CD36 regulates β cell differentiation and influences type 2 diabetic sepsis

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Research Article

Keywords: type 2 diabetes, type 2 diabetic sepsis complications, CD36, β -cells, defferentiation

Posted Date: August 31st, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-851347/v1>

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Abstract

Background

This study aimed to investigate the diagnostic function of CD36 in type 2 diabetic (T2DM) sepsis complications (T2DSC) and its effect on β -cell differentiation.

Methods

First, Age - and sex-matched T2DM patients, T2DSC patients and healthy people (50 cases each) were included. Quantitative polymerase chain reaction was used to measure CD36, FOXO1, PDX1, MAFA, insulin, SOX9, Neurog3 and NANOG expression in blood samples. Second, cultured human β -cell line EndoC- β H1 and the interference and overexpression of CD36. Cell clone, apoptosis, inflammatory cytokine, oxidative stress and β -cell differentiation related proteins were also analysed. Third, examined the role of CD36 in high glucose, LPS-induced β -cell.

Results

CD36 mRNA, and endocrine progenitor β -cell biomarkers SOX9, Neurog3 and NANOG were significantly increased in T2DM than control group, whereas the β -cell maturation biomarkers FOXO1, PDX1, MAFA and insulin were significantly decreased. Compared with the T2DM group, CD36 and FOXO1 were significantly increased in T2DSC, but PDX1, insulin, MAFA, SOX9, Neurog3 and NANOG were significantly decreased. The receiver operating characteristic curve revealed that CD36 was useful for distinguishing T2MD and T2DSC from the control group. Furthermore, CD36 overexpression increased β -cell apoptosis and the secretion of IL-1 β , IL-8 TNF- α , malondialdehyde and reactive oxygen species. CD36 induced cell defferentiation. Lastly, CD36 knockdown could inhibit the high glucose and LPS-induced cell apoptosis, inflammatory, oxidative stress and cell defferentiation.

Conclusion

Significant increase in CD36 can be used as a biomarker for T2MD and T2DSC. CD36 promotes T2MD or T2DSC development by inducing β -cell inflammatory and oxidative stress and defferentiation.

1 Introduction

The increasing rate of type 2 diabetes mellitus (T2DM) in many countries is attributed to the intake of many high sugar foods, sugar desserts, drinks or high-fat foods and threatens the health of individuals [1, 2]. Hyperglycaemia and pancreatic β -cell dysfunction are focused clinical indications of diabetes. β -cell apoptosis is an important factor for beta-cell loss and T2DM onset because an acquired reduction in β -cells results in failure to secrete sufficient insulin to compensate the body's demand [3]. However, β -cell

dedifferentiation leading to β -cell dysfunction plays an important role in T2DM [4, 5]. Oxidative stress, endoplasmic reticulum stress and inflammation can also lead to the dedifferentiation of mature β cells, which is an important factor in functional cell reduction and insulin secretion deficiency in patients with diabetes [6, 7].

Fatty acid translocase cluster determinant 36 (CD36), a scavenger receptor, is expressed in pancreatic β -cells and α -cells and regulates lipid metabolic and cell functions. Plasma CD36 expression in patients with T2DM increases significantly, especially in patients with a body mass index greater than 30 kg/m² [8, 9]. CD36 overexpression in the β -cells of obese donors with T2DM decreases insulin secretion, impairs exocytosis and reduces granule docking [8]. High glucose induces CD36 expression in β -cells [10–12]. The structure of CD36 contains two transmembrane domains, four palmitoylation sites and 10 glycosylation sites in the extracellular domain and two binding entrances called hydrophobic pocket and free fatty acid (FFA) transport [5]. High glucose exposure enhances CD36 membrane expression by activating the Rac1-NADPH oxidase (NOX) complex, which induces mitochondrial dysfunction, reactive oxygen species (ROS) production and β -cell death [10]. CD36 exacerbates the glucotoxic dysfunction of pancreatic β -cells by increasing FFA inflow, inducing intracellular peroxides and reducing insulin secretion and pancreatic and duodenal homeobox 1 (PDX-1) levels [11, 12]. Excessive FFA inflow further induces endoplasmic reticulum stress, oxidative stress, mitochondrial functional impairment and high ROS production, thereby culminating in β -cell apoptosis [13]. Elevated FFA concentration is closely related to T2DM [14]. FFA is mainly taken up by cells through CD36 mediation and can be up-regulated by insulin [13, 15, 16]. Pro-inflammatory biomarkers tumour necrosis factor- α (TNF- α), interleukin-1 beta (IL-1 β), IL-6 and IL-8 are positively associated with polyunsaturated fatty acid intake in patients with T2DM [17]. CD36 enhances the glucotoxicity, lipotoxicity and glucolipotoxicity of β -cell, thus leading to cell failure and insulin resistance and exacerbating diabetes and its complications.

Diabetes is associated with β -cell number and function failure. High glucose induces oxidative stress by increasing ROS and malondialdehyde (MDA) generation and decreasing superoxide dismutase (SOD) activity [18–20]. β -cell apoptosis is also induced by up-regulating cleaved caspase-1/3/9 and poly ADP-ribose polymerase 1 (PARP-1) [19, 20]. PARP-1 can weaken the DNA binding and transactivation of peroxisome proliferator activated receptor gamma (PPAR γ), and CD36 decreases the transcription of the target genes of PPAR γ [21]. CD36 inhibition blocks high glucose-induced caspase-3 activation and β -cell apoptosis [18]. Additionally, sepsis and acute inflammation stimulate PPAR γ to increase the expression of membrane CD36 and promote energy metabolism [22]. In β -cells, CD36 is expressed in the plasma membrane and insulin secretory granules [23]. Surface protein CD36 regulates inflammatory processes. High CD36 expression promotes the expression of IL-1, IL-6, TNF, interferon (IFN) and other cytokines [24] and acts as a central regulator to activate NLRP3 inflammasome and IL-1 β production [25]. CD36 also inactivates AKT by inducing ceramide accumulation and NF- κ B-TXNIP cascade of inflammatory signals [26]. AKT is a central mediator of insulin-stimulated anabolic metabolism [27]. Forkhead box-O1 (FoxO1) is an established downstream target of insulin signalling in phosphatidylinositol 3-kinase (PI3K)/AKT pathway. CD36 overexpression increases the nuclear localisation of FOXO1 by suppressing the PI3K/AKT

pathway, an important mechanism leading to reduction in exocytotic proteins and insulin secretion and impairment in exocytosis and granule docking [9].

FOXO1 is a multifunctional transcription factor with a potential role in T2DM metabolic stress and β -cell dedifferentiation. CD36-mediated fatty acid flux activates PPAR delta/beta to increase the expression of FOXO1, which in turn recruits CD36 to the plasma membrane and acts as reinforcement for fatty acid utilisation [28]. Multiplication of CD36 plasma membrane level is necessary for FOXO1 to promote fatty acid uptake and oxidation [29]. FOXO1 ablation causes hyperglycaemia and β -cell dedifferentiation but not β -cell death [4]. β -cell dedifferentiation is divided into differentiation into pancreatic endocrine progenitor cells and transdifferentiation into α -cell, both of which lead to reduction in β -cell mass and impairment of insulin secretion and/or insulin resistance in T2DM. PDX1, MAFA and insulin are the biomarkers of mature β -cells, and Neurog3, OCT4, NAGOG, and SOX9 molecules are the biomarkers of endocrine progenitor β -cells [30]. CD36 reduces insulin release by inhibiting PDX1 [11, 12] and induces β -cell glucotoxicity by increasing FOXO1 nuclear translocation to suppress MAFA expression [9, 31]. MAFA also induces Neurog3 positive endocrine precursors to β -cells by enhancing PDX1 and producing β -cells from α -cells [32]. Insulin therapy or PDX1 or MAFA expression promotes the conversion of α -cells to pancreatic β -cells [33]. However, FOXO1 knockdown promotes β -cell dedifferentiation to progenitor-like cell expressing Neurog3, OCT4 and NAGOG [4]. Neurog3 protein is specifically expressed in pancreatic progenitor cell and silenced after birth; thus, interfering Neurog3 prevents the generation of new insulin-positive cells [34]. Hyperglycaemia inhibits nuclear translocation and expression of FOXO1 and induces Neurog3 expression, which is necessary for the development and maintenance of pancreatic endocrine progenitor cells [33]. OCT4 and NAGOG are the characteristics of stem cells. Targeting lipid metabolism such as CD36, lipogenic transcription factor SREBP1c and fatty acid synthase may inhibit the stemness of stem cells [35]. Hyperglycaemia, inflammatory cytokines (IL-1, IL-6 and TNF- α) and oxidative stress enhance β -cell dedifferentiation [33]. CD36 may participate in the regulation of β -cell dedifferentiation by inducing oxidative stress and up-regulating cytokines.

In summary, CD36 may play a key role in β -cell oxidative stress, inflammation and dedifferentiation. Its elevation is associated with the increased production and accumulation of triglycerides (TG) and diacylglycerols, resulting in the reduced insulin sensitivity and onset of T2DM [36]. Deficiency in CD36 protects from sepsis by increasing granulocytic phagocyte survival and reducing peritoneal bacteria counts, systemic inflammation and organ damage [37]. However, the role of CD36 in type 2 diabetic sepsis complications (T2DSC) is unclear. EndoC- β H1 is extremely similar to human islet β cell, especially in terms of insulin secretion stimulated by glucose and incretin [38]. Therefore, EndoC- β H1 cells are used as a screening platform for the identification of novel antidiabetic drug candidates. In this study, the expression and molecular function of CD36 in T2DM and T2DSC was tested in clinical patients and human EndoC- β H1 cell line, respectively. Results showed that the increased CD36 in T2DM and T2DSC might induce β -cell oxidative stress, inflammatory response, apoptosis and dedifferentiation. Therefore, CD36 could be a key molecule to regulate T2DM and sepsis.

2 Material And Methods

2.1 Patients

A total of 100 patients with T2DM and T2DSC at the Ming Dong Hospital Affiliated to Fujian Medical University were included from February 20, 2019 to January 15, 2020. Fifty patients with T2DM aged 40–84 years have a course of 0.2–14 years and a body mass index (BMI) of 21.71–23.04 kg/m². Fifty patients with T2DSC have infections in respiratory system, urinary system or digestive system, and their age was 44–85 years with a BMI of 20.79–22.07 kg/m². Fifty healthy people aged 40–84 years with BMI of 22.75–24.23 kg/m² were recruited from the Department of Medical Examination Center at Ming Dong Hospital Affiliated to Fujian Medical University, Ningde City. The healthy control patients were without sepsis, T2DM or metabolic syndrome.

All patients with T2DM fulfilled the ‘Guidelines for the Prevention and Treatment of Type 2 Diabetes in China (2017 Edition)’ [39] and were treated with metformin or a combination of glibenclamide and metformin. Specifically, these individuals have typical symptoms of diabetes (polydipsia, polydipsia, polyuria, polyphagia and unexplained body weight drop) and fasting plasma glucose (FPG) ≥ 7.0 mmol/L, or random plasma glucose (RPG) ≥ 11.1 mmol/L. The exclusion criteria for T2DM group included a history of cardiovascular events, cancer, acquired immunodeficiency syndrome, kidney or liver disease, pregnancy, smoking, substance abuse and alcohol consumption. Sepsis was diagnosed following the criteria of ‘The Third International Consensus Definitions for Sepsis and Septic Shock’ [40], specifically the ‘Quick Sequential [Sepsis-related] Organ Failure Assessment (qSOFA) criteria: respiratory rate ≥ 22 /min, altered mentation and systolic blood pressure ≥ 100 mm Hg (range, 0–3 points, with 1 point each). T2DSC treatment followed the ‘Guidelines for the Emergency Treatment of Sepsis/Septic Shock in China (2018)’, that is, administration with crystal solution for early resuscitation, subsequent volume replacement therapy, use of carbapenems or a combination of broad-spectrum penicillin/ β -lactamase inhibitors for anti-infective therapy and de-escalation therapy after clinical symptoms have been sufficiently improved. The exclusion criteria for T2DSC group included long-term immune-suppressive medication, chronic or acute active rheumatoid inflammatory disease, drug or alcohol abuse and pre-existing essential haemodialysis.

This study was approved by the Ethics Committee of the Ming Dong Hospital Affiliated to Fujian Medical University. The batch number was [2019] NingMin Medical Ethics No (0116-2). Written informed consent was obtained from all participants, and the experiment was conducted in compliance with the World Medical Association Declaration of Helsinki (2013).

2.2 Blood sample collection

Blood samples were collected from the patient’s cubital vein (10 mL) by using Monovette Sarstedt tubes (Sarstedt, Nümbrecht, Germany). The blood was allowed to stand at room temperature for 1–2 hours, and the serum was then separated through centrifugation (3000 rpm, 10 min, room temperature). Biochemical indexes, such as cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C) and high

density lipoprotein cholesterol (HDL-C), were analysed immediately by using an auto analyser (Hitachi 912 Autoanalyser, Germany). The other serum samples were stored at -80°C for further analyses.

2.3 Quantitative polymerase chain reaction (qPCR) assay

Total RNA was isolated by the TRIzol (Invitrogen, USA) method. Primers were resuspended by adding RNase free water. Reverse transcription was performed by using the PCR Nucleotide Mix (Promega, USA). The qPCR mixtures were prepared on ice using a previous method and assayed by the ABI 7500 PCR system (ABI, USA). The reaction conditions were 42°C for 60 min, followed by cooling to 4°C.

For qPCR, a final volume of 25 µL was used including 2.0 µL of reverse transcription product, 12.5 µL of SYBR Green qPCR Master Mix (A6001, Promega, USA), 2.0 µL of forward and reverse primers and 9.5 µL of nuclease-free water. Forty cycles of PCR amplification with initial incubation at 95°C for 10 min and final extension at 72°C for 5 min were performed. Each cycle comprised denaturation at 95°C for 10 s, annealing at 60°C for 30 s and extension at 72°C for 30 s. The mRNA expression levels of CD36, FOXO1, PDX1, MAFA, insulin, SOX9, Neurog3 and NANOG were normalised to GAPDH and calculated by the $2^{-\Delta\Delta C_t}$ method.

The primers were designed and synthesised by Sangon Biotech (Shanghai, China). The gene primer sequence is as follows: CD36 primer AACCACACACTGGGATCTGAC (F) and CTGCAGGAAAGTCCTACACTG (R), FOXO1 primer GGCGTCCGTCCGTCCTTC (F) and CTTAACTTCGCGGGG CCATC (R), PDX1 primer GTTCCGAGGTAGAGGCTGTG (F) and TGAAGGTC ATACTGGCTCGTG (R), MAFA primer CTCTTTGGACTAGCCGGGAG (F) and CTCCGAAAACGGGCAGATCC (R), insulin primer TCAGAAGAGGCCATC AAGCAG (F) and CGCACAGGTGTTGGTTCAC (R), SOX9 primer GGAGACTTC TGAACGAGAGCG (F) and CCGTTCTTCACCGACTTCCTC (R), Neurog3 primer CCGGTAGAAAGGATGACGCC (F) and GGTCACTTCGTCTTCCGAGG (R), NANOG primer CAATGGTGTGACGCAGGGATG (F) and TGCACCAGGTCTG AGTGTTTC (R) and GAPDH primer GTCATCCCTGAGCTGAACGG (F) and CCACCTGGTGCTCAGTGTAG (R).

2.4 Cell culture and transfection

Human EndoC-βH1 cell lines were purchased from Zolgene Biotechnology Co., Ltd (Fuzhou, China) and placed in the coated vessels with Matrigel/fibronectin (100 µg/mL + 2 µg/mL). The cells were cultured in DMEM (Gibco, USA) medium (5.6 mM glucose + 2% BSA + 10 mM nicotinamide + 50 µM β-mercaptoethanol + 5.5 µg/mL transferrin + 6.7 ng/mL sodium selenite + 100 IU/mL penicillin + 100 µg/mL streptomycin) at 37°C in a humidified atmosphere with 5% CO₂ incubator (SANYO, Osaka, Japan). T2DM model was prepared using 30 mM high glucose DMEM medium incubation for 24 hours, and T2DMS model was established by applying 30 mM high glucose DMEM medium and 10 µg/mL LPS induction 24 hours. High glucose and LPS reduced the number and masses of EndoC-βH1 cells.

shRNA sequences targeting CD36 were designed according to CD36 gene information to inhibit CD36 expression, and an unrelated sequence was designed for negative control. The shRNA sequence is the top strand 5'- CACCGCACACAGCT- GTATCCAAATCGAAATTTGGATACAGCTGTGGTGC-3' and the bottom

strand 5'-AAAAGCACACAGCTGTATCCAAATTTGATTGGATACAGCTGTGGTGC-3' and it was synthesised by Sangon Biotech (Shanghai, China). The single-stranded shRNA upstream and downstream fragments were annealed to form a double-stranded DNA fragment to establish a carrier of pLVX-shRNA2-Puro-CD36. The overexpression CD36 carrier of PcDNA3.1-CD36 was established. All plasmids were provided by Zolgene Biotechnology Co., Ltd (Fuzhou, China). The cells with a density of 5×10^5 were cultivated in a six-well plate, cultivated to a density of 60–70% and transfected by Lipofectamine™2000 Transfection Reagent (Invitrogen, USA). The shRNA plasmid-control and overexpression vector (Zolgene Biotechnology Co., Ltd, Fuzhou, China) was used. After 48 hours, the transfected cells were harvested for the next analysis.

2.5 Cell viability assay

CCK-8 kit (CK04, Dojindo) was used to assay the cell viability. Concentration of 1×10^5 cells/mL cells were adjusted in 96-well plates. After transfection and high glucose or/and LPS intervention, the cells were treated with 100 μ L of 10% CCK-8 reagent at 0, 24, 48, and 72 hours and cultured at 37°C for an additional 1–2 hours. Optical density (OD) was detected at 450 nm wavelength using a Microplate Reader (PERLONG Co., Ltd., Beijing, China).

2.6 Cell apoptosis examination

A total of $1 \cdot 10^5$ cells/well were seeded in 24-well plates. The cells were harvested after plasmid transfection or high glucose and LPS intervention. Cell apoptosis was assayed by using the Annexin V-ALEXA 647/PI Kits (CA1040, Beijing Solaibao Technology Co., Ltd., China) according to the specification and calculated the apoptosis rate in each group by flow cytometry (Beckman Coulter, Brea, USA) analysis.

2.7 Cytokine assay

A total of $3 \cdot 10^5$ cells/well were seeded in six-well plates. Cell culture medium was collected after plasmid transfection or high glucose and LPS intervention. The medium was then centrifuged at 3000 rpm/min for 10 min at 4°C to remove floating cells and cell debris, and the supernatant was collected. The levels of IL-1 β , IL-8, TNF- α and insulin were detected by ELISA following the manufacturer's instructions. The ELISA kit details were as follows: human IL-1 beta ELISA kit (EK0392, Boster Biological Technology Co., Ltd., USA), human IL-8 ELISA kit (EK0413, Boster Biological Technology Co., Ltd., USA), human TNF α ELISA kit (EK0525, Boster Biological Technology Co., Ltd., USA), and human Insulin ELISA kit (SEKH-0219, Solaibao, China). The OD value was read by a microplate reader at 450 nm wavelength (PERLONG Co., Ltd., Beijing, China).

2.8 Oxidative stress level analysis

A total of $3 \cdot 10^5$ cells/well were seeded in six-well plates. After plasmid transfection or high glucose and LPS intervention, the cells were collected, 5 million cells were counted and 1 mL of extract was added. The cells were crushed by ultrasonic waves at a power 20%, 3 s ultrasonic with 10 s interval repeated for 30 times. The cells were then centrifuged at 4°C and 9000 r/min for 10 min, and the supernatant was

obtained. MDA content was detected by micro malondialdehyde (MDA) assay kit (BC0025, Solaibao, China). The OD value was read by a microplate reader at 450, 532 and 600 nm wavelength.

The cells were collected and incubated in DCFH-DA following the manufacturer's instructions of Reactive Oxygen Species Assay Kit (CA1410, Solaibao, China). The fluorescent signal was measured by a microplate reader at 525 nm wavelength (FLx800, BioTek, USA).

Approximately 2 million cells were collected and added with 1.0 mL of pre-cooled extract. Ultrasound was used to fully break the cells and release the antioxidants. Cytochylema was centrifuged at 4°C 10000 r/min for 5 min, and the supernatant to be measured was obtained. Total antioxidant capacity was detected by Micro Total Antioxidant Capacity (T-AOC) Assay Kit (BC1315, Solaibao, China), and the signal was measured by a microplate reader at 593 nm wavelength.

2.7 Western blot analysis

The protein was extracted from EndoC-βH1 cells with RIPA buffer including the PMSF for 20 min on ice. Centrifugation was conducted at 12,000 rpm for 20 min at 4°C, and the supernatant was obtained. Protein concentration was calculated using the bicinchoninic acid protein assay kit (Epizyme, China). A certain amount of extracted protein was subjected to 10% sodium dodecyl sulphate-polyacrylamide gel step and then immediately transferred on polyvinylidene difluoride membrane (Millipore). After being blocked with 5% BSA for 2 hours, the blots were incubated with the primary antibody for CD36, FOXO1A, PDX1 MAFA, Neurogenin3 (NGN-3), SOX9, cleaved caspase 3, cleaved PARP 1, AKT1 (phospho S473), NADPH oxidase 4 (NOX4) and insulin and β-actin (1:1000, Abcam, England) at 4°C. The HRP-conjugated secondary antibody (1:500, Protein-Tech, USA) was incubated for 2 hours. The signals were detected by chemiluminescence reagents (Thermo, USA). The results were collected by the Versa Doc™ imaging system (Peiqing Technology Co., Ltd., Shanghai, China) and analysed with Image J software.

2.8 Statistical analysis

SPSS 22.0 statistical software was used for data analysis. If the data followed normal distribution, then Student's t-test was used to compare between two groups. One-way ANOVA was employed to compare the differences between multiple groups. If the data did not follow normal distribution, then Mann–Whitney U test was used to compare between two groups. Kruskal–Wallis H (K) test was applied to compare the differences between multiple groups. Chi-square test was performed to analyse counting data. The correlation of CD36 concentrations with β-cell differentiation related genes was determined by Spearman correlation analysis. The clinical accuracy of significant CD36 was assessed using receiver operator characteristic (ROC) analysis, and the area under the curve (AUC) was calculated by DeLong's method. $P < 0.05$ indicated significant difference.

3 Results

3.1 Patients' characteristics

Clinical data for the control, T2DM and T2DSC groups are presented in Table I. A total of 100 patients with T2DM and 50 control cases were recruited. The T2DM group consisted of 46% males with an average age of 62.52 ± 1.72 years. The T2DSC group comprised 42% males with an average age at 65.10 ± 1.53 years. The control group included 50% males with an average age at 61.88 ± 1.75 years. No significant differences in age and sex status was found among the control, T2DM and T2DSC groups ($P = 0.355$, $P = 0.725$, respectively). Fasting blood sugar (FBS), glycated haemoglobin (HbA1c), white blood cell count (WBC), erythrocyte sedimentation rate (ESR), thrombocytocrit (PCT) and C-reactive protein (CRP) gradually increased in control, T2DM and T2DSC groups ($P < 0.001$, respectively). However, body mass index (BMI) was gradually decreased in control, T2DM and T2DSC groups ($P < 0.001$). Total cholesterol (TC) and high-density lipoproteincholesterol (HDL-C) were the highest in T2DM group, followed by control and T2DSC groups. No significant difference in the level of low-density lipoprotein cholesterol (LDL-C) was observed among these three groups ($P = 0.111$). The triglyceride (TG) level in T2DM group was higher than that in T2DSC and control groups ($P < 0.001$).

The disease course for T2DM was up to 7.80 (3.75–11.72) years, and that for T2DSC was up to 8.95 (3.62–11.20) years. No significant difference in the course of diabetes was found between the two groups ($P = 0.654$). Hypertension was the most common complication of T2DM (64%) and T2DSC (58%), followed by nephropathy (36% and 26%, respectively). Approximately 16% of patients with TT2DSC who developed diabetic ketoacidosis. Urinary system was the most common infection area of patients with TT2DSC (19 patients, 38%), followed by respiratory system (22%), digestive system (14%) and skin (12%). The Acute Physiology and Chronic Health Evaluation (APACHE-II) score of patients with TT2DSC was 24.62 ± 0.41 , and the median of Quick Sequential [Sepsis-related] Organ Failure Assessment (qSOFA) was 2 (interquartile range: 1–2).

3.2 Gene expression levels of CD36 and β -cell differentiation-related genes

Figure 1A shows the expression of CD36 and β -cell differentiation-related genes in the serum of patients. The mRNA expression levels of CD36, SOX9, Neurog3 and NANOG were significantly increased in the T2DM group than in the control group ($P < 0.01$), whereas those of FOXO1, PDX1, MAFA and insulin were significantly decreased ($P < 0.01$). CD36 mRNA increased in the T2DSC group than in the control group ($P < 0.01$), whereas the mRNA levels of PDX1, MAFA, insulin, SOX9, Neurog3 and NANOG were markedly decreased ($P < 0.01$). Compared with T2DM group, T2DSC group had significantly promoted CD36 and FOXO1 mRNA but significantly decreased PDX1, MAFA, insulin, SOX9, Neurog3 and NANOG ($P < 0.05$).

Figures 1B–H show the relationship between CD36 and the expression of β -cell differentiation-related genes in all participants. Spearman correlation analysis revealed that CD36 had a weak negative correlation with FOXO1 ($r = -0.168$, $P = 0.04$) (Fig. 1B), PDX1 ($r = -0.340$, $P < 0.001$) (Fig. 1C), MAFA ($r = -0.334$, $P < 0.001$) (Fig. 1D), and insulin ($r = -0.380$, $P < 0.001$) (Fig. 1E). However, the expression of CD36 mRNA was not significantly correlated with SOX9 ($r = 0.044$, $P = 0.594$) (Fig. 1F), Neurog3 ($r < 0.001$, $P = 0.995$) (Fig. 1G) and NANOG ($r = -0.028$, $P = 0.737$) (Fig. 1H).

3.3 ROC analysis for the diagnostic efficacy of CD36

ROC analysis was performed to evaluate the diagnostic efficacy of the serum CD36 expression for T2MD and T2DSC groups as shown in supplementary Fig. 1S. The ROC curve is also called the sensitivity curve and uses the graphical method to accurately reflect the relationship between the sensitivity and specificity of a certain analytical method. This comprehensive indicator of test accuracy is helpful for users to weigh the impact of missed diagnosis and misdiagnosis via setting a number of different critical values for continuous variables to calculate the best cut-off point as a diagnostic reference value. A large area under the curve (AUC) indicates a high diagnostic accuracy. The results revealed that CD36 was useful for distinguishing T2MD (AUC: 0.926, sensitivity: 0.82, specificity: 0.86, cut-off value: 0.94, $P < 0.001$), and T2DSC (AUC: 0.99, sensitivity: 0.98, specificity: 0.96, cut-off value: 1.33, $P < 0.001$) from control group. The diagnostic efficiency of CD36 for T2MD and T2DSC was AUC: 0.67, sensitivity: 0.32, specificity: 0.98, cut-off value: 1.28, $P = 0.003$.

3.4 Effect of CD36 on cell vitality, cytokine level and oxidative stress of EndoC- β H1 cells

Figure 2 shows the regulation of CD36 on cell proliferation, apoptosis, cytokine level and oxidative stress in EndoC- β H1 cells by inhibiting and overexpressing CD36. CD36 inhibition and overexpression significantly down-regulated and up-regulated the mRNA (Fig. 2A) and protein (Fig. 2B) expression levels of CD36, respectively. EndoC- β H1 showed adherent and clumped growth under the microscope. CD36 inhibition increased cell clusters, and its overexpression significantly reduced the number of cells and cell clusters (Fig. 2C). CCK-8 assay also confirmed that cell proliferation was significantly increased after CD36 knockdown and significantly reduced after CD36 overexpression ($P < 0.001$) (Fig. 2D). Flow cytometry showed that cell apoptosis rate was significantly inhibited after CD36 knockdown but significantly enhanced after CD36 overexpression ($P < 0.001$) (Fig. 2E). Additionally, CD36 significantly promoted the secretion of cytokines IL-1 β , IL-8, and TNF- α ; increased the production of oxide MDA and ROS; and inhibited the total antioxidant capacity. These findings suggested that CD36 stimulated cell inflammation response and oxidative stress (Fig. 2F). However, the secretion of insulin was significantly reduced by CD36, suggesting that the function of EndoC- β H1 cells was impaired.

3.5 Effect of CD36 on EndoC- β H1 dedifferentiation

Figure 3 shows the effect of CD36 on EndoC- β H1 differentiation as determined from the detection of key proteins. After CD36 knockdown, the expression of β -cell maturation biomarkers FOXO1, PDX1, MAFA and insulin was increased significantly, whereas that of dedifferentiation biomarkers SOX9, Neurog3 and NAGOG decreased significantly. CD36 overexpression significantly inhibited the expression of PDX1, MAFA and insulin but induced the expression of Neurog3 and NAGOG (Fig. 3A). The protein levels of cleaved-PARP, NOX4 and cleaved-caspase 3 were significantly reduced in the CD36 knockdown group ($P < 0.01$), whereas p-AKT protein levels were significantly increased ($P < 0.001$). However, CD36 overexpression promoted cleaved-PARP, NOX4 and cleaved-caspase 3 but inhibited p-AKT (Fig. 3B).

3.6 Effect of CD36 on cell vitality, cytokine level and oxidative stress in T2DM and T2DSC cell models

In Fig. 4, high glucose was used to induce T2DM model, and high glucose and LPS were applied to establish T2DSC model to clarify the effect of CD36 on T2DM and T2DSC. High glucose and high glucose combined with LPS reduced the number and masses of EndoC-βH1 cells under the microscope (Fig. 4A). Flow cytometry was used to measure cell apoptosis (Fig. 4B). The results showed that the cell apoptosis rate was significantly increased after 30 mM glucose treatment for 24 hours compared with the control group ($P < 0.05$). The use of 10 μg/mL LPS significantly enhanced the effect of cell apoptosis induced by high glucose ($P < 0.05$). CD36 knockdown could inhibit high glucose and/or LPS-induced cell apoptosis ($P < 0.05$), whereas its overexpression could promote the high glucose and/or LPS-induced cell apoptosis ($P < 0.05$) (Fig. 4C). Viability assay revealed that the EndoC-βH1 cell proliferation was significantly inhibited by high glucose ($P < 0.05$) but significantly enhanced by LPS ($P < 0.05$). The inhibition effect of high glucose and/or LPS on cell proliferation was rescued by CD36 knockdown ($P < 0.05$), but was not affected by CD36 overexpression ($P > 0.05$) (Fig. 4D).

In Fig. 4E, high glucose significantly promoted the inflammation response and oxidative stress by increasing the secretion of IL-1β, IL-8 and TNF-α, elevating the production of MDA and ROS and inhibiting total antioxidant capacity ($P < 0.05$). LPS enhanced the impact of inflammation and oxidative stress by high glucose. However, CD36 knockdown rescued the effect of high glucose combined with LPS by decreasing IL-1β, TNF-α and ROS and increasing the total antioxidant capacity ($P < 0.05$). CD36 overexpression enhanced the induction of high glucose and LPS by increasing IL-1β, TNF-α and ROS and reducing the total antioxidant capacity ($P < 0.05$). LPS promoted the inhibitory effect of high glucose on. CD36 knockdown rescued the insulin secretion inhibited by high glucose and LPS, and CD36 overexpression promoted the inhibiting effect of high glucose and LPS on insulin secretion ($P < 0.05$).

3.7 Effect of CD36 on EndoC-βH1 differentiation in T2DM and T2DSC cell models

Figure 5A shows the effect of CD36 on EndoC-βH1 differentiation in high glucose-induced T2DM model and high glucose and LPS- induced T2DSC model. The results showed that high glucose significantly induced the protein expression of FOXO1, SOX9, Neurog3 and NANOG ($P < 0.05$) and significantly decreased those of PDX1, MAFA and insulin ($P < 0.05$). LPS treatment could increase the FOXO1 expression induced by high glucose ($P < 0.05$). However, LPS treatment significantly decreased the protein expression of PDX1, MAFA, insulin, SOX9 and Neurog3 regulated by high glucose ($P < 0.05$). CD36 knockdown could enhance FOXO1 expression induced by high glucose and/or LPS; rescue PDX1, MAFA and insulin expression; and inhibit SOX9, Neurog3 and NANOG protein expression ($P < 0.05$). Compared with high glucose and/or LPS group, CD36 overexpression did not significantly affect the protein expression of FOXO1, insulin and Neurog3 ($P > 0.05$) but significantly enhanced the inhibited expression of PDX1, MAFA and SOX9 ($P < 0.05$).

In Fig. 5B, the protein levels of cleaved-PARP, cleaved-caspase 3, p-AKT and NOX4 in the high glucose group were significantly increased ($P < 0.05$), and this effect was further enhanced by LPS. However, the

effects of high glucose and LPS in inducing the expression of cleaved-PARP, cleaved-caspase 3, p-AKT and NOX4 were inhibited by CD36 knockdown and promoted by CD36 overexpression ($P < 0.05$).

Discussion

T2DM is a highly prevalent disease that significantly threatens the health of people worldwide [1, 2]. People with diabetes are highly susceptible to infection, particularly sepsis, bone and joint infections and cellulitis [41]. According to statistics, 6% of infection-related hospitalisations and 12% of infection-related deaths are attributed to diabetes [41]. Sepsis is a leading cause of mortality in critical care worldwide [42]. However, research on the relationship between infection and diabetes is still insufficient. Here, the hour postprandial blood glucose levels were 16.59 ± 5.82 and 15.24 ± 5.71 mmol/L in the T2DM and T2DSC groups, which were higher than 11.1 mmol/L. CD36 and triglyceride were significantly increased in the T2DM and T2DSC groups. Therefore, the up-regulation of CD36 in patients with diabetes has been confirmed [8, 9]. The increase in CD36 in T2DM and T2DSC groups might be attributed to the high levels of glucose concentration and triglyceride which induce CD36 expression [10–12, 36]. However, the mRNA expression of CD36 and FOXO1 was significantly increased in T2DSC group than in T2DM group, whereas PDX1, insulin, SOX9, Neurog3 and NANOG were significantly decreased. Low-grade inflammation is a common feature of patients with T2DM [43]. C-reactive protein, a recognised marker of inflammatory development, is up-regulated in patients with T2DM [44]. The results showed that the levels of white blood cell count and C-reactive protein in patients with TT2DSC were significantly higher than those in patients with T2DM, suggesting that patients with TT2DSC have heavier systemic inflammatory response. Sepsis and inflammation also promoted CD36 expression [22, 37], and CD36 in turn strengthened the inflammatory response [24, 25]. Inflammation might be the reason why CD36 level in patients with TT2DSC is significantly higher than that in patients with T2DM. Additionally, CD36 expression changes have good diagnostic sensitivity for distinguishing patients with TT2DSC and T2DM from normal people, indicating that CD36 might be a key molecule in regulating T2DM and T2DSC. Reduced FOXO1 and PDX1 in T2DM would lead to a decrease in β -cell, an increase in α -cell, and enhancement of insulin resistance [45, 46]. α -cell transdifferentiation increases when β -cell mass decreases, and β -cell dedifferentiation is an important factor that causes β -cell dysfunction in T2DM [47]. Furthermore, MAFA expression is significantly reduced in patients with T2DM [48]. MAFA transcription factor is a key regulator of pancreatic islet β -cell activity after birth. The decreased FOXO1, PDX1, and MAFA leads to β -cell dysfunction, which reduces insulin secretion. This finding corresponded to the current results on the decreased insulin expression in patients with TT2DSC and T2DM. FOXO1 expression levels were significantly higher in patients with TT2DSC than in those with T2DM, and this finding may be attributed to sepsis. LPS could promote FOXO1 and NF- κ B expression and inflammatory response [49, 50]. High CD36 levels in patients with TT2DSC also stimulate FOXO1 expression [28]. The high expression of SOX9, Neurog3 and NANOG in patients with T2DM might indicate β -cell dedifferentiation. Sepsis could reduce the expression of progenitor cell marker SOX9 [51]. Hyperglycaemia induces the expression of SOX9, Neurog3 and NANOG and inhibits the expression of FOXO1, PDX1, MAFA and insulin, resulting in the apoptosis and dedifferentiation of β -cells [52]. The

decline in β -cell function is most manifested by the decrease in insulin mRNA and secretion. On the basis of these results, these gene alterations could be an important target for regulating diabetes or sepsis.

CD36 could manage T2DM by negatively regulating insulin secretion [53]. CD36 participates in multiple homeostatic and pathological processes. In β -cells, CD36 enhances glucotoxicity dysfunction by increasing the influx of FFAs [11, 12, 23]. CD36 was knocked down and overexpressed in EndoC- β H1 cells to verify its role of CD36 in β -cells. The results showed that CD36 induced β -cell dysfunction by inhibiting cell proliferation and promoting cell apoptosis, inflammation response, oxidative stress and cell dedifferentiation. CD36 siRNA increased the viability of INS-1 β -cell by reducing cleaved-caspase 3 expression [18]. CD36 induced the expression of cleaved-caspase 3, another apoptosis marker protein PARP-1 that was also up-regulated by CD36. Cleaved caspase-3 and cleaved PARP were also increased in palmitate-induced apoptosis in β -cells [54]. PARP1 activation can culminate cell dysfunction and necrosis through the excessive consumption of energy and play a pro-inflammatory effect [55]. CD36 could induce redoxosome (Vav2-Rac1-NOX) formation and activate Rac1-GTP-NADPH oxidase complex, resulting in mitochondrial dysfunction and β -cell apoptosis [56]. CD36 can stimulate β -cell inflammatory and oxidative stress via promoting the secretion of cytokines IL-1 β , IL-8 and TNF- α and the production of oxide MDA and ROS. CD36 enhances NF- κ B activity to induce the secretion of cytokines IL-1 and TNF- α [24–26]. ROS overproduction or antioxidant reduction may cause oxidative stress, which is an important factor in insufficient insulin secretion and increased β -cell apoptosis [57]. Oxidative stress induced β -cell apoptosis by activating caspase-3/8/9 and cleaving PARP [58]. These results were consistent with previous studies that CD36 enhances oxidative stress, induces β -cell apoptosis, and reduces insulin secretion. Insufficient insulin secretion is also related to decreased β -cell function. CD36 could decrease β -cell maturation biomarker PDX1 and insulin expression [11, 12]. However, the role of CD36 in reducing FOXO1 expression was opposite to a previous finding [28] possibly due to the inhibition of AKT activation by CD36 [26]. The low expression of FOXO1 weakened its MAFA up-regulation [9, 31] but promoted the expression of β -cell dedifferentiation markers neurog3 and NAGOG [4]. Additionally, CD36 promoted SOX9 expression, which was consistent with our clinical findings. In summary, CD36 induced the dedifferentiation of mature β -cell into pancreatic endocrine progenitor cells, resulting in decreased insulin secretion.

However, the effect of CD36 on T2DSC cell models remains unclear. Plasma LPS is increased in patients with diabetes and results in endotoxemia [40] mainly because of the β -cell function decline that led to metabolic syndrome and increased LPS plasma concentrations in patients with T2DM or rats [60]. The effects of high glucose and LPS on EndoC- β H1 cells under CD36 knockdown and overexpression were analysed to verify the role of CD36 in T2DSC. Increases in TNF- α , IL-1 β and IL-8 were observed in T2DM cell models, and this trend consistent with that in patients with T2DM [17]. High glucose promoted oxidative stress marker ROS and MDA production [18–20] and up-regulated cleaved caspase-1/3/9 and PARP-1 to induce β -cell apoptosis [19, 20]. LPS enhanced β -cell apoptosis, inflammatory response and oxidative stress induced by high glucose. Additionally, LPS induced the production of IL-1 β by promoting the maturation of procaspase-1 into active capsase-1 and up-regulated caspase-3, PARP-1 and Bax/Bcl-2 expression and ROS production by inducing Toll-like receptor 4 (TLR4) to promote β -cell apoptosis [61,

62]. Experimental results indicated that LPS induced β -cell dysfunction, which was consistent with previous studies. CD36 inhibition could reverse β -cell apoptosis and IL-1 β , IL-8, TNF- α , MDA and ROS production induced by high glucose and LPS. Additionally, cell proliferation and total antioxidant and insulin production are rescued. CD36 inhibition also blocks high glucose-induced β -cell apoptosis by inhibiting caspase-3 expression [18] and protects against sepsis by reducing inflammation and cell damage [37]. However, CD36 induces the defective exocytosis and cell glucolipotoxicity, resulting in β -cell dysfunction [63]. FOXO1, PDX-1, MAFA and insulin are reduced in patients with T2MD [45, 46, 48]. FOXO1 is a target in the insulin pathway whose inhibition could reduce exocytotic gene and protein expression, thereby exacerbating cell dysfunction [9]. PDX1 and MAFA are the key transcription factors of insulin gene promoter [18, 31]. This study revealed that CD36 knockdown enhanced the protein expression of FOXO1, PDX1, MAFA and insulin in the T2DSC cell model. CD36 knockdown increases the expression of FOXO1, PDX1, MAFA and insulin to alleviate β -cell glucotoxicity [9, 11, 12, 31]. In addition, the high glucose and LPS-induced protein levels of SOX9, Neurog3 and NANOG were suppressed by CD36 knockdown. SOX9 and Neurog3 were increased in the islets of T2DM and are indicative of β -cell dedifferentiation [64]. Targeting CD36 may be a novel treatment for septic patients with hyperglycaemia stress.

Conclusion

In summary, a significant increase in CD36 could be used as an effective molecule for the diagnosis of T2DM and T2DSC. CD36 promotes β -cell apoptosis by inducing inflammation and oxidative stress and activates β -cell dedifferentiation by inhibiting FOXO1, PDX1, MAFA and insulin and promoting SOX9, Neurog3 and NAGOG. CD36 inhibition could effectively rescue β -cell dysfunction induced by high glucose and LPS. The results provided new insights, into the possible contribution of CD36 to the treatment of T2DSC.

Abbreviations

Gene name	Full Name
T2DM	type 2 diabetes
T2DSC	type 2 diabetic sepsis complications
CD36	Fatty acid translocase cluster determinant 36
FOXO1	Forkhead box O1
PDX1	Pancreatic and duodenal homeobox 1
TNF- α	Tumor necrosis factor alpha
IL-8	Interleukin 8
MAFA	MAF bZIP transcription factor A
SOX9	SRY-box transcription factor 9
Neurog3	Neurogenin 3
NANOG	Nanog homeobox
IL-1 β	Interleukin 1 β
MDA	Malondialdehyde
ROS	Reactive oxygen species
TAC	Total antioxidant capacity
p-AKT	Phospho-AKT serine/threonine kinase 1
cleaved caspase-3	Cleaved cysteine-aspartic proteases 3
cleaved PARP 1	Cleaved poly (ADP-ribose) polymerase 1
NOX4	Nicotinamide adenine dinucleotide phosphate oxidase 4
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
HG	High glucose
LPS	Lipopolysaccharide
BMI	Body mass index
FBS	Fasting blood sugar
HbA1c	Glycated hemoglobin
WBC	White blood cell count
ESR	Erythrocyte Sedimentation Rate
PCT	Thrombocytocrit

TC	Total cholesterol
HDL-C	High-density lipoprotein cholesterol
LDL-C	Low-density lipoprotein cholesterol
TG	Triglyceride
CRP	C-reactive protein
DKA	Diabetic ketoacidosis
APACHE-II	Acute Physiology and Chronic Health Evaluation
qSOFA	Quick Sequential [Sepsis-related] Organ Failure Assessment
qPCR	Quantitative polymerase chain reaction
ROC	Receiver operator characteristic
AUC	Area under the curve

Declarations

Funding: The study was supported by [the Medical Innovation Projects of Fujian Province's Health and Scientific Research Talent Training Project] under Grant [Grant NO. 2019-CXB-25].

Conflicts of interest All authors have no conflict of interest to declare in relation to the content of this article.

Authors' contributions: Ling Gu and Huogen Liu made substantial contributions to the conception and design of the work. Ling Gu, Yundi Shi, Hailin Shu, Fengming Huang, Huogen Liu, Xin Wan, Ying Liu and Zuchen Lei made substantial contributions to the acquisition, analysis and interpretation of data for the work. Ling Gu participated in drafting the work, Huogen Liu revising it critically for important intellectual content. Each author approved the final version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Tables

Table 1 Basic information of patients

Factors	Control (n=50 case)	T2DM (n=50 case)	T2DSC (n=50 case)	P-value
Age (years)	61.88±1.75	62.52±1.72	65.10±1.53	0.355 ^a
Men (case)	25 (50%)	23 (46%)	21 (42%)	0.725 ^b
BMI (kg/m ²)	23.53±0.07	22.39±0.05	21.43±0.07	<0.001 ^a
FBS (mmol/L)	4.38±0.10	11.21±0.36	13.70±0.51	<0.001 ^a
HbA1c (%)	4.89±0.15	9.01±0.46	10.37±0.40	<0.001 ^a
WBC (10 ⁹ /L)	5.9 (4.87-6.75)	8.05 (3.2-11.55)	15.9(10.17-20.07)	<0.001 ^c
ESR (mm/h)	8.55(4.70-11.35)	27.4(17.35-33.82)	56.10(25.25-69.50)	<0.001 ^c
PCT (ng/mL)	0.05 (0.02-0.08)	0.16(0.10-.023)	10.10(6.45-12.92)	<0.001 ^c
TC (mmol/L)	4.35 (3.57-5.12)	4.45(3.00-5.50)	3.40(2.28-4.52)	<0.001 ^c
HDL-C (mmol/L)	1.30(0.90-1.90)	1.40(0.80-1.80)	0.85(0.60-1.10)	<0.001 ^c
LDL-C (mmol/L)	2.71±0.12	2.42±0.11	2.43±0.09	0.111 ^a
TG (mmol/L)	1.19±0.04	2.46±0.10	2.30±0.10	<0.001 ^a
CRP (mg/mL)	1.95(1.15-3.22)	5.15(3.75-8.60)	80.50(55.00-119.50)	<0.001 ^c
T2DM course (years)	/	7.80(3.75-11.72)	8.95(3.62-11.20)	0.654 ^d
Complication				
hypertension	/	32 (64%)	29 (58%)	0.002 ^b
nephropathy	/	18 (36%)	13 (26%)	
DKA	/	0 (0%)	8 (16%)	
Infection portion				
respiratory system	/	/	11 (22%)	
digestive system	/	/	7 (14%)	
urinary system	/	/	19 (38)	
skin	/	/	6 (12%)	
other	/	/	7 (14%)	
APACHE-II score	/	/	24.62±0.41	

qSOFA score	/	/	2 (1-2)
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Abbreviations: T2DM,Type II diabetes mellitus; T2DSC, type 2 diabetic sepsis complications; BMI, body mass index; FBS, fasting blood sugar; HbA1c, glycated hemoglobin; WBC, [white blood cell count](#); ESR, Erythrocyte Sedimentation Rate; PCT, thrombocytocrit; TC, total cholesterol; HDL-C, high-density lipoproteincholesterol, LDL-C, low-density lipoprotein cholesterol;TG, triglyceride; CRP, C-reactive protein; DKA, diabetic ketoacidosis; APACHE-II score, Acute Physiology and Chronic Health Evaluation; qSOFA, Quick Sequential [Sepsis-related] Organ Failure Assessment. Data are presented as mean ± [standard error](#), the number and percentage (%) and median with interquartile interval [Q1-Q3]. a, one-way ANOVA analysis; b, Chi-square test; c, Kruskal-Wallis H (K) test; d, Mann-Whitney U test. P < 0.05 was considered to suggest a significant difference.

Figures

Figure 1

Gene expression levels of β-cell dedifferentiation and Spearman correlation analysis. (A) Changes in the expression of CD36 and β-cell dedifferentiation-related genes in control, T2DM and T2DMS groups. (B–H) Relationship between the expression of CD36 and β-cell differentiation-related genes in control, T2DM and T2DMS participants. Association of CD36 expression levels with β-cell differentiation-related genes. All data are expressed as the mean ± standard deviation. One-way analysis with Dunnett's post-hoc test

was used for normally distributed data, Mann–Whitney U test was applied for data with a non-normal distribution. Spearman correlation analysis was used to analyze the relationship between genes. T2DM, Type II diabetes mellitus; T2DM and sepsis, type 2 diabetic sepsis complications. Compared with control group, **P < 0.01. Compared with T2DM group, #P < 0.05, ##P < 0.01.

Figure 2

Effect of CD36 on EndoC-βH1 cell vitality, cytokine level and oxidative stress. (A) mRNA expression of CD36 in shRNA plasmid-control, CD36 knockdown, vector-control and CD36 overexpression cells. (B) Protein expression of CD36 in transfection cells. (C) Effect of CD36 on cell growth observed under a microscope (bar=100 μm). (D) Cell proliferation analysis. (E) Cell apoptosis detection. (F) Changes in cytokines IL-1β, IL-8, TNF-α and insulin, oxidative stress factor MDA, ROS and total antioxidant capacity. All data are expressed as the mean ± standard deviation. Student's t-test with two-side was used. Compared with sh-NC or vector group, *P < 0.05, **P < 0.01 and ***P < 0.001.

Figure 3

Effect of CD36 on EndoC-βH1 dedifferentiation and apoptosis. (A) Changes in β-cell maturation marker proteins FOXO1, PDX1, MAFA, insulin and endocrine β-progenitor cell marker proteins SOX9, Neurog3 and NANOG in shRNA plasmid-control, CD36 knockdown, vector-control and CD36 overexpression cells. (B) Protein levels of cleaved-caspase 3, cleaved-PARP, NOX4 and p-AKT. All data are expressed as the mean ± standard deviation. Student's t-test with two-side was used. Compared with sh-NC or vector group, *P < 0.05, **P < 0.01 and ***P < 0.001.

Figure 4

Effect of CD36 on cell vitality, cytokine level and oxidative stress in T2DM and T2DSC cell models. Induced EndoC- β H1 cells with 30 mM high glucose DMEM medium for 24 hours to establish a T2DM cell model. The T2DSC cell model was established by using 30 mM high glucose DMEM medium and 10 μ g/mL LPS for 24 hours. (A) Cell growth observed under a microscope (bar=100 μ m). (B) Cell apoptosis detected by flow cytometry. (C) Analysis of cell apoptosis rate. (D) Cell proliferation analysis. (E) Analysis of cytokines IL-1 β , IL-8, TNF- α , insulin, oxidative stress factor MDA, ROS and total antioxidant capacity. All data are expressed as the mean $\pm$ standard deviation. One-way analysis with LSD post-hoc test was used. *P < 0.05 vs. control group, #P < 0.05 vs. high glucose group, @P < 0.05 vs. high glucose and LPS group.

(A)

(B)

Figure 5

Effect of CD36 on EndoC-βH1 dedifferentiation and apoptosis in T2DM and T2DSC cell models. (A) Protein expression levels of FOXO1, PDX1, MAFA, insulin, SOX9, Neurog3 and NANOG in CD36 transfection cells treated with high glucose and LPS. (B) Expression of cleaved-caspase 3, cleaved-PARP, NOX4 and p-AKT. All data are expressed as the mean ± standard deviation. One-way analysis with LSD

post-hoc test was used. *P < 0.05 vs. control group, #P < 0.05 vs. high glucose group, @P < 0.05 vs. high glucose and LPS group.

Supplementary Files

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- [FigS1.png](#)