RESEARCH

Effect of TNF- α on the expression of ABCA1 in pancreatic β -cells

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Abstract

ATP-binding cassette transporter A1 (ABCA1), a 254-kD membrane protein, is a key regulator of lipid efflux from cells to apolipoproteins. ABCA1 in pancreatic β -cells influences insulin secretion and cholesterol homeostasis. Tumor necrosis factor (TNF)- α is a pleiotropic cytokine that elicits a wide spectrum of physiological events, including cell proliferation, differentiation and apoptosis and is also known to decrease glucose-dependent insulin secretion in pancreatic islets. In the present study, we examined the role of TNF- α on ABCA1 expression in rat pancreatic islets and INS-1 cells. ABCA1 protein levels decreased in response to rising concentrations of TNF- α in pancreatic islets. Real-time polymerase chain reaction analysis showed a significant decrease in ABCA1 mRNA expression. In parallel with its effect on endogenous ABCA1 mRNA levels, TNF- α suppressed the activity of a reporter construct containing the ABCA1 promoter. This effect was abrogated by BIRB796, but not by SB203580 or LY-294002. The constitutively active form of p38 mitogen-activated protein kinase (MAPK) γ suppressed ABCA1 promoter activity but not p38-MAPK (α , β), while a dominant-negative mutant of p38-MAPK γ blocked the effect of TNF- α on ABCA1 promoter activity. BIRB796 inhibited the increased cholesterol ester content induced by TNF- α . However, BIRB796 had no effect on either the decreased insulin content or the ABCA1 suppression caused by TNF- α in INS-1 cells. We checked the influence of TNF- α of insulin secretion and glucose-stimulated insulin secretion in rat pancreatic islet and INS-1 cell. TNF- α suppressed the insulin secretion and glucose-stimulated insulin secretion in both rat pancreatic islet and INS-1 cell. In summary, TNF- α suppressed the expression of endogenous ABCA1 and suppress the insulin secretion in pancreatic islets and INS-1 cells. These findings raise the possibility that TNF- α may affect insulin secretion by controlling ABCA1 expression.

Key Words

- ▶ tumor necrosis factor- α
- ▶ ATP-binding cassette transporter A1
- ▶ p38MAPK
- ▶ pancreatic β cells
- ▶ cholesterol accumulation

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Introduction

Tumor necrosis factor (TNF)- α is a multifunctional pro-inflammatory cytokine that is associated with some pathological processes such as apoptosis, proliferation, inflammation and immunoregulation. A previous report indicated that TNF- α played a role in impaired secretion of

insulin, which frequently occurs in type 2 diabetes (Donath *et al.* 2003). In pancreatic β -cells, TNF- α alone inhibited glucose-stimulated insulin secretion (GSIS) (Zhang & Kim 1995, Dunger *et al.* 1996, Tsiotra *et al.* 2001). In the rat pancreatic β -cell line, INS-1, most molecules involved

in all steps of insulin secretion, from glucose sensing to insulin exocytosis, were not quantitatively altered by TNF- α treatment, nor was the expression of insulin and PDX-1 decreased. There was little change in the glucose-stimulated ATP level between TNF- α -treated and -untreated INS-1 cells, demonstrating that TNF- α does not affect glucose metabolism (Tsiotra *et al.* 2001, Kim *et al.* 2008).

A previous report indicated that TNF- α inhibited GSIS by reducing glucose-stimulated Ca²⁺ influx, possibly through the activation of c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) and NF- κ B inflammatory signals. Thus, our findings suggest that the activation of stress and inflammatory signals can contribute to the inhibition of GSIS during the development of diabetes (Kim *et al.* 2008). However, the detailed mechanisms have not been clarified.

Type 2 diabetes is characterized by progressive β -cell dysfunction and loss of β -cell mass. The reasons for β -cell dysfunction in type 2 diabetes are incompletely understood. Recently, abnormalities in cholesterol metabolism have emerged as a potential contributor to β -cell dysfunction (Brunham *et al.* 2008, Kruit *et al.* 2010). In pancreatic β -cells, lipotoxicity induced by cholesterol accumulation results in apoptosis and impaired insulin secretion (Unger 1995, Zhou & Grill 1995). ATP-binding cassette transporter A1 (ABCA1), a 254-kDa membrane protein, is a pivotal regulator of lipid efflux from cells to apolipoproteins and plays an important role in reverse cholesterol transport (Fielding & Fielding 1995). Previously, it was reported that specific inactivation of the ABCA1 gene in β -cells induced impaired glucose tolerance and defective insulin secretion in mice, but normal insulin sensitivity was retained (Brunham *et al.* 2007). The absence of ABCA1 in pancreatic islets altered cholesterol homeostasis and impaired insulin secretion *in vitro*, indicating that cholesterol accumulation may contribute to β -cell dysfunction in type 2 diabetes (Brunham *et al.* 2008).

In this study, we hypothesized that TNF- α might increase cholesterol accumulation via the ABCA1 transporter to induce lipotoxicity in pancreatic β -cells, thus attenuating insulin secretion.

Materials and methods

Cell culture

The INS-1 cells originated from a rat insulinoma cell line developed and propagated at the Division of Biochimie Clinique (courtesy of C B Wollheim, Geneva, Switzerland). The present experiments were performed using cell passages 9–35, the cells being trypsinized every 7 days.

These cells were cultured in RPMI1640 media (SIGAMA, Tokyo, Japan) containing 11.2 mmol/L glucose and supplemented with 10% heat-inactivated fetal bovine serum (Dainippon Pharmaceutical Co., Ltd. Tokyo, Japan), 50 μ mol/L 2-mercaptoethanol, 100 U/mL penicillin and 100 μ g/mL streptomycin. All cells were incubated in humidified 5% CO₂ at 37°C. When 80% confluent, the cells were washed twice and incubated with 0.5% fetal bovine serum RPMI 1640 media for 6 h. Then the cells were treated with varying doses of angiotensin II for 24 h before harvesting as described previously (Yu *et al.* 2004).

Western blot analysis

The 15–30 μ g proteins were separated by a 7.5% SDS-PAGE and transferred to PVDF membrane for immunoblotting. After blocking with 7.5% skim milk in PBS (pH 7.2) overnight at 4°C, the membrane was incubated with 0.1% Tween 20 in PBS (PBS-T) containing the anti-ABCA1 antibody, anti-insulin (Santa Cruz Biotechnology Inc; diluted to 1:200) overnight at 4°C or anti-GAPDH antibody (Biomol Research, Plymouth Meeting, PA; diluted to 1:5000) for 1 h at room temperature as described previously (Nishiuchi *et al.* 2010). Membrane was washed three times with PBS-T, 10 min each, and then incubated for 1 h at room temperature with the appropriate horseradish peroxidase-linked secondary antibody (DakoCytomation; diluted to 1:2000). Membranes were again washed three times, 10 min each, and antigen–antibody complexes were visualized by ECL (GE Healthcare).

Real-time PCR

PCR was performed with a final volume of 20 μ L in LightCycler (Roche) glass capillaries. The sequences of the forward and reverse ABCA1 primers were 5'-CCCGGCGG AGTAGAAAGG-3' and 5'-AGGGCGATGCAAACAAAGAC-3', respectively. Each set of PCR reactions included water as a negative control and 5 dilutions of the standard. Known amounts of DNA were then diluted to make the standards, and a regression curve of crossing points versus concentration was generated with LightCycler as described previously (Miyai *et al.* 2011). GAPDH was used as the housekeeping standard.

Transfection of INS-1 cells and luciferase reporter gene assay

We used a construct (pABCA1-LUC) containing the human ABCA1 promoter obtained using PCR and the luciferase reporter gene as previously described (Nagao

et al. 2006). Purified promoter plasmid was transfected into INS-1 using Lipofectamine (Life Technologies). Transfected cells were maintained in medium containing 20 ng/mL TNF for 24 h with or without pre-treatment with LY (LY294002, 10 μ mol/L), PD (PD98095, 10 μ mol/L), SB (SB203580, 1 μ mol/L), H-89 (1 μ mol/L) or STO609 (1 μ g/mL) to separately inhibit the PI3K, MEK, p38MAPK, PKA or CaMKK signaling pathway for 30 min (Yu *et al.* 2010). Then transfected cells were harvested, and the promoter activity of ABCA1 was measured in an aliquot of the cytoplasmic preparation. Forty microliter aliquots were taken for the luciferase assay, which was performed according to the manufacturer's instructions (ToyoInk, Tokyo, Japan).

Plasmid preparation

An expression vector encoding a constitutively active p38 MAPK (α , β , γ) and a dominant-negative mutant of p38 MAPK (p38-DN) were kindly provided by Dr Z Wu (Hong Kong University of Science & Technology) as described previously (Murao *et al.* 2008).

Cholesterol content assay

To measure cellular cholesterol content concentration, we used a colorimetric assay that utilizes reagents widely used for the measurement of cholesterol in conjunction with a random-access chemistry analyzer ARCHITECT c8000 as described previously (Lyu *et al.* 2016).

Glucose-stimulated insulin secretion (GSIS)

Pancreatic islets or INS-1 cells treated with varying treatments were starved in Krebs-Ringer-bicarbonate (KRB) buffer containing 120 mM NaCl, 5 mM KCl, 1.1 mM MgCl_2 , 2.5 mM CaCl_2 , 25 mM NaHCO_3 and 0.1% bovine serum albumin (pH 7.4) for 1 h. Following, cells were incubated in KRB buffer containing 3.3 mM glucose for 1 h and then the medium was replaced by KRB buffer supplemented with varying glucose concentration (basal 3.3 mM; stimulatory 16.7 mM) together with other test reagents. After 1-h incubation, the supernatant was harvested and used for insulin measurement by ELISA kit (Shibayagi, Japan). All the incubation processes were performed in 5% CO_2 incubator at 37°C.

Animals

All procedures involving animals were in accordance with Japanese laws and approved by the Animal Care Committee of Kagawa University. Seven-week-old Sprague–Dawley

(SD) rats were purchased from UNIMEDIA and housed at controlled temperature (25°C) and lighting (12 h light/dark cycles) in compliance with the Guide for Experimental Animal Research. After 1-week adaptation, animals were killed and pancreas was extracted. Isolation of pancreatic islets from rat was performed as described previously (Hellman *et al.* 2012). Briefly, the pancreas was digested by collagenase type XI at 37°C for 15 min and then islets were purified and collected by Histopaque1077. Finally, islets (100–200 μ m) were picked and placed in RPMI1640 media supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin and 0.1 mg/mL streptomycin in 5% CO_2 incubator at 37°C.

Statistical analysis

Data are expressed as mean $\pm$ S.E.M. Results were analyzed by one-way ANOVA and Student's *t* test. A value of $P < 0.05$ was considered statistically being significant. All experiments were performed at least three times.

Results

Glucose-stimulated insulin secretion was reduced by TNF- α

Previous report suggested that accumulation of cholesterol in pancreatic beta cells resulted in impaired insulin secretion. Thus, we checked the abundance of insulin secretion in INS-1 cells treated with TNF- α . As shown in Fig. 1A, TNF- α significantly decreased the amount of insulin. Then, glucose-stimulated insulin secretion (GSIS) assay showed that high glucose (16.7 mM) increased insulin secretion to compared with basal level (3.3 mM, Fig. 1B). INS-1 cells treated with TNF- α were not able to release insulin response to high glucose stimulation (Fig. 1B). These data demonstrated that TNF- α decreased glucose-stimulated insulin secretion in pancreatic beta cells. So, we measured the insulin secretion in rat pancreatic islet treated with TNF- α . TNF- α significantly decreased the amount of insulin in rat pancreatic islet (Fig. 1C). It also suppressed the GSIS in rat pancreatic islet treated with TNF- α (Fig. 1D).

TNF- α decreased the expression of ABCA1 in rat pancreatic islets

We examined the effect of TNF- α on the expression of ABCA1 in pancreatic islets from rats. Both the protein and mRNA expression of ABCA1 was significantly decreased by treatment with TNF- α (Fig. 2).

Figure 1

Effect of tumor necrosis factor (TNF)- α on insulin secretion and glucose stimulated insulin secretion in INS-1 cells and pancreatic islet. (A) INS-1 cells were incubated with 10 ng/mL TNF- α for 24 h. Fold of insulin secretion has significantly decreased in treated with TNF- α ($P < 0.05$). (B) To compare with 3.3 mM glucose group, 16.7 mM glucose group has significantly increased of insulin secretion. But treated with TNF- α , the increased level of insulin secretion in 16.7 mM glucose has canceled. (C) Rat pancreatic islet was incubated with 10 ng/mL TNF- α for 24 h. Fold of insulin secretion has significantly decreased in treated with TNF- α ($P < 0.05$). (D) To compare with 3.3 mM glucose group, 16.7 mM glucose group has significantly increased of insulin secretion. But treated with TNF- α , the increased level of insulin secretion in 16.7 mM glucose has canceled.

Concentration-response and time course of TNF- α -mediated suppression of ABCA1 in the rat pancreatic β -cell line, INS-1

Total protein was isolated from INS-1 cells treated with varying concentrations (0–20 ng/mL) or varying incubation periods (0–72 h) of TNF- α . Protein samples were subjected to Western blotting to detect the expression of ABCA1. These analyses (Fig. 3A) showed a decreased abundance of ABCA1 protein in response to rising concentrations of TNF- α , with maximum induction observed at 20 ng/mL. The time course of changes in ABCA1 protein levels were also analyzed up to 72 h after treatment with TNF- α by Western blot analysis. In pancreatic islets, there was a decrease in ABCA1 expression after 48 h with levels reaching 50% of control after 72 h (Fig. 3B). Furthermore, the abundance of ABCA1 mRNA also decreased after treatment with TNF- α (Fig. 3C).

Figure 2

Effect of tumor necrosis factor (TNF)- α on the expression of ATP-binding cassette transporter A1 (ABCA1) in rat pancreatic islets. The effect of TNF- α on the expression of ABCA1 protein (A) and mRNA (B) in rat pancreatic islets. The ABCA1/GAPDH ratios are shown as percentages of control. Each data point shows the mean $\pm$ S.E.M. of three separate experiments for each treatment group. *Significantly different compared to the control ($P < 0.05$).

TNF- α decreased the transcription of ABCA1 via the MEK/ERK signaling pathway in INS-1 cells

Because TNF- α lowered the abundance of both ABCA1 protein and mRNA in INS-1 cells, we speculated that TNF- α regulated the activity of the ABCA1 promoter in INS-1 cells. For these studies, luciferase activity was measured in INS-1 cells transfected with pABCA1-LUC and exposed to 10 ng/mL TNF- α (Fig. 4A). In agreement with the protein and mRNA levels, TNF- α treatment also inhibited the activity of the promoter. Together, these results clearly show that TNF- α suppresses the activity of the ABCA1 gene in INS-1 cells.

Next, we used LY294002 (10 μ M), SB203580 (1 μ M) or BIRB796 (1 μ M) to separately inhibit the PI3K, p38-MAPK (α , β) or p38-MAPK (α , β , γ) signaling pathways. The results showed that only inhibition of the p38MAPK γ signaling pathway blocked the effect of TNF- α on ABCA1 promoter activity. This suggests that TNF- α may regulate ABCA1 expression via this pathway (Fig. 4B).

p38-MAPK regulated ABCA1 promoter activity

Because TNF- α suppressed ABCA1 promoter activity, the use of constitutively active forms of p38-MAPK should mimic the effects of TNF- α . To test this hypothesis, we expressed constitutively active forms of p38-MAPK (α , β , γ) in INS-1 cells carrying a reporter gene containing the ABCA1 promoter. The results showed that the constitutively active form of p38-MAPK γ , but not α or β , suppressed ABCA1 promoter activity in INS-1 cells (Fig. 5A). The converse of this study is using a dominant-

Figure 3

Effect of tumor necrosis factor (TNF)- α on the expression of ATP-binding cassette transporter A1 (ABCA1) in INS-1 cells. (A) Dose-dependent suppression of ABCA1 protein by TNF- α . INS-1 cells were seeded in six-well plates and exposed to the indicated amounts of TNF- α for 24 h. ABCA1 in the total cell lysate was detected by Western blot analysis. GAPDH served as the control and is shown at the bottom of each lane. The ABCA1/GAPDH ratios are shown as percentages of control in the lower panel. Each data point shows the mean $\pm$ S.E. of three separate experiments. *Significantly different from control ($P < 0.05$). (B) Time-dependent suppression of ABCA1 protein by TNF- α . INS-1 cells were incubated for the indicated periods of time in the presence of 10 ng/mL TNF- α . GAPDH served as the control and is shown at the bottom of each lane. The ABCA1/GAPDH ratios are shown as percentages of control in the lower panel. Each data point shows the mean $\pm$ S.E. of three separate experiments. *Significantly different from control ($P < 0.05$). (C) Abundance of ABCA1 mRNA in INS-1 cells treated with 10 ng/mL TNF- α . The ABCA1/GAPDH ratios are shown as percentages of control. Each data point shows the mean $\pm$ S.E.M. of three separate experiments for each treatment group. *Significantly different from control ($P < 0.05$).

negative mutant of p38-MAPK γ (p38-DN) to block the effect of TNF- α on ABCA1 promoter activity (Fig. 5B). Consistent with the above results, TNF- α treatment inhibited ABCA1 promoter activity while the expression of p38-DN masked the ability of TNF- α to suppress this activity. Together, these findings support the idea that the p38-MAPK γ pathway is required for TNF- α suppression of ABCA1 promoter activity in INS-1 cells.

Effect of TNF- α on cholesterol content in INS-1 cells

ABCA1 is a cholesterol transporter that facilitates lipid efflux from cells. Thus, we investigated the effect of TNF- α

Figure 4

Effect of tumor necrosis factor (TNF)- α on ATP-binding cassette transporter A1 (ABCA1) promoter activity in INS-1 cells. (A) TNF- α decreases ABCA1 gene transcription. INS-1 cells were transfected with 1 μ g of pABCA1-LUC and treated with TNF- α for 24 h prior to harvesting. All assays were corrected for β -galactosidase activity. The total amount of protein in every reaction was identical. The results are expressed as relative luciferase activity compared with control cells arbitrarily set at 100. Each data point shows the mean $\pm$ S.E. of three separate transfections that were performed. *Significantly different from control ($P < 0.05$). (B) Effects of the phosphatidylinositol 3-kinase inhibitor, LY-294002 (LY), and the p38-mitogen-activated protein kinase (MAPK) inhibitors, SB-203580 (SB) and BIRB796 (BIRB), on ABCA1 transcriptional activity in INS-1 cells treated with TNF- α . The p38-MAPK inhibitors block the actions of TNF- α . Vehicle was 0.1% dimethyl sulfoxide. Each data point shows the mean $\pm$ S.E. of three independent transfections that were performed. *Significantly different from only TNF- α ($P < 0.05$).

on cholesterol ester content in INS-1 cells. Figure 6A shows that INS-1 cells treated with TNF- α have larger and more Oil Red O-stained lipid droplets than untreated control cells. The p38-MAPK inhibitor, BIRB796, blocked the effect of TNF- α on lipid accumulation of INS-1 cells.

We also measured the intracellular cholesterol ester concentration in INS-1 cells quantitatively. TNF- α increased cholesterol content, and the cholesterol ester concentration in cells increased about 25% compared to untreated cells (Fig. 6B). As predicted, the p38-MAPK inhibitor, BIRB796, inhibited the increment of cholesterol ester content by TNF- α (Fig. 6B) but had no significant effect on basal cholesterol ester levels in the absence of TNF- α .

Effect of TNF- α on the insulin content in INS-1 cells

A previous study reported that the pancreatic knockout of ABCA1 resulted in cholesterol accumulation and, subsequently, impaired insulin secretion (Brunham *et al.* 2007). To test this possibility in our system, we measured the insulin content in INS-1 cells treated with TNF- α . These cells showed a decreased insulin content in parallel with ABCA1 suppression (Fig. 7). However, BIRB796 prevented neither the decreased insulin content nor ABCA1 suppression caused by TNF- α .

Discussion

In this study, we found that TNF- α inhibited the expression of ABCA1 in the pancreatic β -cell line, INS-1 and isolated rat islets. TNF- α alone, or in combination with other cytokines, is associated with type I and type II diabetes mellitus (Argilés *et al.* 1994). While destruction of β -cells by cytokines may be responsible for type I diabetes, cytokines appear to play a multifaceted role in type II diabetes.

Previous reports suggested that the cytokines, interleukin-1, interferon- γ and TNF- α , alone or combination, decreased GSIS (Zhang & Kim 1995). While the insulin content in cytoplasm was not changed by treatment with TNF- α (Zhang & Kim 1995), the reduction of glucose utilization via decreased GLUT2 or glucokinase was suggested to be a cause for TNF- α -induced inhibition of GSIS (Park *et al.* 1999). However, Kim *et al.* demonstrated that a defect in glucose metabolism was not the main cause of TNF- α -induced GSIS inhibition. Furthermore, they showed that most molecules involved in the steps from glucose sensing to insulin exocytosis were not quantitatively altered by TNF- α treatment and that the expression of insulin and PDX-1 was not decreased. The minor change in the glucose-stimulated ATP level between TNF- α -treated and -untreated INS-1 cells demonstrates that TNF- α does not affect glucose metabolism (Kim *et al.* 2008).

Elevated cholesterol levels in pancreatic islet cells, either in ob/ob mice that lack ApoE and have diabetogenic obesity or in transformed β -cell lines directly overloaded with cholesterol, reduce GSIS (Lenzen & Klöppel 1978). This correlation is consistent with that between the reduction in insulin secretion and the elevation of pancreatic islet cell cholesterol levels in mice lacking β -cell ABCA1 (Brunham *et al.* 2007, 2008). This suggests that cholesterol has a direct effect on reducing β -cell functions.

In the current study, we showed that treatment with TNF- α increased the cholesterol ester level and decreased insulin secretion. The important role of β -cell ABCA1 in glucose homeostasis was further underscored by the finding that rosiglitazone increased β -cell ABCA1 expression (Brunham *et al.* 2007). Furthermore, we previously found that exendin-4, a glucagon-like peptide-1 analog, and insulin-like growth factor-I, have stimulatory effects on ABCA-1 expression at the transcriptional level, thereby influencing insulin secretion in pancreatic β -cells (Li *et al.* 2010, Lyu *et al.* 2016).

One goal of the present study was to examine in more detail the signaling pathways activated by TNF- α that

Figure 5

Role of the p38-mitogen-activated protein kinase (MAPK) signal transduction pathway on ABCA1 promoter activity inhibited by tumor necrosis factor (TNF)- α . (A) Effects of p38-MAPK isoforms on ABCA1 promoter activity. INS-1 cells were transfected with pABCA1-LUC and either an empty vector (control) or expression vectors of constitutively active forms of p38-MAPK (α , β , γ) for 24 h prior to cell harvesting. All assays were corrected for β -galactosidase activity. The total amount of protein in each reaction was identical. The results are expressed as relative luciferase activity compared with control cells arbitrarily set at 100. Each data point shows the mean $\pm$ s.e. of four separate transfections that were performed on different days. *Significantly different from control (* P <0.05). N.S., no significant difference. (B) Dominant-negative p38-MAPK γ (p38 γ -DN) blocks the TNF- α -induced inhibition of ABCA1 transcription. INS-1 cells were transfected with pABCA1-LUC and either the empty vector or p38 γ -DN, then treated with TNF- α for 24 h prior to cell harvesting. The results are expressed as relative luciferase activity compared with control cells arbitrarily set at 100. Each data point shows the mean $\pm$ s.e. of four separate transfections that were performed on different days. *Significantly different from control (P <0.05). N.S., no significant difference.

affect ABCA1 gene expression. TNF- α -induced responses are mediated through the TNF- α receptor (Locksley *et al.* 2001). The levels of phospho-JNK, phospho-p38 and NF- κ B are enhanced by TNF- α treatment, and inhibitors of these MAPK and inflammatory signals reduce TNF- α -induced GSIS inhibition (Kim *et al.* 2008). MAPKs contribute to direct cellular responses to various stimuli. p38/MAPK, a member of the MAPK superfamily that transduces extracellular responses, is known for its regulatory roles in apoptosis, cytokine production, transcriptional regulation and cytoskeletal reorganization (Zarubin & Han 2005). p38, a class of serine/threonine MAPKs, is composed of four isoforms (α , β , γ and δ) with more than 60% overall sequence homology and over 90% identity within the kinase domains (Cuenda & Rousseau 2007).

The use of BIRB796 decreases the activity of the four p38-MAPK subunits by almost 100%, while SB203582 fails to exert an obvious effect on the activities of the γ and δ isoforms (Kuma *et al.* 2005). Furthermore, the amounts of p38-MAPK β and p38-MAPK δ are only 10.6 and 0.08% of that of p38-MAPK α , respectively (Dingar *et al.* 2010). Previous studies, as well as the present study, have focused on the α and γ subunits of p38-MAPK. The present study found that BIRB796 treatment inhibited the effects of TNF- α , including the enhancement of glucose uptake, increasing the cholesterol content and decreasing ABCA1

Figure 6

Effect of tumor necrosis factor (TNF)- α on cholesterol accumulation in INS-1 cells. INS-1 cells were pretreated with dimethyl sulfoxide or BIRB796 (BIRB) for 30 min, then incubated with 10 ng/mL TNF- α for 24 h. Cells were then stained with Oil Red O (A) or lysed to measure intracellular cholesterol content (B). Percentages of cholesterol content per cell relative to control are shown as means $\pm$ S.E.M. of three separate experiments. *Significantly different compared to control ($P < 0.05$). #Significantly different compared to dimethyl sulfoxide plus TNF- α ($P < 0.05$). A full colour version of this figure is available at <https://doi.org/10.1530/JME-18-0167>.

expression, while SB203580 treatment did not exert any inhibitory effects. Because the distribution of the β and δ subunits is significantly lower than that of the α subunit, while γ is similar to α (LU *et al.* 2015), it is assumed that the functional effect of TNF- α in cells is mainly mediated via the p38-MAPK γ subunit. These results clearly demonstrate that the p38-MAPK signaling pathway, particularly p38-MAPK γ , may have an important role in TNF- α -mediated downregulation of ABCA1 expression. This was confirmed using CA-p38-MAPK γ or DN-p38-MAPK γ (Fig. 5).

In type 2 diabetes, p38/MAPK can be activated by high concentrations of glucose and advanced glycation end products and may play important roles in its pathogenesis (Chen *et al.* 1994). We previously examined glucose-mediated regulation of ABCA1 gene expression in vascular smooth muscle cells (Yu *et al.* 2010). The results showed that the expression of ABCA1 mRNA and protein decreased

Figure 7

Effect of tumor necrosis factor (TNF)- α on ABCA1 expression and insulin synthesis in INS-1 cells. (A) INS-1 cells were pretreated with dimethyl sulfoxide or BIRB796 (BIRB) for 30 min and then incubated with 10 ng/mL TNF- α for 24 h. Protein extracts were subjected to Western blot analysis to determine ABCA1 expression. The ABCA1/GAPDH ratios are shown as mean $\pm$ S.E.M. percentages of control from three separate experiments for each treatment group. (B) Abundance of insulin in INS-1 cells treated with TNF- α . INS-1 cells were pretreated with dimethyl sulfoxide or BIRB for 30 min, and then incubated with 10 ng/mL TNF- α for 24 h. Protein extracts were subjected to Western blot analysis to determine insulin expression. The insulin/GAPDH ratios are shown as mean $\pm$ S.E.M. percentages of control from three separate experiments for each treatment group. *Significantly different compared to DMSO ($P < 0.05$). #Significantly different compared to DMSO + TNF- α ($P < 0.05$).

after the cells were treated with 22.4 mM glucose for 48 h, and also high glucose-induced ABCA1 suppression was sensitive to p38-MAPK inhibitors. On the other hand, glucose-dependent insulinotropic polypeptide potentiated GSIS, insulin biosynthesis and β -cell proliferation and survival. Importantly, a previous report discovered that glucose-dependent insulinotropic polypeptide suppressed p38-MAPK and JNK via Akt-mediated changes in the phosphorylation state of apoptosis signal-regulating kinase 1 in INS-1 cells and human islets, resulting in the inhibition of its activity (Widenmaier *et al.* 2009).

Tangier disease (TD) is a rare autosomal recessive disease caused by mutations in the *ABCA1* gene. Patients with TD exhibit high-density lipoprotein deficiency, resulting in the accumulation of cholesteryl esters in many tissues, such as liver, tonsils, spleen, gastrointestinal mucosa, lymph nodes and peripheral nerves (Mott *et al.* 2000). Furthermore, ABCA1 is expressed in pancreatic β -cells (Brunham *et al.* 2007), suggesting that ABCA1 deficiency, along with abnormal lipid rafts, might lead to the dysfunction of pancreatic β -cells in TD patients. Koseki *et al.* reported that all patients with TD suffered from type 2 diabetes. A characteristic of diabetes in TD patients might be the extremely low insulinogenic index value, suggesting that ABCA1 plays a critical functional role in insulin secretion from β -cells (Koseki *et al.* 2009). In the current study, INS-1 cells treated with TNF- α suppressed ABCA1-decreased insulin secretion in response to changes in the glucose concentration.

In summary, our studies show that TNF- α suppressed the expression of endogenous ABCA1 in pancreatic islets and INS-1 cells. These findings raise the possibility that TNF- α may affect insulin secretion by controlling ABCA1 expression in pancreatic β -cells, which may be of therapeutic value in the treatment of diabetes mellitus.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

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