact, the culture supernatant of endotoxin-stimulated RAW 264.7 cells induced higher Xaf1 gene (▶ **Fig. 3a**) and protein expression (▶ **Fig. 3b, c**) in βTC6 cells. IFNβ stimulation of pancreatic β cells resulted in higher Xaf1 gene expression (▶ **Fig. 3d**) and protein synthesis levels in an IFNβ-dose dependent manner (▶ **Fig. 3e, f**). These results suggest that macrophage-derived IFNβ stimulated β cells to synthesize Xaf1 at high levels. Xaf1 specifically inhibits Xiap, which inhibits caspase 3 activation and suppresses the apoptosis cascade [18, 19]; thus, as a result, Xaf1 promotes cell death [19]. In contrast, the Xiap-independent apoptosis-promoting action by Xaf1 has been reported [20, 21]. In βTC6 cells co-cultured with macrophages in the presence of endotoxin, Xiap gene expression was unchanged (data not shown). Thus, enhanced expression of Xaf1 in pancreatic β cells by activated macrophage-derived IFNβ may promote β-cell apoptosis.

IFNβ promoted apoptotic cell death in βTC6 cells

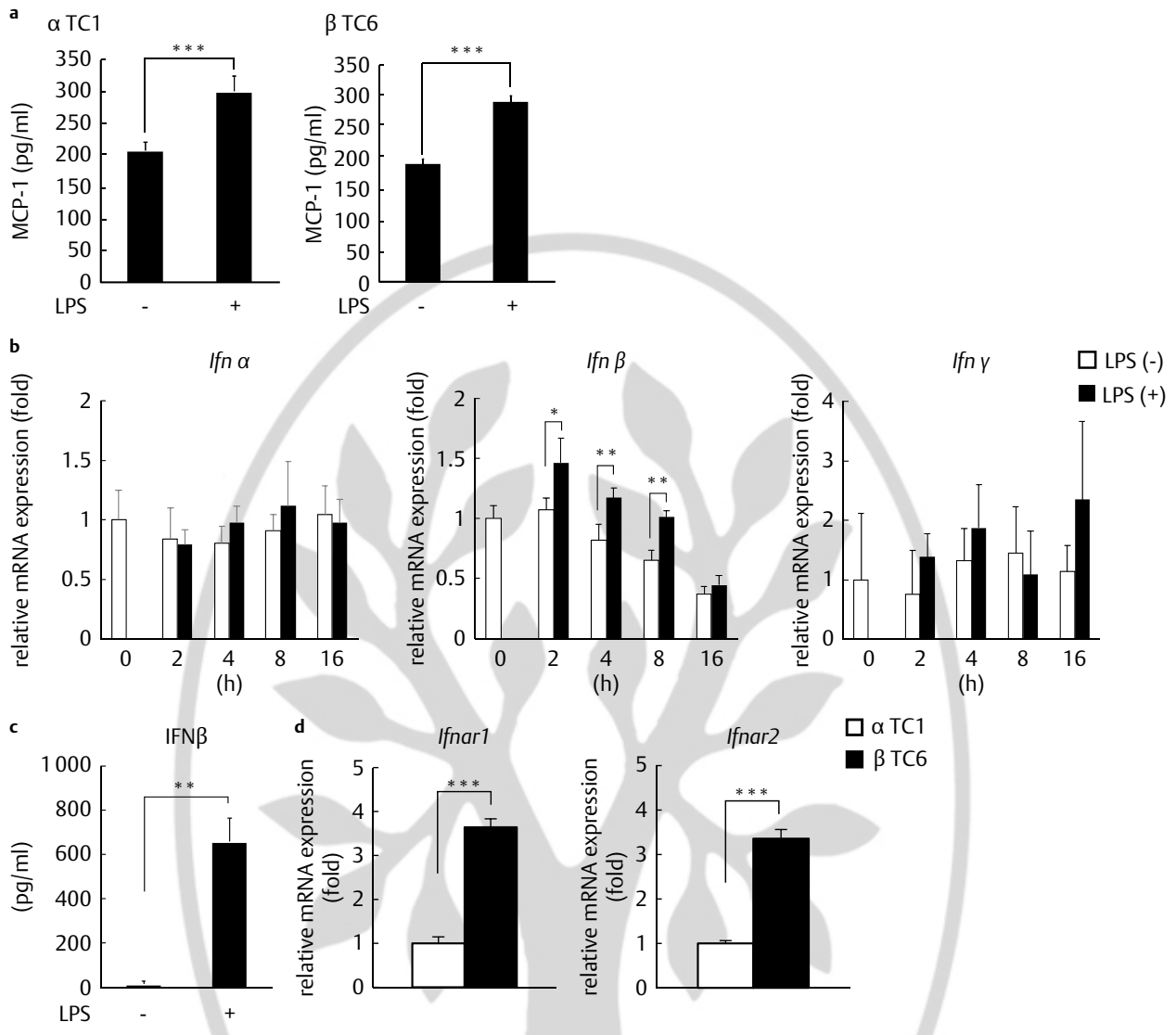
We therefore valuated whether IFNβ upregulated caspase 3 expression in pancreatic β cells, and the results are shown in ▶ **Fig. 4a, b**. IFNβ significantly upregulated the expression levels of cleaved caspase 3 protein. It is known that saturated fatty acids such as palmitate are involved in β-cell death [8, 22]. Interestingly, co-stimulation with both IFNβ and palmitate further upregulated cleaved caspase 3 expression in β cells (▶ **Fig. 4a, b**). Apoptotic cells were detected by TUNEL staining. As shown in ▶ **Fig. 4c**, in agreement with cleaved caspase 3 expression, TUNEL-positive cell numbers increased following IFNβ stimulation. Furthermore, co-stimulation with IFNβ and palmitate increased the number of cells undergoing cell death by up to 50 % (▶ **Fig. 4c, d**). These results indicate that activated macrophage-derived IFNβ caused activation of caspase 3 by upregulating Xaf1 expression and accelerating β-cell apoptosis induced by palmitate. βTC6 cells transfected with Xaf1 siRNA showed a significant reduction in the number of IFNβ-induced apoptotic cells as compared with the cells transfected with scrambled siRNA (▶ **Fig. 4e, f**). In contrast, cells transfected with Xiap siRNA showed a tendency to increase the number of IFNβ-induced apoptosis as compared to the cells transfected with scrambled siRNA (▶ **Fig. 4e, g**). Transfection of Ifnar1 or Ifnar2 siRNA significantly decreased the apoptosis by 10–15 % (▶ **Fig. 4e-i**). These results suggest that suppression of IFNβ signaling downregulated Xaf1 expression and subsequent apoptosis.

Discussion

In this study, we first compared the changes in gene expression levels under low-grade inflammatory conditions; under metabolic endotoxemia-associated conditions, it appeared that β cells were influenced more profoundly than α cells. IFNβ secretion from activated macrophages was enhanced. Because the basal expression of the type I IFN receptor, IFNAR1/2 was higher in β cells than in α cells, IFN signals may be more intensively transduced in β cells. When type I IFN binds to IFNAR 1/2, Janus kinase-signal transducer and activator of transcription is activated, resulting in the tran-



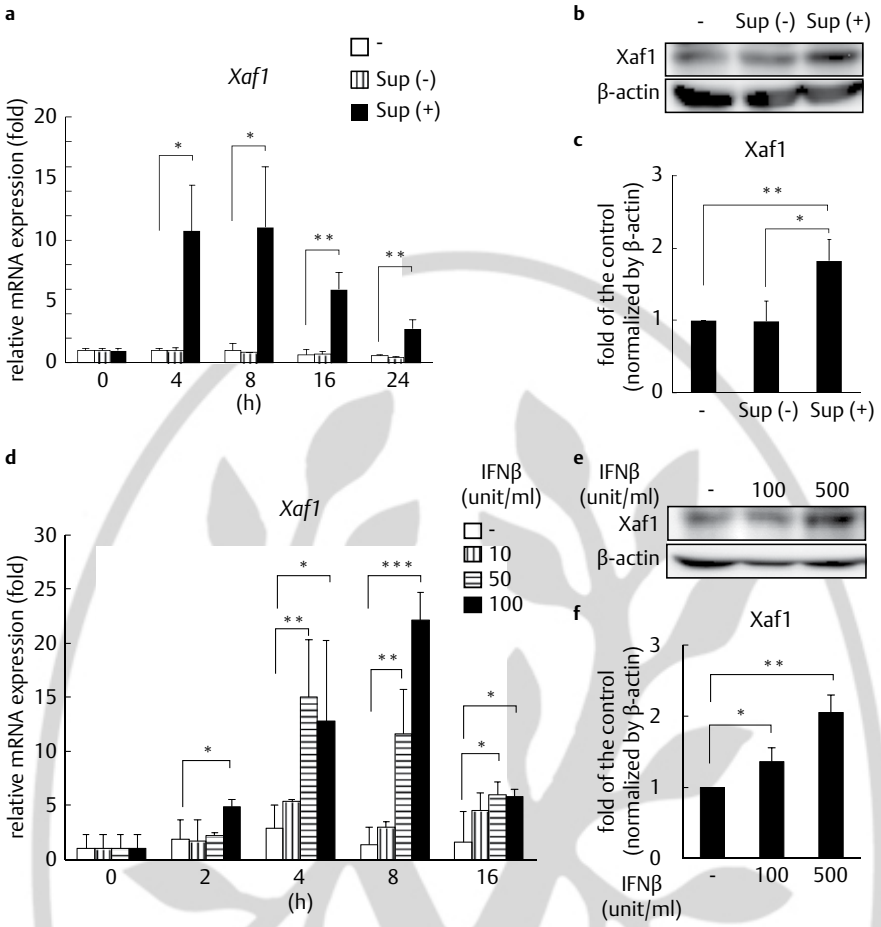
▶ **Fig. 1** Expression of interferon-associated genes in both α and β cells co-cultured with endotoxin-stimulated macrophages: **a**, **b**: Time course by DNA microarray analysis for highly expressed genes in αTC1 **a** and βTC6 **b** cells co-cultured with RAW 264.7 macrophages in the presence or absence of *E. coli* LPS (1 ng/ml) stimulation. Data are expressed as fold changes of the genes expressed with LPS stimulation against those without LPS stimulation. **c**, **d**: Real-time PCR analysis of representative genes from the microarray analysis that were highly expressed in αTC1 **c** and βTC6 **d** cells co-cultured with RAW264.7 cells following *E. coli* LPS (1 ng/ml) stimulation. mRNA was extracted 0, 4, 8, and 16 h following stimulation. Representative data from three independent experiments are shown. ** $p<0.01$; * $p<0.05$; $n=4$; Student's *t*-test.



▶ **Fig. 2** Higher expression of interferon-responsive genes was induced in β cells than in α cells: **a** MCP-1 protein concentration of the respective culture supernatants was determined 16 h following LPS stimulation. **b** Relative gene expression levels of Ifnα, Ifnβ, and Ifnγ in RAW264.7 macrophages stimulated with or without 1 ng/ml E. coli LPS for indicated times. **c** IFNβ protein concentration in the culture supernatants of RAW264.7 macrophages incubated with LPS for 12 h. **d** Relative gene expression levels of type I IFN receptor genes in αTC1 and βTC6 cells. Representative data from three independent experiments are shown. * * * $p<0.001$; * * $p<0.01$; * $p<0.05$; $n=4$; Student's t-test.

scription initiation of a series of genes with promoters containing interferon-stimulated response element-binding sites. Most genes induced by the activation of interferon-stimulated response elements are associated with antivirus, anti-proliferative, and immunosuppressive activities, and thus, type I IFN may be useful for the treatment of several infections caused by viruses and autoimmune diseases [23, 24]. This is the first study to show that IFNβ upregulated the expression of Xaf1 in β cells. It has also been shown that IFNβ enhances Xaf1 expression in a colon cancer cell line [25]. Xaf1 promotes apoptosis by inhibiting Xiap [19]. In addition, it may be associated with p53-mediated cell death [26]. p53-mediated β-cell death has been reported in mice with constitutively active glucokinase and in subjects with persistent hyperinsulinemic hypoglycemia of infancy with mutated genes regulating insulin secretion [27]. Previous reports indicated that both receptor subunits are required for IFN signal transduction despite the fact that IFNAR2 binds more strongly than IFNAR1 to type I IFNs [28]. We consider that suppression of either type of IFNAR1 or IFNAR2 was enough to downregulate IFNβ signaling, followed by the suppression of Xaf1 expression and reduced apoptosis.

In this study, we found that IFNβ upregulated caspase 3 expression in β cells and promoted cell apoptosis, suggesting that macrophage-derived IFNβ is a critical trigger of β-cell death. In addition, under this condition, saturated fatty acids further upregulat-



▶ **Fig. 3** Macrophage-derived IFNβ is a primary molecule upregulating Xaf1 in βTC6 cells: **a**–**c** RAW264.7 macrophages were stimulated with or without 1 ng/ml *E. coli* LPS for 12 h, then the culture medium was added to βTC6 cells. Sup (-), supernatant from RAW 264.7 macrophages without stimulation; Sup (+), supernatant from LPS stimulated RAW 264.7 macrophages. **a** Relative expression level of *Xaf1* in βTC6 cells at 0, 4, 8, 16, and 24 h after addition of the supernatant from LPS stimulated RAW 264.7 macrophages. **b**, **c** Representative immunoblots **b** of Xaf1 expression in βTC6 cells incubated with or without supernatant of RAW264.7 macrophages. βTC6 cells were collected 24 h following addition of the supernatant to examine with immunoblotting. Quantitative data from three independent experiments are presented as bar graphs **c**. **d** Relative gene expression levels of *Xaf1* in βTC6 cells stimulated with or without IFNβ. **e**, **f** Representative immunoblots **e** of Xaf1 expression in βTC6 cells stimulated with or without IFNβ. βTC6 cells were collected 24 h following addition of IFNβ to examine with immunoblotting. Quantitative data from four independent experiments are presented as bar graphs **f**. * * * $p<0.001$; * * $p<0.01$; * $p<0.05$; $n=4$; Student's *t*-test.

ed β-cell death. Because both α and β cells secreted MCP-1 when co-cultured with endotoxin-stimulated macrophages, metabolic endotoxemia may have influenced both α and β cells, and these cell-derived chemokines further acted to recruit immune cells into islets and evoked IFN signals into β cells.

A recent report valuating islets of type 2 diabetes model mice showed that expression of microRNA (miR)-200 family members is highly induced; miR-200 negatively regulates anti-apoptotic and stress-resistant networks via several mechanisms including the negative regulation of caspase inhibiting Xiap, and β-cell specific overexpression of miR-200 induces β-cell death and fatal diabetes [29]. Although further studies are necessary to understand the molecular mechanisms of β-cell death enhanced by Xaf1, inhibition of IFNβ-mediated pancreatic β-cell death may be a therapeutic strategy for type 2 diabetes.

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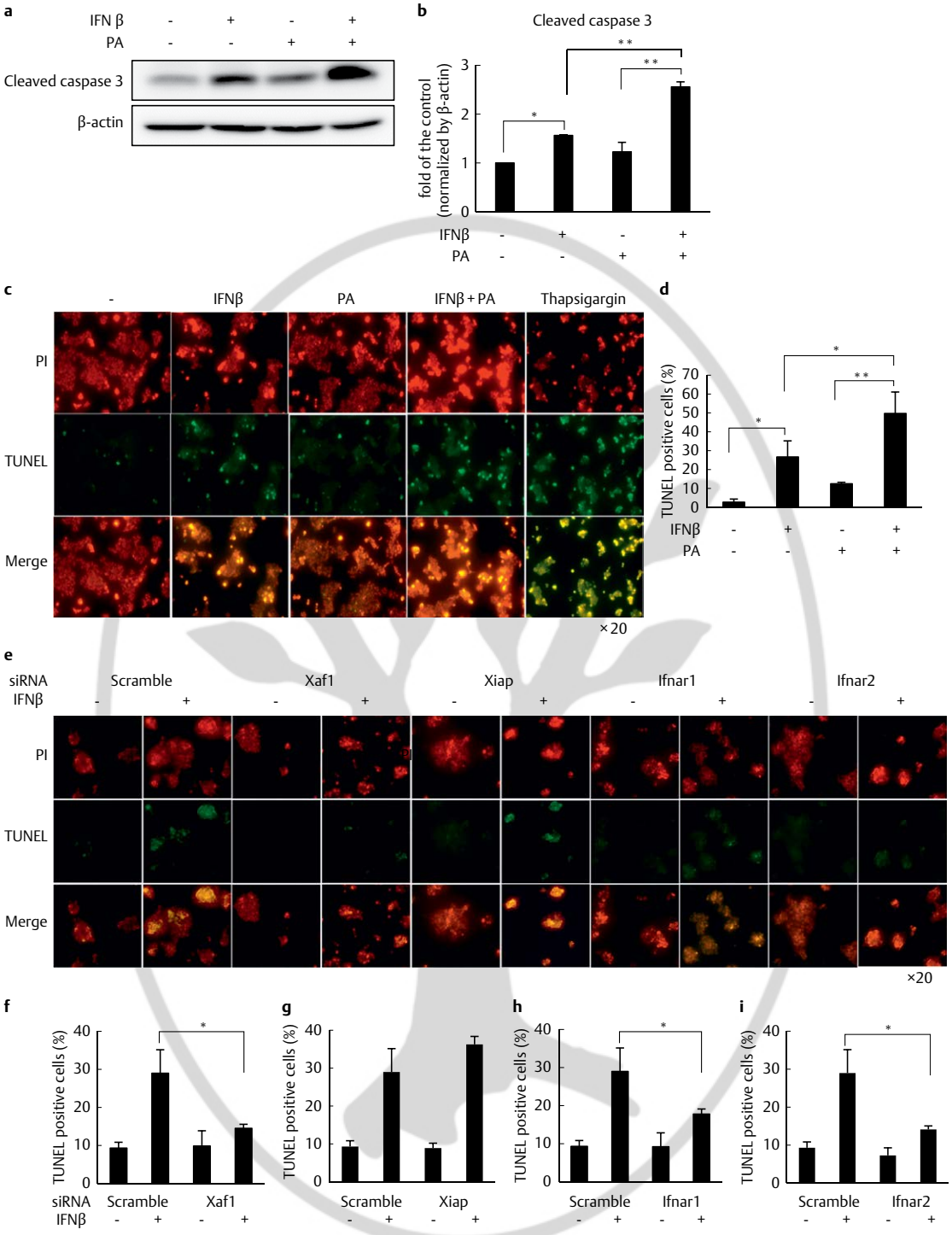

▶ **Fig. 4** IFNβ promotes apoptotic cell death in βTC6 cells: **a**, **b** Representative immunoblots **a** of Xaf1 in βTC6 cells stimulated with or without IFNβ and/or palmitate (PA) for 24 h. βTC6 cells were collected 24 h following addition of IFNβ (500 unit/ml) and/or 400 μM palmitate (PA) to examine with immunoblotting. Quantitative data from four independent experiments are presented as bar graphs **b**. **c** Images of TUNEL staining from βTC6 cells. βTC6 cells were stimulated with IFNβ (500 unit/ml) and/or palmitate (PA, 400 μM) for 24 h. Thapsigargin (1 μM) was used as a positive control. **e** Images of TUNEL staining from βTC6 cells. βTC6 cells were transfected with siRNAs against mouse Xaf1, Xiap, Ifnar1, and Ifnar2. Forty-eight hours after siRNA transfection, cells were stimulated with IFNβ (500 unit/ml) for 24 h. **c**–**i** Evaluation of apoptosis was performed with TUNEL staining. PI was used to visualize the nuclei. Stained cells were photographed and analyzed using a microscope. The ratio of cell apoptosis was expressed as the percentage of TUNEL staining positive cells versus PI staining positive cells. Five random fields from each sample were selected and quantitated. Representative photos from four independent experiments are shown **c**, **e** and quantified data are shown as bar graphs **d**, **f**–**i**. ** $p < 0.01$; * $p < 0.05$; n = 4; Tukey's test **b, d**; Student's t-test **f**–**i**.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006; 444: 860–867

[2] Suganami T, Ogawa Y. Adipose tissue macrophages: Their role in adipose tissue remodeling. J Leukoc Biol 2010; 88: 33–39

[3] Schrover IM, Spiering W, Leiner T, Visseren FL. Adipose tissue dysfunction: Clinical relevance and diagnostic possibilities. Horm Metab Res 2016; 48: 213–225

[4] Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, Almeida M, Arumugam M, Batto JM, Kennedy S, Leonard P, Li J, Burgdorf K, Grarup N, Jørgensen T, Brandslund I, Nielsen HB, Juncker AS, Bertalan M, Levenez F, Pons N, Rasmussen S, Sunagawa S, Tap J, Tims S, Zoetendal EG, Brunak S, Clément K, Doré J, Kleerebezem M, Kristiansen K, Renault P, Sicheritz-Ponten T, de Vos WM, Zucker JD, Raes J, Hansen T, MetaHIT consortium.Bork P, Wang J, Ehrlich SD, Pedersen O. Richness of human gut microbiome correlates with metabolic markers. Nature 2013; 500: 541–546

[5] Amar J, Serino M, Lange C, Chabo C, Iacovoni J, Mondot S, Lepage P, Klopp C, Mariette J, Bouchez O, Perez L, Courtney M, Marre M, Klopp P, Lantieri O, Doré J, Charles M, Balkau B, Burcelin R.D.E.S.I.R. Study Group. Involvement of tissue bacteria in the onset of diabetes in humans: Evidence for a concept. Diabetologia 2011; 54: 3055–3061

[6] Oslowski CM, Urano F. A switch from life to death in endoplasmic reticulum stressed β-cells. Diabetes Obes Metab 2010; 12: 58–65

[7] Tachibana K, Sakurai K, Yokoh H, Ishibashi T, Ishikawa K, Shirasawa T, Yokote K. Mutation in insulin receptor attenuates oxidative stress and apoptosis in pancreatic beta-cells induced by nutrition excess: Reduced insulin signaling and ROS. Horm Metab Res 2015; 47: 176–183

[8] Fonseca SG, Gromada J, Urano F. Endoplasmic reticulum stress and pancreatic β-cell death. Trends Endocrinol Metab 2011; 22: 266–274

[9] Donath MY. Targeting inflammation in the treatment of type 2 diabetes: Time to start. Nat Rev Drug Discov 2014; 13: 465–476

[10] Jurgens CA, Toukatly MN, Fligner CL, Udayasankar J, Subramanian SL, Zraika S, Aston-Mourney K, Carr DB, Westermark P, Westermark GT, Kahn SE, Hull RL. β-cell loss and β-cell apoptosis in human type 2 diabetes are related to islet amyloid deposition. Am J Pathol 2011; 178: 2632–2640

[11] Mizukami H, Takahashi K, Inaba W, Tsuboi K, Osonoi S, Yoshida T, Yagihashi S. Involvement of oxidative stress-induced DNA damage, endoplasmic reticulum stress, and autophagy deficits in the decline of β-cell mass in Japanese type 2 diabetic patients. Diabetes Care 2014; 37: 1966–1974

[12] Tessem JS, Jensen JN, Pelli H, Dai XM, Zong XH, Stanley ER, Jensen J, DeGregori J. Critical roles for macrophages in islet angiogenesis and maintenance during pancreatic degeneration. Diabetes 2008; 57: 1605–1617

[13] Ehses JA, Perren A, Eppler E, Ribaux P, Pospisilik JA, Maor-Cahn R, Gueripel X, Ellingsgaard H, Schneider MK, Biollaz G, Fontana A, Reinecke M, Homo-Delarche F, Donath MY. Increased number of islet-associated macrophages in type 2 diabetes. Diabetes 2007; 56: 2356–2370

[14] Eguchi K, Manabe I, Oishi-Tanaka Y, Ohsugi M, Kono N, Ogata F, Yagi N, Ohto U, Kimoto M, Miyake K, Tobe K, Arai H, Kadowaki T, Nagai R. Saturated fatty acid and TLR signaling link β cell dysfunction and islet inflammation. Cell Metab 2012; 15: 518–533

[15] Yamashita A, Soga Y, Iwamoto Y, Yoshizawa S, Iwata H, Kokeguchi S, Takashiba S, Nishimura F. Macrophage-adipocyte interaction: Marked interleukin-6 production by co-cultures stimulated with lipopolysaccharide. Obesity 2007; 15: 2549–2552

[16] Quackenbush J. Microarray data normalization and transformation. Nat Genet 2002; 32 (suppl): 496–501

[17] Wu P, Yang L, Shen X. The relationship between GPR40 and lipotoxicity of the pancreatic β-cells as well as the effect of pioglitazone. Biochem Biophys Res Commun 2010; 403: 36–39

[18] Salvesen GS, Duckett CS. IAP proteins: Blocking the road to death's door. Nat Rev Mol Cell Biol 2002; 3: 401–410

[19] Liston P, Fong WG, Kelly NL, Toji S, Miyazaki T, Conte D, Tamai K, Craig CG, McBurney MW, Korneluk RG. Identification of XAF1 as an antagonist of XIAP anti Caspase activity. Nat Cell Biol 2001; 3: 128–133

[20] Xia Y, Novak R, Lewis J, Duckett CS, Phillips AC. Xaf1 can cooperate with TNFalpha in the induction of apoptosis, independently of interaction with XIAP. Mol Cell Biochem 2006; 286: 67–76

[21] Chung SK, Lee MG, Ryu BK, Lee JH, Han J, Byun DS, Chae KS, Lee KY, Jang JY, Kim HJ, Chi SG. Frequent alteration of XAF1 in human colorectal cancers: Implication for tumor cell resistance to apoptotic stresses. Gastroenterology 2007; 132: 2459–2477

[22] Jeffrey KD, Alejandro EU, Luciani DS, Kalynyak TB, Hu X, Li H, Lin Y, Townsend RR, Polonsky KS, Johnson JD. Carboxypeptidase E mediates palmitate-induced beta-cell ER stress and apoptosis. Proc Natl Acad Sci U S A 2008; 105: 8452–8457

[23] Borden EC, Sen GC, Uze G, Silverman RH, Ransohoff RM, Foster GR, Stark GR. Interferons at age 50: Past, current and future impact on biomedicine. Nat Rev Drug Discov 2007; 6: 975–990

[24] Zitvogel L, Galluzzi L, Kepp O, Smyth MJ, Kroemer G. Type I interferons in anticancer immunity. Nat Rev Immunol 2015; 15: 405–414

[25] Sun Y, Qiao L, Xia HH, Lin MC, Zou B, Yuan Y, Zhu S, Gu Q, Cheung TK, Kung HF, Yuen MF, Chan AO, Wong BC. Regulation of XAF1 expression in human colon cancer cell by interferon beta: Activation by the transcription regulator STAT1. Cancer Lett 2008; 260: 62–71

[26] Lee MG, Han J, Jeong SI, Her NG, Lee JH, Ha TK, Kang MJ, Ryu BK, Chi SG. XAF1 directs apoptotic switch of p53 signaling through activation of HIPK2 and ZNF313. Proc Natl Acad Sci U S A 2014; 111: 15532–15537

[27] Tornovsky-Babeay S, Dadon D, Ziv O, Tzipilevich E, Kadosh T, Schyr-Ben Haroush R, Hija A, Stolovich-Rain M, Furth-Lavi J, Granot Z, Porat S, Philipson LH, Herold KC, Bhatti TR, Stanley C, Ashcroft FM, In't Veld P, Saada A, Magnuson MA, Glaser B, Dor Y. Type 2 diabetes and congenital hyperinsulinism cause DNA double-strand breaks and p53 activity in β cells. Cell Metab 2014; 19: 109–121

[28] Domanski P, Colamonici OR. The type-I interferon receptor. The long and short of it. Cytokine Growth Factor Rev 1996; 7: 143–151

[29] Belgardt BF, Ahmed K, Spranger M, Latreille M, Denzler R, Kondratiuk N, von Meyenn F, Villena FN, Herrmanns K, Bosco D, Kerr-Conte J, Pattou F, Rülicke T, Stoffel M. The microRNA-200 family regulates pancreatic beta cell survival in type 2 diabetes. Nat Med 2015; 21: 619–627

Supplementary Material

Table 1S Primer sequences used for real-time PCR.

Gene	Forward	Reverse
Gapdh	5′-AATGTGTCCGTCGTGGATCTGA-3′	5′-GATGCCTGCTTCACCACCTTCT-3′
Mcp-1	5′-AGGTCCCTGTGATGCTTCTG-3′	5′-TCTGGACCCATTCCTTCTTG-3′
Cxcl1	5′-CACCCAAACCGAAGTCATAG-3′	5′-AAGCCAGCGTTCACCAGA-3′
Cxcl10	5′-TGAGAGACATCCCGAGCCAA-3′	5′-GAGGCAGAAAATGACGGCAG-3′
Isg15	5′-AGTGCTCCAGGACGGTCTTA-3′	5′-CGTGTCTACAGTCTGCGTCA-3′
Irf9	5′-TTCAGGATGGCCTCAGGCAAAGTA-3′	5′-GAACAAGTCTATTTCCATGGAGACG-3′
Ifi27	5′-GTGACTTCAGATTTTCCTTCCCC-3′	5′-ACTGTCATGGCTCCCCCAA-3′
Ifit3	5′-CTGAACTGCTCAGCCCACA-3′	5′-TCAGCTTGCCCTAAGCACTC-3′
Xaf1	5′-GCAGACCAAGGAAAGCCAAC-3′	5′-ACTTGGAGTGTGATGGGCTG-3′
Ifnα	5′-TTGAAGGTCCTGGCACAG-3′	5′-GAGGTTCAAGGTCTGCTGA-3′
Ifnβ	5′-CTGGCTTCCATCATGAACAA-3′	5′-CATTTCCGAATGTTCGTCCT-3′
Ifnγ	5′-TCAAGTGGCATAGATGTGGAAGAA-3′	5′-TGGCTCTGCAGGATTTTCATG-3′
Ifnar1	5′-TTTAATCCTGCCGTAGCCCC-3′	5′-GCCAGCTCCTCCAGTTAGTG-3′
Ifnar2	5′-GCCGCCAGAATTTGAGATCG-3′	5′-GGGCTCGTGCTTCTTCCTAA-3′

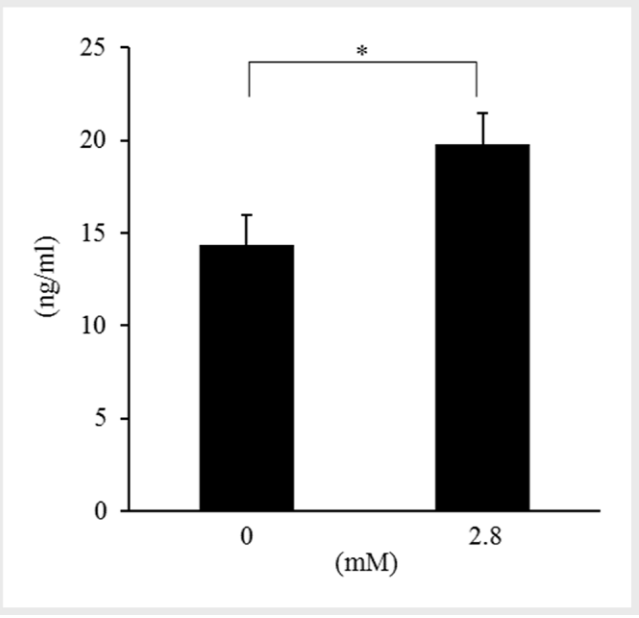

Fig. 1S Glucose-stimulated insulin secretion in βTC6 cells. βTC6 cells were stimulated by 0 or 2.8 mM glucose. Data represent means ± SEM of three to five independent experiments. * $p < 0.05$; $n = 4$; Student's t-test