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Syndecan-4 is regulated by IL-1 β in β -cells and human islets

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ABSTRACT

Syndecans (SDC) are important multifunctional components of the extracellular matrix mainly described in endothelial cells. We studied the expression and regulation of SDC in cultured MIN6B1 cells and pancreatic islets. qRT-PCR revealed that syndecan-4 (SDC4) was the predominant isoform expressed in MIN6B1 cells and islets compared to other forms of SDC. Immunofluorescence in mouse and human pancreas sections revealed that SDC4 is mainly expressed in β -cells compared to other pancreatic cells. Exposure of MIN6B1 and human islets to IL-1 β dose-dependently induced a rapid and transient expression of SDC4 while SRC and STAT3 inhibitors decreased this effect. Exposure of human islets to IL-1 β caused an increase of SDC4 shedding, however treatment with STAT3 and SRC inhibitors inhibited this effect. These results indicate that SDC4 is upregulated by IL-1 β through the SRC-STAT3 pathway and this pathway is also involved in SDC4 shedding in islets.

1. Introduction

Type 1 diabetes (T1D) is a chronic autoimmune disease resulting from a selective destruction of pancreatic β -cells leading to an individual's inability to regulate their glycaemia. This disease is characterized by an infiltration of pancreatic islets by lymphocytes and other inflammatory cells (Donath et al., 2003). Even though the precise role of these cells in the T1D initiation is not yet understood, the activation of inflammatory cells by several antigens, synthesized in part by pancreatic islet β -cells, seems to be crucial for the disease progression. Extravasation of inflammatory cells from the postcapillary venules surrounding the islets and their subsequent infiltration leads to β -cell destruction. Mechanisms involved in inflammatory cell-mediated β -cell destruction are only partially understood. It is known that inflammatory cells around islets mediate their effects through interactions with the extracellular matrix, direct cell-to-cell contact with islet cells and via soluble mediators. However finer mechanistic studies are crucial prior to further investigation into therapies that target molecules involved in the activation or modulation of inflammation in islets.

The interface between endocrine islet cells and blood vessels is composed of an interstitial matrix and a basement membrane (BM), which play an important role in β -cell function and survival (Miao et al., 2013; Pinkse et al., 2006). BM consists of a collagen IV network,

which forms a scaffold with other BM components including laminins and heparan sulfate proteoglycan (HSPG) (Yurchenco, 2011). HSPGs are composed of a core protein and glycosaminoglycan heparan sulfate (HS) chains. They cooperate with adhesion molecules to facilitate cell attachment and cell-cell interactions, and bind chemokines, cytokines, and growth factors (Bernfield et al., 1999).

Among the HSPGs, syndecans (SDC) are a family of transmembrane proteoglycans and constitute the major source of cell surface HS on all cell types (Bernfield et al., 1999; Alexopoulou et al., 2007; Gotte, 2003). The SDC family is composed of four members, SDC1, -2, -3 and -4. SDC1-3 expressions are rather tissue specific whereas SDC4 expression is ubiquitous (Oh and Couchman, 2004). SDC have three distinct domains: an ectodomain where several HS chains are attached, a single transmembrane domain and a short cytoplasmic domain. The SDC ectodomain mediates several cell-cell and cell-matrix interactions via the HS chains. The SDC ectodomain is constitutively shedding in some cultured cells, as part of normal turnover, but this shedding is increased in pathophysiological conditions such as inflammation (Visse and Nagase, 2003; Page-McCaw et al., 2007). The release of SDC ectodomain can downregulate the signal transduction and convert the membrane-bound receptors into soluble effectors or agonists. Soluble SDC ectodomain can also compete with intact SDC for extracellular ligands in the pericellular environment (Manon-Jensen et al., 2010). The

Abbreviations: SDC, Syndecan; T1D, Type 1 Diabetes; HSPG, Heparan Sulfate Proteoglycan; HS, Heparan Sulfate; MMP, Matrix MetalloProtease; PP, Pancreatic Polypeptide; HUVEC, Human Umbilical vein endothelial cells; CMRL, Connaught Medical Research Laboratories

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shedding is a highly regulated process that requires the direct action of enzymes such as matrix metalloproteases (MMPs). MMPs are involved in many physiological processes and are important in normal matrix turnover but also have an essential role in pathological tissue remodelling during inflammatory disease (Manon-Jensen et al., 2010). Many studies show that SDC4, a particular isoform of SDC, plays an important role in various inflammatory processes. Silencing experiments in synoviocytes showed that SDC4 plays a role in initiating the inflammation in rheumatoid arthritis (Cai et al., 2019). In mice, intestine SDC4 expression is reduced during colitis and the loss of SDC4 expression aggravates colitis symptoms (Frohling et al., 2018). Inflammation increases expression of SDC4 and shedding of its ectodomain in cardiomyocytes and fibroblasts. Cardiac inflammation induced by lipopolysaccharide correlates with shedding of the SDC4 ectodomain (Strand et al., 2015). Furthermore, it has been shown that the inflammation observed in atrial fibrillation is promoted by SDC4 shedding (Wu et al., 2015). In vitro, endothelial cells exposed to inflammatory mediators, such as interleukin-1 beta (IL-1 β) and tumour necrosis factor alpha (TNF α), undergo a rapid expression of SDC4 (Okuyama et al., 2013; Vuong et al., 2015). It is not well understood if increased expression of SDC4 has protective or worsening effects on tissues prone to inflammatory events. Considering that inflammation is above all a response to damage, which prevents the spreading of the injury, production of SDC4 and/or its shedding may be also involved in these healing mechanisms. To this regard, different experimental observations demonstrate the beneficial effects of SDC4. Particularly suggestive are results obtained with SDC4 knockout (SDC4 $^{-/-}$) mice. First, injection of LPS into SDC4 $^{-/-}$ mice results in increased mortality compared to control mice injected with LPS (Ishiguro et al., 2001). Furthermore, intratracheal administration of LPS to control mice induces a rapid lung expression of SDC4 and pulmonary inflammation and, interestingly, the same treatment results in a higher pulmonary injury in SDC4 $^{-/-}$ mice. In vitro experiments show that LPS and TNF α increase the expression of SDC4 and IL-8 in macrophages and human bronchial epithelial cells, but when these cells are pretreated with SDC4 expression, the cytokines decrease. The authors suggest that syndecan-4 in the lung is produced in reaction to cytokines to limit the extent of inflammation and injury (Tanino et al., 2012).

HSPGs have been poorly studied in pancreatic islets. It has been suggested that HS has a role in postnatal islet maturation and β -cell function (Takahashi et al., 2009) and that HSPGs are part of the islet peri-capsule in mice. Regarding SDC4, only a couple of research papers described the expression of this molecule in islet cells, suggesting that it may be involved in the regulation of insulin secretion (Takahashi et al., 2018; Cheng et al., 2012). With regard to islet inflammation, it has been suggested that the peri-islet capsule containing HSPGs may function as a physical barrier against lymphocytes, but no report thoroughly explored the role of HSPGs or SDC in islet inflammation.

The purpose of this study was to better characterize expression of SDC isoforms in pancreatic islets, to investigate whether SDC expression is affected in response to inflammatory conditions and to gain an insight into the molecular mechanism involved in its regulation.

2. Material and methods

2.1. MIN6B1 cell culture

MIN6B1 cells, a subclone of the parental MIN6 insulinoma cell line (Miyazaki et al., 1990), were cultured as previously described (Lilla et al., 2003).

2.2. Animals

Two-month-old male C57BL/6 mice were purchased from Janvier (Le Genest-St-Isle, France). All animals were kept in the animal facilities at the University of Geneva with free access to food and water. All

experiments were conducted under protocols reviewed and approved by the University of Geneva Institutional Animal Care and Use Committee.

2.3. Human islet isolation and culture

Human islets were isolated between 2014 and 2016 from brain-dead multiorgan donors using Ricordi's automated method (Ricordi et al., 1988) with local modifications (Bucher et al., 2005). Islets were used for research only if not suitable for transplantation. Experiments with human islets were approved by our local institutional ethical committee, and islet preparation expenses were in part supported by the European Consortium for Islet Transplantation (ECIT), sponsored by the Juvenile Diabetes Research Foundation. After isolation, islets were incubated in CMRL 1066 medium containing 5.6 mM glucose and supplemented with antibiotics, HEPES and 10% FCS, first at 37 °C for 18–24h and subsequently at 24 °C before being used in experiments.

2.4. Islet and MIN6B1 cell treatment with IL-1 β and inhibitors

Aliquots of 1000 human islets in 1 ml CMRL or 3×10^5 MIN6B1 cells in 1 ml DMEM were incubated in the absence or presence of 0.1–10 ng/ml recombinant human (cross reactivity with mouse) IL-1 β (Prepro Tech, London, UK), added to culture media, for 2, 4, 8 and 24h. To inhibit specific signalling pathways, islets or cells were pretreated (before IL-1 β treatment) either 1h with 5 μ M BAY-117085 ((E)3-[(4-*t*-Butylphenyl)sulfonyl]-2-propenenitrile) or 10 μ M SM-7368 (3-Chloro-4-nitro-N-(5-nitro-2-thiazolyl)-benzamide) (Calbiochem, San Diego, USA) or 4h with 5 μ M WP1066 (S,E)-3-(6-Bromopyridin-2-yl)-2-cyano-N-(1-phenylethyl)acrylamide, 10 μ M IDR-E804 (indirubin derivative E804) or 1 μ M DBI 2-(1,1-Dimethylethyl)-9-fluoro-3,6-dihydro-7H-benz[h]-imidaz[4,5-f]isoquinolin-7-one (Calbiochem, San Diego, USA) or 10 μ M PD98059 (2'-Amino-3'-methoxyflavone) or 5 μ M NFAT inhibitor (11R-VIVIT).

2.5. Pancreas sections and immunofluorescence

C57BL/6 mice were euthanised and their pancreata harvested. These pancreata and small biopsies of human pancreata processed for islet isolation were fixed 24h in 10% neutral buffered formalin. Then tissue was dehydrated, embedded in paraffin and sectioned at 5 μ m. Sections were deparaffinized, rehydrated with a series of alcohol solutions of decreasing concentrations, washed with phosphate buffer saline (PBS) and treated for 15 min with citrate buffer. They were washed again with PBS and successively treated for 10 min with 0.5% Triton X100 in PBS and 30 min with 0.5% BSA in PBS at room temperature. Then, sections were exposed for 2 h to different primary antibodies, used in combination as indicated in the "Results" section. Primary antibodies used were a rabbit anti-SDC4 H-140 antibody (1:50, sc15350, Santa Cruz Biotechnology, Dallas, Texas, USA), a rabbit anti-SDC4 antibody (1:100, abcam 24511, Abcam, Cambridge, UK) a guinea pig anti-insulin antibody (1:200; Dako, Carpinteria, CA, USA), mouse anti-glucagon antibody (1:100, Sigma Aldrich, St. Louis, Missouri, USA), a goat anti-pancreatic polypeptide antibody (1:150, ab77192, Abcam, Cambridge, UK), and a rat anti-somatostatin M09204 antibody (1:100, ab30788, Abcam, Cambridge, UK). Then, sections were rinsed and exposed for 1 h to adequate combinations of different fluorescent-conjugated secondary antibodies, following manufacturer instructions. Finally, sections were rinsed and mounted for microscopic analysis.

2.6. Microscopic analysis

Microscopic sections were analyzed using a confocal laser scanning, LSM800 (Carl Zeiss microscopy, GmbH). Images were recorded using ZEN 2.3 software for microscopy.

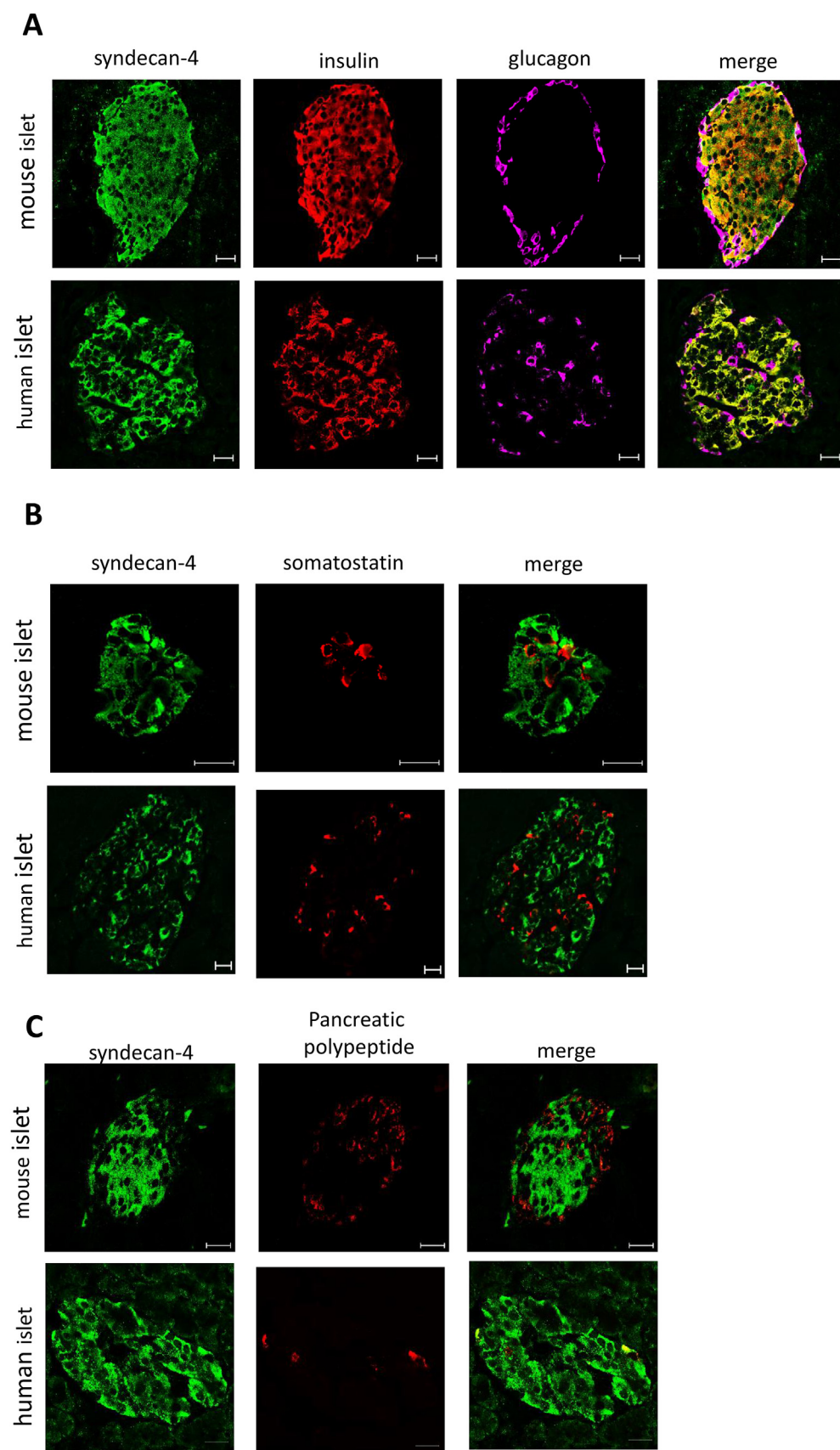


Fig. 1. Localization of SDC4 in human and murine islets.

(A) Representative confocal images of mouse and human pancreatic sections showing islets immunostained for SDC4 (green), insulin (red) and glucagon (pink). Merged images are shown in the right panels (Merge). (C) Representative confocal images of mouse and human pancreatic sections showing islets immunostained for SDC4 (green) and somatostatin (red). (D) Representative confocal images of mouse and human pancreatic sections showing islets immunostained for SDC4 (green) and PP (red). The colour was done using the Zen image editor software, Carl Zeiss Microscopy GmbH. Scale bar, 100 μ m.

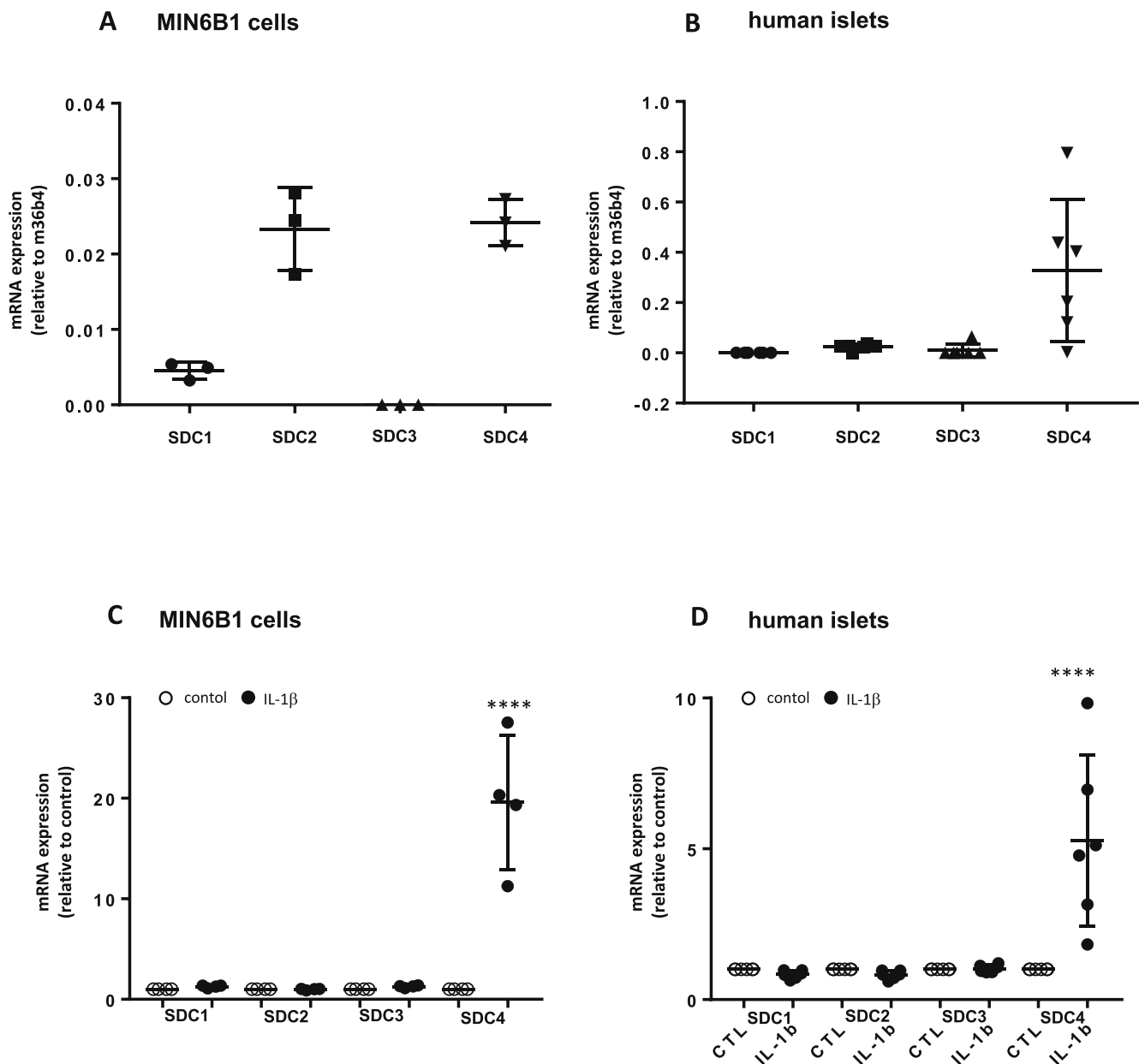


Fig. 2. Expression of SDC isoform in MIN6B1 cells and human islet.

(A and B) Gene expression levels of *Sdc* family in MIN6B1 (A) and human islets (B) were analyzed by qRT-PCR and normalized to the internal control genes, $n = 3$. (C and D) MIN6B1 (C) and human islets (D) were treated with IL-1 β (1 ng/ml; 2h) and gene expression levels of *Sdc4* were analyzed by qRT-PCR and normalized to the internal control genes *36b4* (MIN6B1) or *RPLP0* (human islets), $n = 3$. Data are presented as the means \pm SD, ANOVA one-way; **** $p < 0.0001$.

2.7. Quantitative real-time PCR

After the various treatments with IL-1 β and/or inhibitors, total RNA of human islets or MIN6B1 cells was isolated and purified by using RNeasy mini kit (Qiagen, Courtaboeuf, France) according to the manufacturer's instructions. RNA concentrations were measured using NanoDrop™. Equal amounts of RNA from each sample were then reverse transcribed in an adequate volume using High Capacity RNA to cDNA Reverse transcription kit (ThermoFischer Scientific, Waltham, Massachusetts, USA). For quantitative PCR, gene amplification was performed on a CFX96 connect™ system (Biorad, Hercules, California, USA) using qPCR Master mix for Sybr Green I (Eurogentec, Liege, Belgium), and primers (see below). In all conditions, the housekeeping genes *m36b4* or *RPLP0* were used as an internal control. The cycling conditions for the amplification reaction were as follows: initial denaturation at 95 °C for 5 min, followed by 40 cycles at 95 °C for 15 s and

60 °C for 1 min. The threshold cycles (Ct) for each sample, run in triplicate, were determined using the Bio-Rad CFX Manager. Murine and human mRNA data were normalized by *m36b4* and *RPLP0* respectively. The relative expression of target genes in treated samples compared to control samples was calculated using $\Delta\Delta Ct$ method (Livak and Schmittgen, 2001).

The following oligonucleotide primer sets were used:

sdcl-F(5'-GCGGCACCTTCTGTCATCAAA-3'), *sdcl*-R(3'-TGAAGTCTT GTTCTCCAGAGCC-5'), *sdcl*-F(5'-CTTCCCCAAAGTGAAACC-3'), *sdcl*-R(3'-GTCAGTTTCTTCAGGTGACTCAG-5'), *sdcl*-F(5'-GGACACC GCCACCAT-3'), *sdcl*-R(3'-AGCGTTGAGCCCCCG-5'), *sdcl*-F(5'-GCCTTGAGATGAGTCTGCCA-3'), *sdcl*-R(3'-ACTGGACTT AGAATGCCCC-5'), *36b4*-F(5'-TCACTGTGCCAGCTCAGAAC-3'), *36b4*-R(3'-AATTTCAAT GGTGCCTCTGG-5'),

SDC1-F(5'-GAGCTGAAAGGCCGGAA-3'), SDC1-R(3'-AGCTCCGCC TGCTTGG-5'),
 SDC2-F(5'-GTCCCTGGGGAATATGCGG-3'), SDC2-R(3'-GTCAGCTCT GCTCTCGACT-5'),
 SDC3-F(5'-GCTCGTAGCTGTGATTGTGG-3'), SDC3-R(3'-GCTGCCCT CATCTTTTCT-5'),
 SDC4-F(5'-CAAGGTGTCAATGTCCAGCA-3'), SDC4-R(3'-AGAGGAT GCCACGATGC-5'),
 RPLP0-F(5'-AAGGTGTAATCCGCTCCACAGA-3'),
 RPLP0-R(3'-TGCATCAGTACCCATTCTATCAT-5'),
 MMP9-F(5'-AGA CCT GGG CAG ATT CCA AA-3'),
 MMP9-R(5'-CGG CAA GTC TTC CGA GTA GT-3').

2.8. ELISA for Syndecan-4 and MMP9

After incubation of human islets in the absence or presence of IL-1 β and inhibitors, human syndecan-4 released in culture medium was analyzed using a human syndecan-4 ELISA kit (27188, IBL, Gunma, Japan) and a human MMP9 quantikine ELISA kit (DMP900, R&D Systems, Minneapolis, USA), according to the manufacturer's instructions.

2.9. Data and statistical analyses

Data are expressed as means \pm SD for n independent experiments. Statistical analyses were performed using GraphPad Prism 7 for Windows (GraphPad software, San Diego, USA) by one-way ANOVA or by Student's t -test. P -values less than 0.05 were considered significant.

3. Results

3.1. Distribution of SDC4 in pancreatic islets

To determine which islet cells expressed SDC4, human and mouse pancreas sections were analyzed by immunofluorescence using antibodies against SDC4, insulin, glucagon, somatostatin and pancreatic polypeptide (PP). By triple immunofluorescence for SDC4, insulin and glucagon, we observed that SDC4 co-localized with insulin and not with glucagon, in both mouse and human islets (Fig. 1A). Double staining for SDC4 and somatostatin (Fig. 1B) in mouse and human pancreas showed that SDC4 was not expressed in delta cells. Double staining for SDC4 and PP in mouse pancreas showed that SDC4 was not expressed in PP cells, but few cells showed a co-localization of SDC4 and PP in human pancreas (Fig. 1C). However, no staining was observed in either mouse or human pancreas exocrine tissue.

3.2. SDC expression in MIN6B1 and human islets

The relative expression of four SDC isoforms was investigated by quantitative RT-PCR analysis in MIN6B1 cell line and human islets. In MIN6B1, expression levels of *Sdc2* and *Sdc4* were similar and higher than that of *Sdc1* (Fig. 2A), while expression of *Sdc3* was not detected. In human islets, expression of *SDC4* was much higher than expressions of *SDC2* and *SDC3*, whereas expression of *SDC1* was undetectable (Fig. 2B).

3.3. IL-1 β upregulates SDC4 mRNA in MIN6B1 and human islets

To address the possible involvement of SDC4 in the inflammatory response, MIN6B1 and human islets were tested against exposer and non-exposure to IL-1 β for 2h and then analyzed for SDC mRNA. In response to IL-1 β the expression of SDC4 was up-regulated in both MIN6B1 and human islets (Fig. 2C and D). Interestingly, expressions of other SDC isoforms (SDC1-3) were not affected by IL-1 β . We further investigated whether the effect of IL-1 β on SDC4 expression was concentration- and/or time-dependent using MIN6B1. Exposure of these

cells for 2h to increasing concentrations of IL-1 β demonstrated a maximal effect at 1 ng/ml (Fig. 3A). Moreover, time course analysis performed with 1 ng/ml IL-1 β showed a maximum upregulation of *Sdc4* after 2h of treatment. An upregulation persisted for 4h and then vanished afterwards (Fig. 3B). Similar results were obtained when MIN6B1 were exposed to TNF α (supplementary figure 1).

3.4. Src-STAT3 pathway regulates SDC4 mRNA expression

To determine the mechanisms by which IL-1 β induces the regulation of SDC4, different specific inhibitors of pathways involved in IL-1 β signalling were tested. MIN6B1 and human islets were pretreated first in the presence of NF κ B inhibitor BAY-117085 or SM-7368 for 1h followed by stimulation with 1 ng/ml IL-1 β . Exposure of MIN6B1 and human islets to BAY-117085 or SM-7368 did not prevent the upregulation of *Sdc4* induced by IL-1 β (Fig. 4A and B). Similar results were observed when NFAT and ERK1/2 pathways were inhibited in MIN6B1 (Supplementary figure 2). In contrast, the inhibition of the STAT-3 pathway by WP1066 inhibitor decreased the upregulation of *Sdc4* induced by IL-1 β compared to the control (1.5-fold in MIN6B1 and 2.2-fold in human islets) (Fig. 4A–C). Exposure to SRC inhibitor (IDR-E804) also decreased upregulation of *Sdc4* compared to the control (2.7-fold in MIN6B1 and 6-fold in human islets). The inhibition of JAK by DBI inhibitor did not prevent the upregulation of *Sdc4* after IL-1 β stimulation (Fig. 4A–C). Taken together, these results indicate that the regulation of SDC-4 by IL-1 β is through the SRC-STAT3 pathway in MIN6B1 and in human islets.

3.5. IL-1 β induces shedding of cell surface SDC4 in human islets

To determine whether IL-1 β was involved in SDC4 shedding, human islets were exposed to 1 ng/ml IL-1 β for 2h and SDC4 released in the culture medium was analysed by ELISA. The level of extracellular SDC4 increased 3-fold after IL-1 β stimulation, compared to the islets without stimulation (Fig. 5A).

To determine whether the SRC-STAT3 pathway was involved in SDC4 shedding, islets were pretreated with STAT3, SRC or Janus kinase (JAK) inhibitors followed by exposure to 1 ng/ml IL-1 β for 2h. In the presence of STAT3 or SRC inhibitors SDC4 shedding was inhibited (Fig. 5A). These results indicate that the SRC-STAT3 pathway is involved in the SDC4 shedding in human islets.

3.6. IL-1 β upregulates MMP9 expression and release in human islets

To address the possible involvement of MMP9 in shedding of SDC4 in human islets, we analyzed whether MMP9 expression and release were also affected by IL-1 β exposure. We observed that after 2h exposure to IL-1 β , the expression of *MMP9* was up-regulated 1.8-fold, compared to the control (Fig. 5B). In addition, MMP9 quantified by ELISA was 1.5 higher in the human islets medium that was exposed for 2h to IL-1 β , compared to the control (Fig. 5C).

4. Discussion

In this study, we studied the expression and regulation of SDC in the MIN6B1 cell line and pancreatic islets. We showed that in MIN6B1 cells SDC4 are expressed at the same level than SDC2 and at a higher level than SDC1 and SDC3. Because in human islets SDC4 is more highly expressed relative to SDC1, SDC2 and SDC3, we mainly focused our study on this isoform. Given the reported correlation between SDC4 expression and inflammatory process (Okuyama et al., 2013; Vuong et al., 2015; Strand et al., 2013), we aimed to assess whether the expression of SDC in islets was affected by inflammatory events associated with diabetes, such as insulinitis and cytokine production.

A previous study has shown that SDC4 is present in β -cells and probably not in α -cells (Cheng et al., 2012). Here, we confirmed the

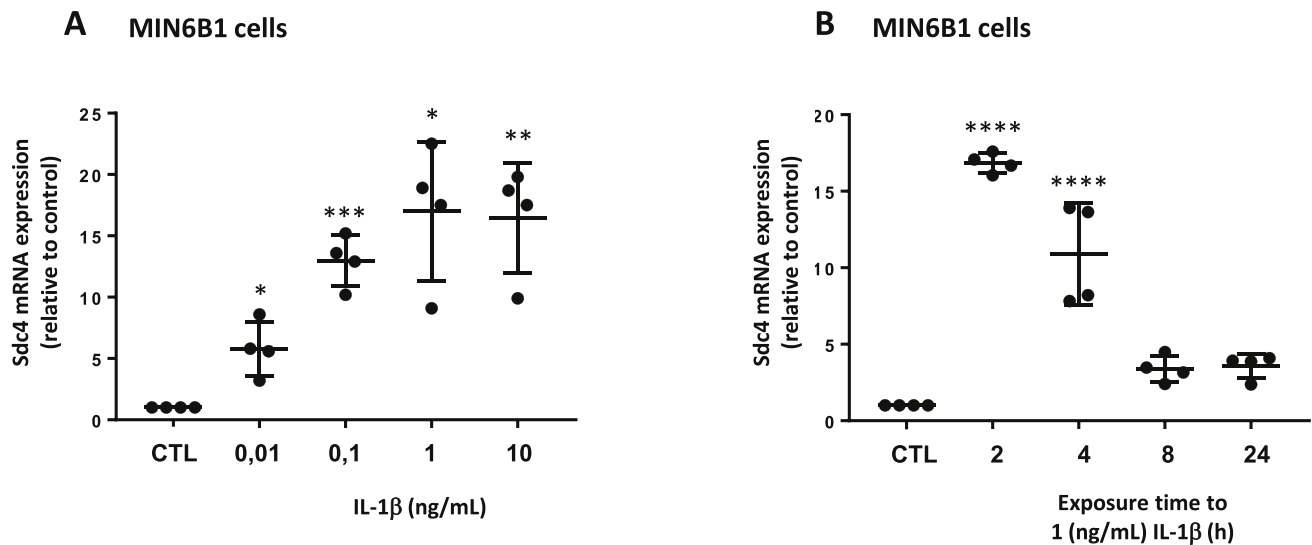


Fig. 3. IL-1 β up-regulated *Sdc4* mRNA in MIN6B1.

(A) MIN6B1 were treated with IL-1 β (0, 0.1, 1, 5 ng/ml; 2h) and *Sdc4* gene expression levels were analyzed by qRT-PCR, normalized to the internal gene *36b4* and expressed relative to the control, $n = 3$. (B) MIN6B1 were treated with IL-1 β (1 ng/ml; 2h, 4h, 8h and 24h), and *Sdc4* gene expression levels were analyzed by qRT-PCR, normalized to the internal gene *36b4* and expressed relative to the control (0 min), $n = 4$. Data are presented as mean \pm SD; ANOVA one-way; **** $p < 0.0001$, *** $p < 0.005$, ** $p < 0.01$; * $p < 0.05$.

presence of SDC4 in β -cells, and its absence in α -cells. We further demonstrated that SDC4 is undetectable exocrine pancreatic cells and other islet cells types, even if some few PP cells were positive for SDC4 immunostaining. In comparison with the work of Cheng et al. (2012), here we used a different combination of antibodies allowing co-staining of SDC4 and glucagon in the very same α -cells. The fact that SDC4 in the pancreas is mainly, if not only, expressed in β -cells suggests an important and specific role of SDC4 in these cells. Recently, a correlation between expression of SDC4 and secretory activity has been shown in the mouse beta cell line MIN6 (Takahashi et al., 2018), but no other functions were investigated in this cell line or in primary β -cells. In other systems, it was shown that SDC4 expression correlates with inflammatory processes. For instance, expression of SDC4 increases after exposure to pro-inflammatory cytokines IL-1 β and TNF α in cardiac myocytes, fibroblasts (Strand et al., 2013; Zhang et al., 1999), and human vascular endothelium-like cell line (Okuyama et al., 2013). SDC4 is also regulated by LPS and IL-1 β in human umbilical vein endothelial cells (HUVEC) (Vuong et al., 2015) and in glomerular endothelial cells (Reine et al., 2019) suggesting that the mechanism of SDC4 regulation is conserved. Our results show that in MIN6B1 and human islets SDC4 is affected by IL-1 β and TNF α . SDC4 expression is dose dependently upregulated by IL-1 β with a maximum effect at 1 ng/ml IL-1 β . The effect of IL-1 β on SDC4 expression is also time dependent. Indeed, the expression of SDC4 showed a rapid and strong response, with a 17-fold increase after 2h exposure to IL-1 β . Furthermore, the stimulation persisted after 4h, with a significant 11-fold increase expression relative to control, then after 8h expression of SDC4 returns to a basal level.

IL-1 β overproduction leads to a chronic inflammation and contributes to the pathogenesis of many inflammation and autoimmune diseases (Lukens et al., 2011). In T1D, IL-1 β production correlates with the recruitment of immune cells in islets and β -cell damages (Donath et al., 2003; Mandrup-Poulsen, 1996), but the exact link between these events and whether other components are involved are still unknown. Our hypothesis is that SDC4 production induced by IL-1 β may play a role in these events. In cardiomyocytes (Strand et al., 2013; Zhang et al., 1999) and in an endothelium cell line (Okuyama et al., 2013), the regulation of SDC4 by IL-1 β involves the transcription factor NF κ B. By contrast, our results show that NF κ B is not involved in the regulation of SDC4 by IL-1 β in MIN6B1 and in human islets. We rather found that the

transcription factor STAT3, via SRC kinase, is a candidate in this regulation. STAT proteins are activated by cytokine stimulation via JAK family proteins (Darnell, 1997) and can be activated by SRC in NIH3T3 cells (Cao et al., 1996; Turkson et al., 1998). Interestingly, STAT3 has been shown to be involved and regulated by IL-1 β in rheumatoid arthritis (Mori et al., 2011).

Our results show an increase of SDC4 shedding in human islets after 2h of treatment by IL-1 β , but not when the SRC-STAT3 pathway is inhibited. It is known that SDC4 ectodomain can be shed from the cell surface and this proteolytic process converts SDC4 from a cell surface receptor to a soluble HS effector. Once released, this soluble ectodomain can circulate and compete with cell surface binding (Sanderson et al., 2005). Shedding occurs in response to external triggers such as growth factors (Subramanian et al., 1997), chemokines (Li et al., 2002; Brule et al., 2006; Charnaux et al., 2006), insulin (Wang et al., 2009), heparanase (Yang et al., 2007) and cell stress (Fitzgerald et al., 2000). Shedding occurs also as a constitutive process in cultured cells (Fitzgerald et al., 2000). SDC4 shedding can be mediated by matrix metalloproteases such as MMP9 in endothelial cells (Ramnath et al., 2014), HeLa cells and human macrophages (Brule et al., 2006). Furthermore, a treatment by IL-1 β induces an increase of MMP9 expression (Reine et al., 2019; Cheng et al., 2010). Taken together, these results suggest that in islets IL-1 β induces SDC4 expression and its subsequent intracytoplasmic transportation to the cell and also MMP9 expression and release. This lead to cleavage and shedding of SDC4 resulting in the disruption of β -cell glycocalyx and thus their protection against lymphocyte infiltration. The fate of the SDC4 ectomain remains to clarify. In summary, our study highlights a new signalling Src-STAT3 pathway for SDC4 expression in the β -cell line and human islets. Even though the function of the SDC4 ectodomain after shedding is not yet understood, it will be interesting to investigate whether it can be used as a marker of T1D progression.

E.B and D.B designed the study and wrote the manuscript. E.B performed and analysed the experiment. M.A. and V.L. helped with the experiments. T.B. contributed to discussions and reviewed the manuscript. All authors approved the final version. D.B is the guarantor of this work.

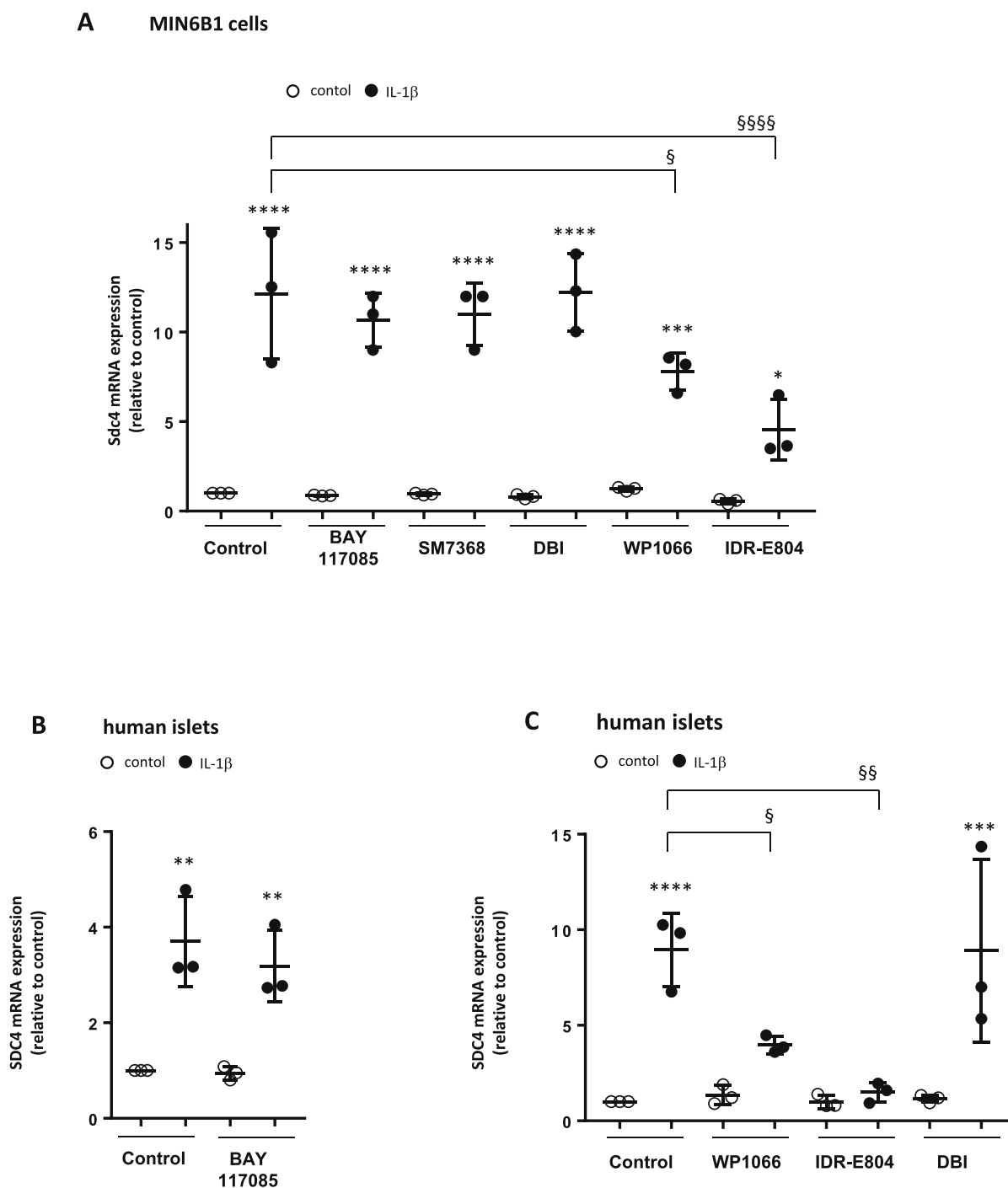


Fig. 4. SDC4 expression is regulated by SRC-STAT3 pathway.

(A)

MIN6B1 cells were preincubated with BAY117085 (5 μ M, 1h), SM7368 (10 μ M, 1), DBI (1 μ M, 4h), WP1066 (5 μ M, 4h) or IDR-E804 (10 μ M, 4h) then stimulated with IL-1 β (0–1 ng/ml; 2h) and *Sdc4* gene expression was analyzed by qRT-PCR, normalized to the internal gene *36b4* and expressed relative to the control, n = 3. (B and C) Human islets were preincubated with BAY117085 (5 μ M, 1h) (A) and with WP1066 (5 μ M, 4h), IDR-E804 (10 μ M, 4h) or DBI (1 μ M, 4h) (B) before stimulation with IL-1 β (0–1 ng/ml; 2h) and SDC4 gene expression was analyzed by qRT-PCR, normalized to the internal gene RPLP0 and expressed relative to the control, n = 3. Data are presented as mean \pm SD; ****p < 0.0001 vs. control, ***p < 0.005 vs. control, **p < 0.01 vs. control, §§§§p < 0.0001 vs. IL-1 β , §§§p < 0.005 vs. IL-1 β , and §p < 0.05 vs. IL-1 β .

Declaration of competing interestCOI

None.

CRediT authorship contribution statement

Estelle Brioude: Writing - original draft, Formal analysis. **Thierry Berney:** Writing - review & editing. **Domenico Bosco:** Writing -

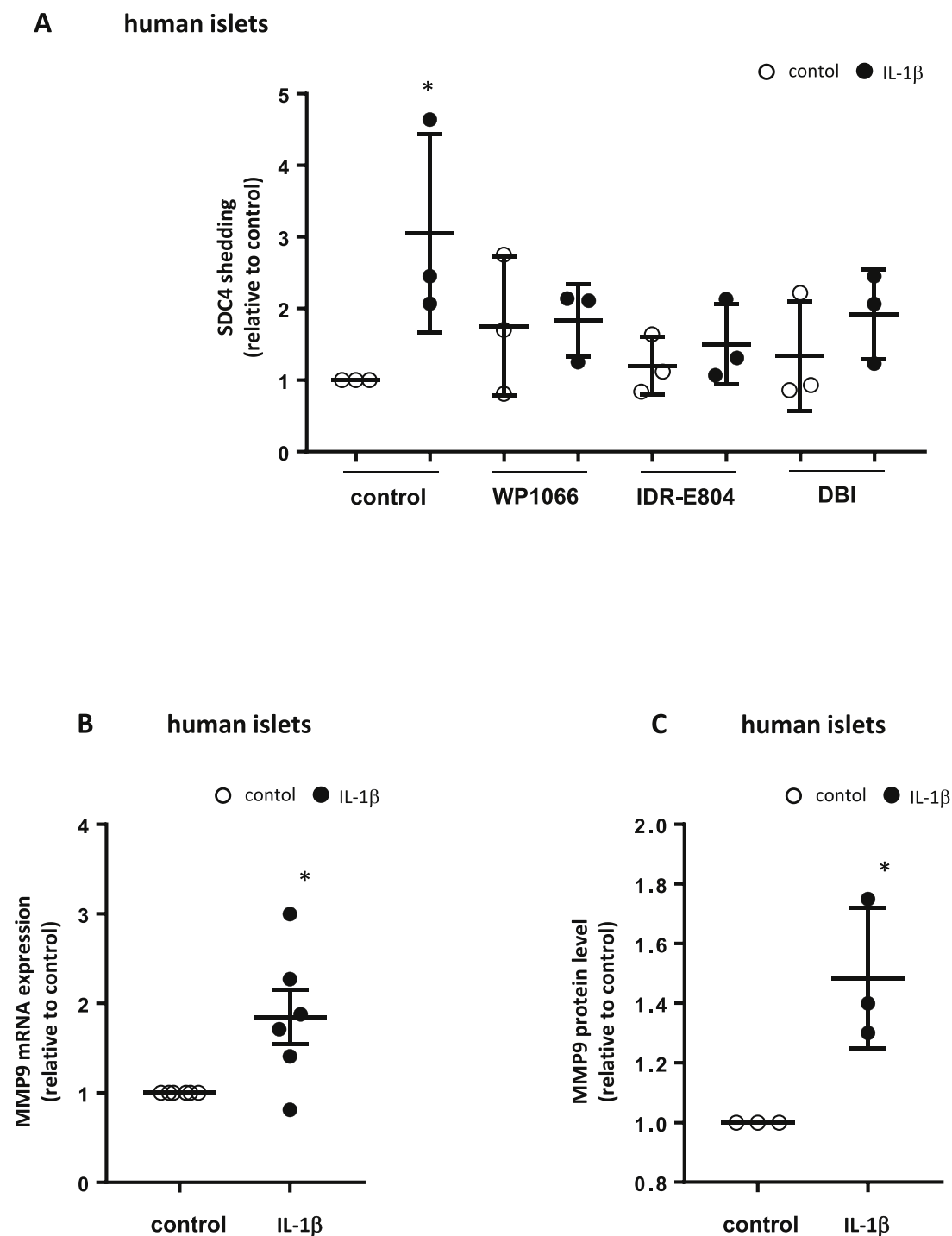


Fig. 5. IL-1 β induces SDC4 shedding in human pancreatic islets.

(A) Human islets were preincubated with WP1066 (5 μ M, 4h), IDR-E804 (10 μ M, 4h) or DBI (1 μ M, 4h) before stimulation with or without IL-1 for 2h and SDC4 shedding was measured by ELISA and normalized to the total protein content, $n = 3$. Data presented as mean \pm SD; ANOVA one-way, * $p < 0.05$. (B) Human islets were treated with or without IL-1 β for 2h and MMP9 gene expression level was analyzed by qRT-PCR, normalized to the internal gene RPLP0 and expressed relative to the control, $n = 3$. (C) Human islets were treated with IL-1 β and protein level was measured by ELISA and normalized to the total protein content, $n = 3$. Data presented as mean \pm SD; t -test, * $p < 0.05$.

original draft.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mce.2020.110815>.

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