Inhibitory effect of TCF7L2 on pancreatic β-cell dedifferentiation via ERK/MAPK signaling pathway in diabetes

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Research Article

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Abstract

Background

Transcription factor 7-like 2 (TCF7L2) variants seem to affect diabetes susceptibility through β-cell dysfunction, underlying basis of which has been considered to be β-cell dedifferentiation rather than apoptotic β-cell death. However, little is known about how TCF7L2 variation contributes to β-cell dedifferentiation and how pancreatic β-cell dedifferentiation changes during diabetes progression.

Methods

To clarify the effects of TCF7L2 on β-cell dedifferentiation and insulin secretion, MIN6 cells were transfected with TCF7L2 shRNA or lenti-TCF7L2 virus for 48h and then the degree of β-cells dedifferentiation and insulin concentrations in supernatant were measured respectively. To further determine whether the effects are mediated by ERK/MAPK signal pathway, MIN6 cells were administrated with ERK phosphorylation inhibitor U0126 prior to TCF7L2 shRNA virus transfection. Subsequently, changes of TCF7L2 expression and pancreatic β-cell dedifferentiation were measured respectively in db/db mice after 2, 6 and 10 weeks of ND or HFD feeding.

Results

Our present study demonstrated that stable shRNA-mediated knockdown of TCF7L2 significantly increased β-cell dedifferentiation and drastically decreased insulin secretion of MIN6 cells. The opposite results were observed following lenti-TCF7L2 virus transfection. Interestingly, TCF7L2 exerted an inhibitory effect on the activation of ERK/MAPK signal and the effects of TCF7L2 on β-cells dedifferentiation and insulin secretion were totally attenuated when the phosphorylation of ERK was blocked using its chemical inhibitor U0126. Additionally, the declined TCF7L2 expression in paralleled with sustained activation of ERK/MAPK signal and increased pancreatic β-cell dedifferentiation were observed simultaneously in db/db mice. All animals showed impaired glucose tolerance during intraperitoneal glucose tolerance tests.

Conclusion

The pancreatic β-cell dedifferentiation which mediated by ERK/MAPK signaling pathway might be the essential component of TCF7L2 variants to develop diabetes.

Introduction

Type 2 diabetes mellitus (T2DM) is known as one of the most common metabolic diseases which characterized by chronic hyperglycemia mainly due to pancreatic β-cell destruction or dysfunction [1].
The underlying mechanism of β-cell failure in diabetes is controversial, which was almost exclusively attributed to apoptotic β-cell death originally. However, an important concept regarding β-cell failure has been proposed that it was not only due to apoptotic β-cell death but also due to differentiation of insulin-producing mature β-cells into the endocrine progenitor cells [2]. The process, known as dedifferentiation, leads to severe dysfunction of insulin secretion which is a major contributor to hyperglycemia in diabetes [3–4]. There are good reasons to think that dedifferentiation might be reversible because of the prevention or reversion of β-cell dysfunction by good glycaemia control in T2D patients [5–6]. In addition, insulin-producing mature β-cells might also convert to other endocrine cells, including α-cells.

Various transcription factors are involved in pancreatic β-cell differentiation and the maintenance of mature β-cell function. During the dedifferentiation process, β-cell loss its identity and function with a decrease in the expressions of beta cell specific markers, such as MafA, Ucn3, FoxO1 and Glp1r [7–8]. Such dedifferentiated β-cells intent to express endocrine progenitor cell markers, such as Neurog3, Sox9, Hes1 and L-Myc [9].

TCF7L2 (transcription factor 7-like 2), a basic component of Wnt signaling pathway, genetic variants of which are considered to be definitively associated with T2DM. The association has been consistently replicated in multiple populations with diverse genetic origins [10–12]. However, the cellular mechanism through which abnormal TCF7L2 activity contributes to β-cell dysfunction remains conflicted. Previous analyses have demonstrated that TCF7L2 might modulate β-cell function through affecting β-cell growth or differentiation [13–14]. There is also evidence to suggest that TCF7L2 may mediate β-cell proliferation and silencing of TCF7L2 in cell lines and primary islets lead to increased apoptosis [15–16]. However, β-cell dedifferentiation has been considered as a major contributor to β-cell dysfunction, we therefore question what effects of TCF7L2 exert on β-cell dedifferentiation and whether the impaired insulin secretion in diabetes be explained by altered β-cell differentiation state.

The Extracellular regulated protein kinases/Mitogen-activated protein kinase signaling pathway (ERK/MAPK signaling pathway), a most thoroughly studied MAPK signaling cascade, has been confirmed to be significantly associated with multiple basic cellular processes and signal transduction network [17–18]. Previous studies have demonstrated that sustained activation of ERK/MAPK signaling is known to promote differentiation and initiate cell proliferation, reduced activation of ERK/MAPK signaling in islet and MIN6 cells is associated with apoptosis [19–20]. The ERK/MAPK signaling pathway has also been confirmed to play an important role in cellular dedifferentiation [21–22]. Interestingly, previous experiments also found that impaired TCF7L2 significantly reduced ERK phosphorylation in human colorectal cancer cells and treatment of ERK phosphorylation inhibitor U0126 inhibited TCF7L2-induced endogenous LCN2 expression in ESCC cells [23–24]. These findings raised the possibility that TCF7L2 might serve as a modulator of ERK/MAPK signaling pathway.

In our present study, we investigated the effects of TCF7L2 on β-cell dedifferentiation and further determined whether the effects were mediated by ERK/MAPK signaling pathway. Using the db/db mouse
model, we aimed to clarify how TCF7L2 expression and pancreatic β-cell dedifferentiation changes during diabetes progression.

Methods

Cell lines and antibodies

All cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C in a humidified atmosphere containing 5% CO2. After 65% cells attached, we synchronized them by the serum-free medium for 8 h.

Antibodies targeting the following proteins were obtained from Cell Signaling Technology, Inc. (Danvers, MA, USA): Sox9 (cat. no. 82630), Hes1 (cat. no. 11988), FoxO1 (cat. no.2880), total-ERK (cat. no.4696) and phospho-ERK (cat. no. 4370). TCF7L2 antibody (cat. no.DF7622) was purchased from Anity Biosciences, OH, USA. The specific inhibitor of ERK phosphorylation (U0126) was purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany).

Cell transfection

When the cells were 60–70% confluent, they were transfected with lenti-TCF7L2 or TCF7L2 shRNA virus respectively. Cell transfections were performed using Lipofectamin 3000™ (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol.

Animals

Adult male db/db mice (20–30g, 6 weeks old) (Jackson Laboratory, Bar Harbor, Maine) were housed in a light (12h/12h on/off) and temperature controlled environment. All the animal protocols were approved by the Animal Welfare and Ethics Committee at Fudan University. The mice were fed a normal diet (ND; Harlan Teklad Rodent Diet 8604, containing 12.2, 57.6 and 30.2% energy from fat, carbohydrate and protein, respectively) or a high-fat/high-sucrose diet (HFD; Surwit Research Diets, New Brunswick, NJ, USA containing 58, 26 and 16% energy from fat, carbohydrate and protein, respectively ) for 2, 6 and 10 weeks.

Intraperitoneal glucose tolerance test and insulin measurement

For intraperitoneal glucose tolerance test (IPGTT), mice were fasted 12h overnight and injected intraperitoneally with glucose at a dose of 2 g/kg body weight. Blood samples were obtained at time-points 0, 30, 60, 90 and 120 min. Glucose concentration was determined using a glucometer (Roche, Indianapolis, IN). For insulin and c-peptide measurements, blood samples were centrifuged and measured using a mouse ELISA kit for insulin and c-peptide (Mercodia, Sweden).

Western blots
Total proteins were extracted with RIPA lysis buffer (Beyotime Inc, China). The cell lysates were clarified by centrifugation and protein concentrations were measured by using BCA Protein Assay Kit (Beyotime Inc, China). The protein samples were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), transferred to polyvinylidene fluoride (PVDF) membranes, blocked in 4% milk for 2h at room temperature followed by the incubation of primary antibodies overnight at 4°C. Protein expression levels were quantified by scanning the immunostaining bands and analyzed by using Lab Work 45 Image Software.

**Real-time Quantitative PCR**

Total RNA was extracted from cell lines by using Trizol Reagent (Thermo Fisher Scientific, Inc.) according to the manufacturer’s instruction. Reverse transcription was performed by using Reverse Transcription Kit (Thermo Fisher Scientific, Inc.). The qPCR was performed in triplicate with SYBR Green Master mix (Takara Bio, Inc., Otsu, Japan) on the ABI 7000 sequence detection system (Thermo Fisher Scientific, Inc.). The conditions for real-time PCR are listed as follows: 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 5 sec and elongation at 60°C for 30 sec. The $2^{-\Delta\Delta}$ method was used to calculate threshold cycle. GAPDH was used as internal control for normalization. All primers used here are listed in Table 1.

**Statistical analysis**

Data was exhibited as mean ± standard deviation and the comparisons were evaluated using the nonparametric Mann–Whitney U test. Data were statistically analyzed using Graph Pad software (version 6.0c; Graph Pad Software, Inc., La Jolla, CA, USA). P < 0.05 was considered to indicate a statistically significant difference. Each experiment was performed for more than 3 times.

**Result**

**Impaired TCF7L2 resulted in increased β-cells dedifferentiation and decreased insulin secretion of MIN6 cells**

MIN6 cells were transfected with TCF7L2 shRNA for 48 h and then the level of β-cells dedifferentiation and insulin concentrations in supernatant were measured respectively. The raised expressions of Hes1 and Sox9 in paralleled with the significantly declined FoxO1 protein whose localization is restricted to β-cells indicating that impaired TCF7L2 lead to increased dedifferentiation of MIN6 cells (Fig. 1A-B, *P < 0.05 and **P < 0.01 as indicated). Meanwhile, a decreased insulin concentration was also presented in our experiment (Fig. 1C, **P < 0.01 as indicated.). The opposite results were observed following lenti-TCF7L2 virus transfection (Fig. 1D-F, *P < 0.05 and **P < 0.01 as indicated). Primers used for target amplification are shown in Table 1. GAPDH was used as internal control.

Table 1: Primers used for target amplification in real-time PCR.
Impaired TCF7L2 lead to sustained activation of ERK/MAPK signaling pathway

To clarify the effects of TCF7L2 exert on ERK/MAPK signaling pathway, MIN6 cells were transfected with TCF7L2 shRNA or lenti-TCF7L2 virus for 48 h and then the expressions of total and phosphorylated ERK were measured respectively. The increased expression of p-ERK following TCF7L2 shRNA virus transfection suggesting that a failure in TCF7L2 lead to sustained activation of ERK/MAPK signaling pathway (Fig. 2A-B, *p < 0.05 as indicated). In parallel, a decreased expression of p-ERK was observed following lenti-TCF7L2 virus transfection (Fig. 2C-D, *p < 0.05 as indicated).

The effects of TCF7L2 on β-cell dedifferentiation and insulin secretion are mediated by ERK/MAPK signaling pathway

To further determine whether the effects of TCF7L2 on β-cell dedifferentiation and insulin secretion are mediated by ERK/MAPK signaling pathway, MIN6 cells were administrated with ERK phosphorylation inhibitor U0126 prior to TCF7L2 shRNA virus transfection. Interestingly, we found that the changes of β-cells dedifferentiation and insulin secretion induced by impaired TCF7L2 were totally attenuated when the phosphorylation of ERK was blocked. (Fig. 3A-B, *p < 0.05, **p < 0.01).

Glucose tolerance worsened with time during diabetes progression

Six-week-old male db/db mice were fed either ND or HFD for 2, 6 and 10 weeks. Body weight and glucose tolerance following IPGTT were investigated respectively. As previously expected, body weight increased with time on the diet and the differences between ND and HFD feeding were statistically significant (Fig. 4A, *P < 0.05 and **P < 0.01 as indicated). Impaired fasting glucose became apparent after 2 weeks of HFD feeding, compared with that of ND feeding. Glucose tolerances following IPGTT became impaired in response to 2 weeks of ND and HFD feeding (*P < 0.05, Fig. 4B-D). However, the animals did not show significant changes in glucose tolerance after 10 weeks of ND and HFD feeding, which can be explained by progressively aggravated β-cells dysfunction induced by glucose toxicity during diabetes progression.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Forward</th>
<th>Reverse</th>
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<tbody>
<tr>
<td>GAPDH</td>
<td>5’-GCACCACACCTCTTCAAATGA- 3'</td>
<td>5’-GTAGGGAGGATAGCC- 3’</td>
</tr>
<tr>
<td>FoxO1</td>
<td>5’-AGTTTTCTAAGTGGCTGAGTC- 3'</td>
<td>5’-CCCCATCTCCAGGTGATCC- 3’</td>
</tr>
<tr>
<td>Hes1</td>
<td>5’-TCAACAGACACCCGACAAAC- 3'</td>
<td>5’-ATGCCGGAGCTATCTTTCCT- 3’</td>
</tr>
<tr>
<td>Sox9</td>
<td>5’-AGTACCCAGCATCGACAAAC- 3’</td>
<td>5’-ACGAAGGTTCTTCCTCGCT- 3’</td>
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</table>
Reciprocal changes of pancreatic TCF7L2 protein and β-cells dedifferentiation during diabetes progression

To determine whether TCF7L2 expression is increased or decreased during diabetes progression, pancreatic levels of TCF7L2 protein in db/db mice were measured after 2, 6 and 10 weeks of ND or HFD feeding. All animals showed impairment in glucose tolerance during IPGTT. Consist with previous findings [25], the expressions of TCF7L2 protein decreased with time on the diet, both in ND and HFD group. Since we previously found that TCF7L2 might serve as a modulator of ERK/MAPK signaling, the phosphorylation of ERK were measured subsequently which observed to be increased with time on the diet, opposite to the changes of TCF7L2 in the same mice. These changes suggesting a direct interplay between TCF7L2 variants and the activation of ERK/MAPK signaling pathway. However, the increased expressions of Sox9 in paralleled with the declined FoxO1 protein indicating that pancreatic β-cells dedifferentiation increased gradually during diabetes progression (Fig. 5).

Discussion

Of the possible SNPs that have been associated with T2DM, TCF7L2 is the most potent locus for diabetes risk and the first locus to have been repeated demonstrated by genome-wide association studies [26]. Evidences have shown that TCF7L2 variants seem to affect diabetes susceptibility through β-cell dysfunction because TCF7L2 silencing exert a strong inhibitory effect on glucose-mediated insulin secretion [27]. TCF7L2 itself could promote beta cell proliferation, protect against apoptosis and improve insulin secretion [28]. However, the underlying basis of β-cell dysfunction has been considered to be β-cell dedifferentiation rather than apoptotic β-cell death [29], we therefore question whether TCF7L2 variants participate in the regulation of pancreatic β-cell dedifferentiation which further lead to impaired insulin secretion in diabetes.

It has been demonstrated that β-cell dedifferentiation might be linked to decreased specific mature β-cell markers and increased endocrine progenitor cell markers. In our present study, we demonstrated that stable shRNA-mediated knockdown of TCF7L2 reduced the expression of FoxO1 protein whose localization is restricted to β-cells and its decline in T2DM patients is paralleled with the loss of insulin immunoreactivity [30]. We also discovered that following TCF7L2 shRNA transfection, the expressions of endocrine progenitor cell markers were increased, such as Sox9 and Hes1. Taken together, induction of TCF7L2 could have a positive effect on β-cells dedifferentiation which represent an alternative mechanism to explain loss of β cells function.

Previous data demonstrated that both SAPK/JNK and ERK/MAPK signaling play an essential role in diabetes and increased level of ERK phosphorylation in macrophages mostly occur by exerting a protective effect to the chronic high glucose environment [32–33]. There is also evidence suggest that sustained activation of ERK/MAPK signaling in adipocytes is associated with the pathogenesis of T2DM and its selective blocked by MEK inhibitor might be a promising approach to the treatment of insulin resistance in diabetes [34]. However, little is known about the mechanism of ERK/MAPK signaling.
pathway contribute to β-cell dysfunction and the regulation of TCF7L2 in ERK/MAPK signaling are controversial. Previous findings demonstrated that stable knockdown of TCF7L2 significantly reduced ERK phosphorylation in acute lymphoblastic leukemia and RAS-induced activation of ERK signaling in colorectal cancer cells was mediated by TCF7L2-regulated gene expression [24, 35]. These data indicated that TCF7L2 might serve as a positive modulator of ERK/MAPK signaling pathway. However, our experiments demonstrated that a failure in TCF7L2 lead to sustained activation of ERK/MAPK signaling, increased β-cell dedifferentiation and decreased insulin secretion which further contribute to hyperglycemia in diabetes. The contradictory effects of TCF7L2 on ERK/MAPK signaling can potentially be explained by the different genetic background. Additionally, the effects of TCF7L2 on pancreatic β-cell dedifferentiation and insulin secretion were totally attenuated following the administration of ERK phosphorylation inhibitor U0126, indicating that β-cell dedifferentiation induced by impaired TCF7L2 was mediated by ERK/MAPK signaling which further lead to a loss of functioning β-cells.

It is generally believed that developing therapies modulating TCF7L2 expression may provide opportunities to treat T2DM patients carrying a TCF7L2 risk allele. Multiple previous studies have investigated the changes of TCF7L2 expression in diabetes which was observed to be almost undetectable in pancreatic sections of T2DM patients [25] and its protein expression were consistently impaired in various rodent models for T2DM [28, 31]. However, nobody know exactly how pancreatic β-cell dedifferentiation changes during diabetes progression and the contribution of ERK/MAPK signaling pathway to diabetes remains to be determined. Here, we investigated the changes of TCF7L2 protein, ERK signaling activation and β-cell dedifferentiation in db/db mice after 2, 6 and 10 weeks of ND or HFD feeding. All animals showed impaired glucose tolerance during intraperitoneal glucose tolerance tests. Together with an induction of TCF7L2 protein, sustained activation of ERK signaling and increased β-cell dedifferentiation were simultaneously observed in both ND and HFD feeding group. Such data indicated a tight correlation of TCF7L2 variation, ERK signaling activation and β-cell dedifferentiation in diabetes. However, the changes of β-cell dedifferentiation became inconspicuous after 6 weeks of feeding, no matter in ND or HFD group, which can be explained by progressively declined β-cells function induced by prolonged exposure to chronic hyperglycemia. Considering T2DM is a possible reversible disease, it is possible to improve β-cell function by reestablishing cellular maturation and good glycaemia control during the disease progression.

**Conclusion**

Our present study demonstrated that pancreatic β-cell dedifferentiation which mediated by ERK/MAPK signaling pathway might be the essential component of TCF7L2 variants to develop diabetes. However, more functional and physiological studies are still needed to gain insight into the underlying mechanisms.

**Abbreviations**

TCF7L2
Transcription factor 7-like 2
T2DM
Type 2 diabetes mellitus
ERK/MAPK signaling pathway
Extracellular regulated protein kinases/Mitogen-activated protein kinase signaling pathway
PCR
Polymerase Chain Reaction

Declarations

Authors’ contribution

JW conceived of the study, participated in its design and helped to draft the manuscript. H-HW, Q-WM and Y-ML performed most experiments and drafted the manuscript. XW, X-CW, BL and Z-YZ carried out part of the experiments and participated in the completion of the final manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data generated and/or analyzed during this study are available from the corresponding author upon reasonable request.

Consent for publication

Not applicable.

Ethics approval and consent to participate

All the animal protocols were approved by the Animal Welfare and Ethics Committee at Fudan University.

Funding

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References


Molecular Mechanism of Pancreatic β-Cell Failure in Type 2 Diabetes Mellitus


Figures

Figure 1

MIN6 cells were transfected with TCF7L2 shRNA or lenti-TCF7L2 virus for 48 h and then the expressions of TCF7L2, Hes1, Sox9 and FoxO1 were evaluated by western blot (A and C) and real-time PCR respectively (B and E). The insulin concentrations in supernatant were measured by ELISA (C and F). (n =3 samples per group, *P<0.05 and **P<0.01 as indicated. NC, negative control).
MIN6 cells were transfected with TCF7L2 shRNA or lenti-TCF7L2 virus for 48 h and then the expressions of total and phosphorylated ERK were evaluated by western blot respectively (A-D). (n = 3 samples per group, *P<0.05 as indicated. NC, negative control).

Figure 2
Figure 3

MIN6 cells were cultured with 10uM ERK inhibitor U0126 for 30min prior to TCF7L2 shRNA virus transfection. Then the expressions of Hes1 and Sox9 were evaluated by real-time PCR (A) and insulin concentrations in supernatant were measured by ELISA (B). (n = 3 samples per group, *P<0.05 and **P<0.01 as indicated).

Figure 4

Six-week-old db/db mice were fed either ND or HFD for 2, 6 and 10 weeks, changes of body weight (A), glucose tolerance (B), serum insulin(C) and c-peptide (D) following IPGTT were investigated respectively.
(*P<0.05 and **P<0.01 as indicated. HFD compared with ND).

**Figure 5**

Pancreatic TCF7L2, FoxO1, Sox9 and p-ERK expressions of db/db mice were measured respectively after 2, 6 and 10 weeks of ND or HFD feeding. (*P<0.05 and **P<0.01 as indicated).