Current Science
CS 6 (2), 6059-6073 (2026)
Received 13 February 2025 | **Accepted 24 March** 2025 | **Online Available 26 March** 2026
https://doi.org/10.52845/CS/2026-6-2-7

Current Science

Current Science

OPEN ACCESS

ISSN (O) 2795-8639

Original Article

Molecular Mechanisms of TGF-β1 Regulating Mesenchymal Stem Cell Function and angiogenesis through Upregulation of SDC4

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Abstract:

Diabetes mellitus is characterized as a chronic metabolic disorder marked by persistent hyperglycemia. This sustained elevation in blood glucose compromises angiogenesis and contributes to endothelial dysfunction.This study investigates the molecular mechanisms by which transforming growth factor-β1 (TGF-β1) regulates syndecan-4 (SDC4) to modulate Mesenchymal Stem Cell (MSC) function and promote vascular endothelial angiogenesis in type 2 diabetes (T2D). Serum analysis of T2D patients revealed a significant positive correlation between TGF-β1 and SDC4 levels, suggesting a potential regulatory relationship. The results demonstrate that TGF-β1 upregulates SDC4 expression in MSCs through Smad3 activation, which enhances MSC viability and migration. SDC4-mediated suppression of the Wnt/β-catenin signaling pathway led to the observed reduction in β-catenin protein levels. On one hand, SDC4 mitigates vascular endothelial inflammation and oxidative stress by inhibiting the expression of IL-6, IL-1β, and VCAM-1. On the other hand, it is notable that SDC4 modulation can partially counteract the TGF-β1-induced downregulation of key angiogenic factors like VEGF, bFGF, and HGF. This study indicates that SDC4, as a key regulatory molecule in the vascular endothelial self-protection mechanism, can respond to VEGFA signals and interact with vascular endothelial cadherins, thereby promoting angiogenesis. This study demonstrates TGF-β1-induced SDC4 expression in MSCs as a novel therapeutic avenue for managing diabetic vascular complications.

Keywords: Diabetes, TGF-β1, mesenchymal stem cells, angiogenesis, vascular endothelial dysfunction, Wnt/β-catenin signaling.

Introduction

Diabetes mellitus represents a widespread metabolic disorder, currently impacting over 415 million adults globally. This number is estimated to rise to approximately 700 million by the year 2045 (1). Persistent high blood sugar, as a typical feature of this disease, triggers a series of pathological and physiological changes, leading to complications in multiple tissues and blood vessels throughout the body. Dysregulation of vascular endothelial cell generation is a key factor in the progression of clinical manifestations related to diabetes (2). Physiological angiogenesis formation improves perfusion and nutrient supply, promoting tissue angiogenesis, while high blood sugar in diabetes disrupts this important process through endothelial dysfunction (3). This functional disorder is clinically manifested as delayed wound healing, impaired formation of collateral vessels in the coronary artery, vascular malformations in pregnant diabetic fetuses, and

increased incidence of transplant rejection.

Mesenchymal stem cells (MSCs) not only have the potential for multi-directional differentiation to promote tissue regeneration, but also can act on recipient cells through paracrine effects to promote tissue angiogenesis and angiogenesis (4). Studies have shown that the proteins secreted by MSCs can promote vascular endothelial formation, thereby accelerating the healing process of diabetic wounds (5). Further research has found that the conditioned medium of MSCs has a angiogenesising effect on vascular endothelial damage induced by high sugar environment. Proteomics sequencing analysis revealed that in the secreted proteins of MSCs with vascular angiogenesis function, the expression of SDC4 was significantly upregulated, suggesting that SDC4 may be a key mediator for restoring vascular endothelial dysfunction (6).

The syndecan (SDC) family belongs to the heparan sulfate proteoglycans (HSPGs) in mammals, consisting of four different genes encoding transmembrane proteins (SDC1-4). Among them, SDC4 (Syndecan-4) is the most widely distributed subtype in this family (7), closely related to the metabolic syndrome phenotype (8-10), and exerts a pivotal influence over energy homeostasis by governing lipid and glucose metabolic processes (11). Studies have shown that SDC4 can act as a downstream mediator of the vascular endothelial growth function response mediated by VEGFA. This protein is located at the junction of endothelial cells and interacts with vascular endothelial cadherin, becoming an important component of the response to the VEGFA signaling pathway (12). It is noteworthy that in SDC4-/- cells and tissues, reintroducing SDC4 can restore normal angiogenic responses (12), indicating that SDC4 may have important intervention potential in angiogenesis disorders caused by high sugar environment. Zhu et al. (13) reported that after knockdown of SDC4, inflammatory factors related to endothelial injury (IL-6, IL-1β, and VCAM-1) significantly increased, leading to pathological changes such as thickening of the vascular wall, infiltration of inflammatory cells, and formation of a transparent membrane, highlighting the critical function of SDC4 in modulating endothelial inflammatory responses. It should be noted that vascular endothelial dysfunction is often accompanied by inflammatory cascades, and the role of SDC4 in angiogenesising vascular endothelial damage and promoting angiogenesis may mainly depend on its precise regulation of the inflammatory process.

Astudillo et al. (14) studied that SDC4 can inhibit the Wnt/β-catenin signaling pathway. This inhibitory effect is achieved by regulating LRP6 at the cell membrane level. When SDC4 levels are low, the ligand R-spondin3 tends to enhance the classical Wnt/β-catenin signaling pathway, indicating that SDC4 can regulate the regulatory ability of R-spondin on different Wnt signaling pathways. A large amount of evidence shows that the Wnt/β-catenin signaling pathway is involved in the vascular endothelial damage process, and its activation mainly leads to endothelial dysfunction through inducing oxidative stress and inflammatory responses (15-17). Therefore, it can be inferred that SDC4 may help alleviate vascular endothelial dysfunction by inhibiting the Wnt/β-catenin pathway.

Although SDC4 plays a significant role in the angiogenesis of vascular endothelial damage, its involvement in the physiological and pathological changes of diabetes has received little attention. The regulatory mechanisms controlling SDC4 expression, particularly by cytokines or growth factors like TGF-β1, are still poorly defined. Therefore, this study investigates the regulatory effect of TGF-β1 on SDC4 and seeks to elucidate the molecular mechanisms by which SDC4 modulates mesenchymal stem cell function and influences angiogenesis.

Materials and Methods

ELISA

This study retrospectively analyzed data on serum concentrations of TGF-β1 and SDC4, which were determined by ELISA in the clinical laboratory of The Affiliated Huaian No.1 People's Hospital of Nanjing Medical University from September to October 2025, including samples from 20 diabetic patients (aged 6–30 years) and 20 age-matched healthy controls.

Extraction and Culture of BMSCs

After euthanizing rats by cervical dislocation, we isolated bone marrow mesenchymal stem cells (BMSCs, is explicitly defined as a subtype of MSCs) from rat femurs by flushing the marrow cavity with a syringe. All animal procedures, including the isolation of primary cells, were approved by the Department of Experimental Animal Resources (Approval No.: DWSY-2024-03-25) and conducted in accordance with relevant guidelines. The harvested cells were then maintained in α-MEM medium containing 10% fetal bovine serum (Gibco, USA, A5669801) at 37°C with 5% CO_2, and the medium was refreshed every 2-3 days. Using cells with a fusion rate of 80%-90% and which have been stably cultured for 3 to 5 generations for subsequent experiments. Flow cytometry was used to detect relevant markers (CD11b, CD34, CD44, CD45, CD73 and CD90) to identify the characteristics of BMSCs.

TGF-Beta Treatment

For the induction experiments, cells were treated with recombinant human TGF-beta 1 protein (Gibco, Human TGF-beta 1 Recombinant Protein, PeproTech®, USA, 100-21-50UG) at a concentration of 5 ng/mL for 24 to 48 hours.

Cell Transfection

Knockdown of SDC4 gene expression was performed using LipofectamineTM 2000 (Invitrogen, USA, 11668500), according to the protocol provided by the manufacturer. Briefly, cells were reversely transfected with SDC4 siRNA (Rongyou, China, si-3588451) for 5 h followed by a 5 h forward transfection with a 16 h interval between the two transfections. The sequences of si-3588451 is 5'-UCUUCAA CGGGUGAGAUUCUC-3' and 5'-GAAUCUCACCCGUUGAAGAGA-3'. Another siRNA duplex, synthesized and annealed by Rongyou Biotechnology Co., Ltd. with the target sequences 5’-UCUUCAUACGGUACAUGAGCA-3’ and 5’-CUCAUGUACCGUAUGAAGAAG-3’, was also used to confirm the effect of syndecan-4 knockdown in BMSCs.The sequences of siCTRL is 5'-UUCUCCG AACGUGUCACGUTT-3' and 5'-ACGUGACACGUUCGGAGAATT-3'.

In the meantime, the shRNA sequences is shSDC4-1-F-5’-CCGGTCCCTTGG TGCCTCTAGATAACTCGAGTTATCTAGAG GCACCAAGGGATTTTTG-3’; shSDC4-1-R-5’-AATTCAAAAATCCCTTGGTGCCTCTAGAT AACTCGAGTTATC

TAGAGGCACCAAGGGA-3’. shSDC4-2-F-5’-CCGGGGCAGGAATCTGATGACT TTGCTCGAGCAAAGTCATCAGATTCCTGC CTTTTTG-3’; shSDC4-2-R-5’-AAT TCAAAAAGGCAGGAATCTGATGACTTTGC TCGAGCAAAGTCATCAGATTCCTGCC-3’. shCtrl-F-5’-CCGGCAACAAGATGAAGAGCACCAACTC GAGTTGGT GCTCTTCATCTTGTTGTTTTTG-3’; shCtrl-R-5’-AATTCAAAAACAACAAGATG AAGAGCACCAACTCGAGTTGGTGCTCTTC ATCTTGTTG-3’.

For overexpression, the full-length cDNA of human SDC4 was cloned into the pcDNA3.1(+) provided by Chengdu Rongyou Biotechnology Co., Ltd.), designated as the pcDNA3.1(+)-SDC4 plasmid. The empty vector was used as a control.

RT-qPCR

Total RNA was isolated from cells with the PureLink™ RNA Microextraction Kit (Invitrogen, USA, 12183016). Following RNA quantification, the expression levels of SDC4 were assessed by RT-qPCR with the TaqPath™ 1-Step RT-qPCR Master Mix (Applied Biosystems, USA, A15299), using β-actin as the internal reference. The relative mRNA expression was calculated based on the 2-ΔCt method. The primer sequences used in this study were as follows:

β-actin(Fw:CCACCATGTACCCTGGCATT;Rv:GTCCTCGGCCACATTGTGAA),
SDC4(Fw:TACTTCTCCGGAGCCCTACC;Rv:AGACCGGCTGTGAAAGGAAG).

Western Blot

Protein extraction was performed using cell lysis buffer (Invitrogen, USA, FNN0011). Protein concentration was quantified using the Pierce™ BCA Protein Assay Kit (Thermo Scientific, USA, 23225), followed by SDS-PAGE and transfer to PVDF membranes. The membranes were incubated with the following primary antibodies: Anti-Syndecan 4 (abcam, UK, ab74139), Anti-Smad3 (abcam, UK, ab40854), Anti-SMAD3 (pS423/425) (abcam, UK, ab52903), Anti-beta Catenin non-phospho (Ser37/Thr41) (abcam, UK, ab246504), and Anti-GAPDH (abcam, UK, ab8245). Protein bands were detected using the ECL Plus Western Blotting Substrate (Thermo Scientific, USA, 34579).

CCK-8

Cells were plated in 96-well plates at a density of 5,000 cells per well in 100 μL of medium and cultured for 24 hours under standard conditions (37°C, 5% CO_2). Cell viability was determined using the Cell Counting Kit-8 (MCE, USA, HY-K0301). Briefly, 10 μL of CCK-8 reagent was introduced into each well, and the plates were incubated for an additional 1 hour. Absorbance at 450 nm was then recorded with a microplate reader to evaluate cell viability.

Cell Cycle Detection

Flow cytometric analysis of cell cycle distribution was carried out using the Cell Cycle and Apoptosis Analysis Kit (PI staining; MCE, USA, HY-K1071), according to the manufacturer's instructions.

Transwell Assay

A Transwell-based assay was used to assess cell migration. The upper chamber received 6×10^4 cells suspended in 400 μL serum-free medium, and the lower chamber contained 600 μL complete medium to induce migration. Cells were first incubated for 24 h, then allowed to migrate for 12 h. Non-migrated cells remaining on the upper side of the membrane were swabbed away. Subsequent steps included fixation in 4% formaldehyde (10 min), two PBS washes, staining with 0.5% crystal violet (5-8 min), and final quantification of migrated cells by microscopy.

Statistical Analysis

Statistical comparisons between two groups were performed with a two-tailed Student's t-test. For experiments involving three or more groups, one-way ANOVA followed by Bonferroni’s post-hoc test was applied. All analyses were carried out using GraphPad Prism software (v10.0). A p-value of less than 0.05 was considered statistically significant, with specific thresholds denoted as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$.

Results

P1: Elevated Serum Levels of TGF-β1 and SDC4 in Type 2 Diabetes Patients

Serological studies have shown that the concentrations of TGF-β1 and SDC4 in patients with type 2 diabetes (T2D) have both increased, and this phenomenon is independent of the occurrence of complications (18, 19). However, whether there is an association between these two factors in expression remains unclear. To explore

the possible regulatory relationship between TGF-β1 and SDC4, We performed statistical analysis on the obtained ELISA data. Consistent with previous reports, serum levels of both TGF-β1 and SDC4 were markedly elevated in T2D patients relative to healthy controls (Figure 1A). Further analysis of the correlation between the two factors in the two groups of samples revealed that in the healthy population, TGF-β1 and SDC4 only showed a weak correlation, while in the T2D group, there was a significant positive correlation (Figure 1B), suggesting that there may be some regulatory connection between the two factors in the T2D pathological environment.

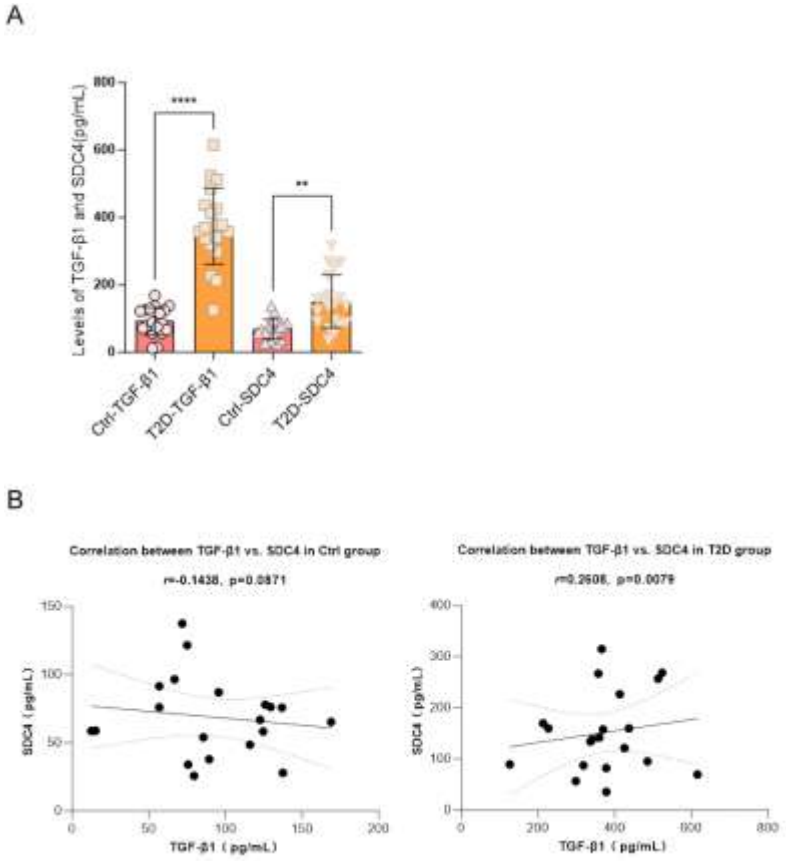

Figure 1. Elevated Serum TGF-β1 and SDC4 Levels in Type 2 Diabetes (T2D) and Their Positive Correlation

A. TGF-β1 and SDC4 Levels in T2D vs. Healthy Controls were measured by performing ELISA. **P < 0.01(Ctrl-SDC4 vs T2D-SDC4); ****P < 0.001(Ctrl-TGF-β1 vs T2D-TGF-β1).

B. the Correlation between TGF-β1 and SDC4 protein levels in control group (left) or in T2D group (right) were presented.

P2: TGF-β1 Treatment Activates the Smad3/SDC4 Signaling Pathway in MSCs

When the vascular endothelial tissue is damaged, bone marrow mesenchymal stem cells (BMSCs) can be recruited to the circulatory system and participate in the angiogenesis process at the damaged site (20, 21). Based on the promoting effect of TGF-β1 on the migration ability of BMSCs, this study further explored whether the interaction between TGF-β1 and SDC4 was involved in the angiogenesis of vascular endothelium. We first isolated BMSCs from the femoral bone marrow of mice and after 7 days of culture, the cells showed the typical spindle-shaped morphology of mesenchymal stem cells (Figure 2A). Flow cytometry detection of cell surface markers revealed that the positive rates of

CD73 and CD90 were 95.6% and 93.6% respectively, while CD11b, CD34, CD44 and CD45 were negative, confirming that the obtained cells had the phenotypic characteristics of MSCs (Figure 2B). After treatment with TGF-β1 for 24 to 48 hours, RT-qPCR results showed that the expression of SDC4 mRNA was significantly upregulated at both time points (Figure 2C). At the same time, the levels of SDC4 protein in cell culture supernatants and total cell lysates (Figure 2D&E) also increased simultaneously, indicating that TGF-β1 not only promotes the expression of SDC4 at the transcriptional level, but also enhances its protein secretion.

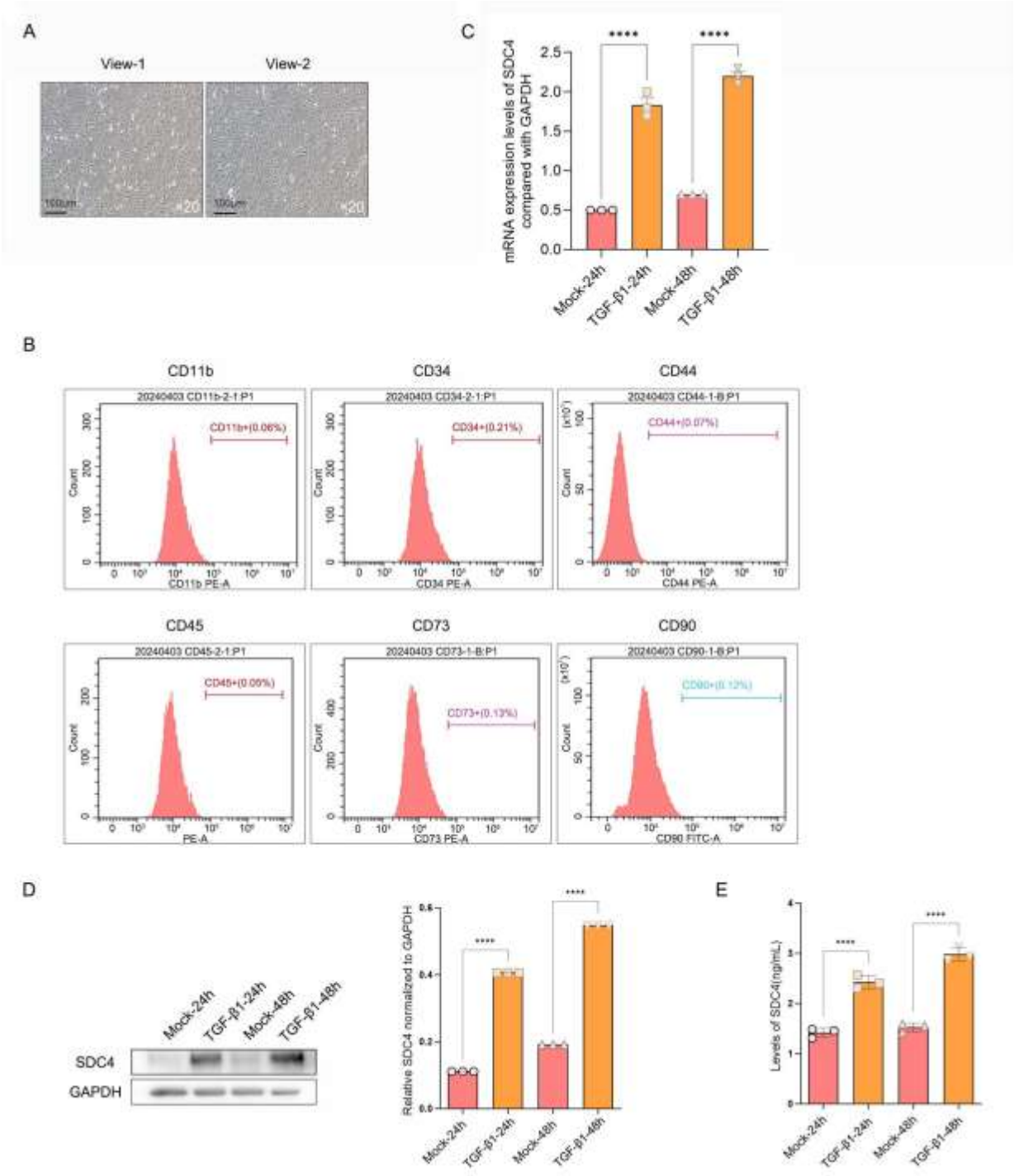

Figure 2. TGF-β1 Upregulates SDC4 Expression and Modulates BMSC Phenotype
A. BMSC Morphology and Surface Marker Characterization. Cultured BMSCs exhibited spindle-shaped morphology after 7 days (scale bar = 100 μm).

B. Flow Cytometry: CD73 (95.6% ± 2.1%) and CD90 (93.6% ± 3.4%) were highly expressed, confirming MSC identity. CD11b, CD34, CD44, and CD45 were minimally detected (<5%).

C. TGF-β1 Induces SDC4 mRNA Expression in a Time-Dependent Manner. RT-qPCR analysis revealed significant SDC4 mRNA upregulation at 24 h and 48 h post-TGF-β1 treatment (5 ng/mL). ****P < 0.001(Mock-24h vs TGF-β1-24h; Mock-48h vs TGF-β1-48h).

D. TGF-β1 Upregulates Total SDC4 Protein in BMSCs

Western blot confirmed elevated SDC4 protein levels in TGF-β1-treated BMSCs (normalized to GAPDH: 1.8 ± 0.2-fold). ****P < 0.001(Mock-24h vs TGF-β1-24h; Mock-48h vs TGF-β1-48h).

E. TGF-β1 Enhances SDC4 Protein Secretion. ELISA demonstrated a dose-dependent increase in secreted SDC4. ****P < 0.001(Mock-24h vs TGF-β1-24h; Mock-48h vs TGF-β1-48h).

P3: TGF-β1 Partially Enhances MSC Viability and Migration by Upregulating SDC4

To assess whether TGF-β1 has a regulatory effect on SDC4, we added TGF-β1 to bone marrow mesenchymal stem cells for treatment, and detected the changes in protein expression using Western blot. As shown in Figure 3, TGF-β1 treatment significantly upregulated the expression level of SDC4 in the cells. Further experiments revealed that after co-treatment with the Smad3 phosphorylation inhibitor SB431542, the increase in SDC4 protein caused by TGF-β1 was effectively inhibited, suggesting that the upregulation of SDC4 by TGF-β1 may mainly be achieved by activating the Smad3 pathway.

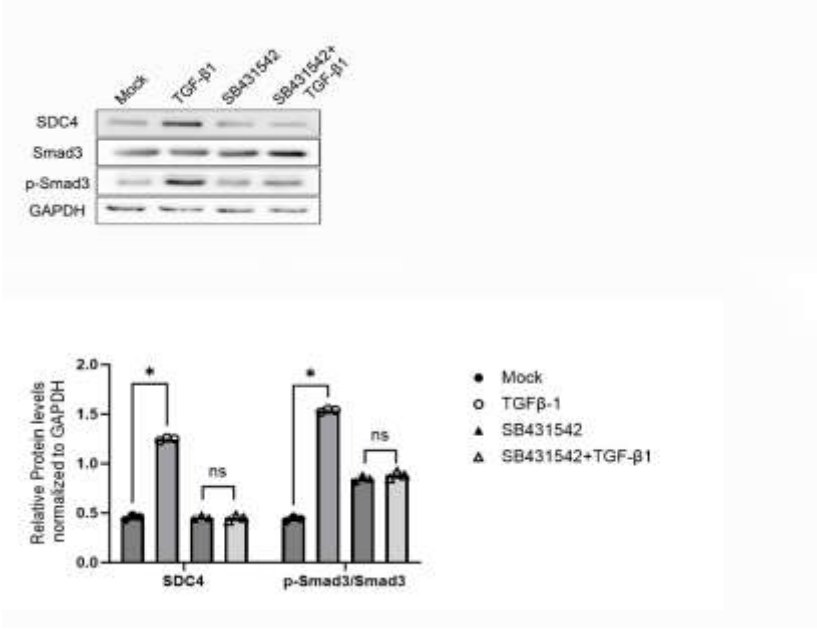

Figure 3. TGF-β1 Upregulates SDC4 via Smad3 Phosphorylation in Mesenchymal Stem Cells After being treated with TGF-β1, the SDC4, Smad3 and phosphorylation of Smad3 (p-Smad3) were measured by performing western blot. ****P < 0.001(Mock vs TGF-β1; SB431542 vs SB431542+TGF-β1).

P4: TGF-β1 Treatment of MSCs Reduces Pro-Angiogenic Factors VEGF, bFGF, and HGF

To deeply investigate the effect of SDC4 on the behavior of mesenchymal stem cells, we first established cell models with overexpression and knockdown of SDC4 (Figure 4A). The cell proliferation experiment showed that inhibiting

SDC4 expression could significantly enhance the proliferation ability of MSCs, while overexpressing SDC4 significantly inhibited cell proliferation (Figure 4B). Cell cycle detection revealed that SDC4 knockdown caused a G2 phase arrest of the cells, and overexpression of SDC4 could significantly alleviate this arrest phenomenon (Figure 4C). In the cell migration experiment, the expression of SDC4 significantly enhanced the migration ability of the cells (Figure 4D), which was in line with the expected results.

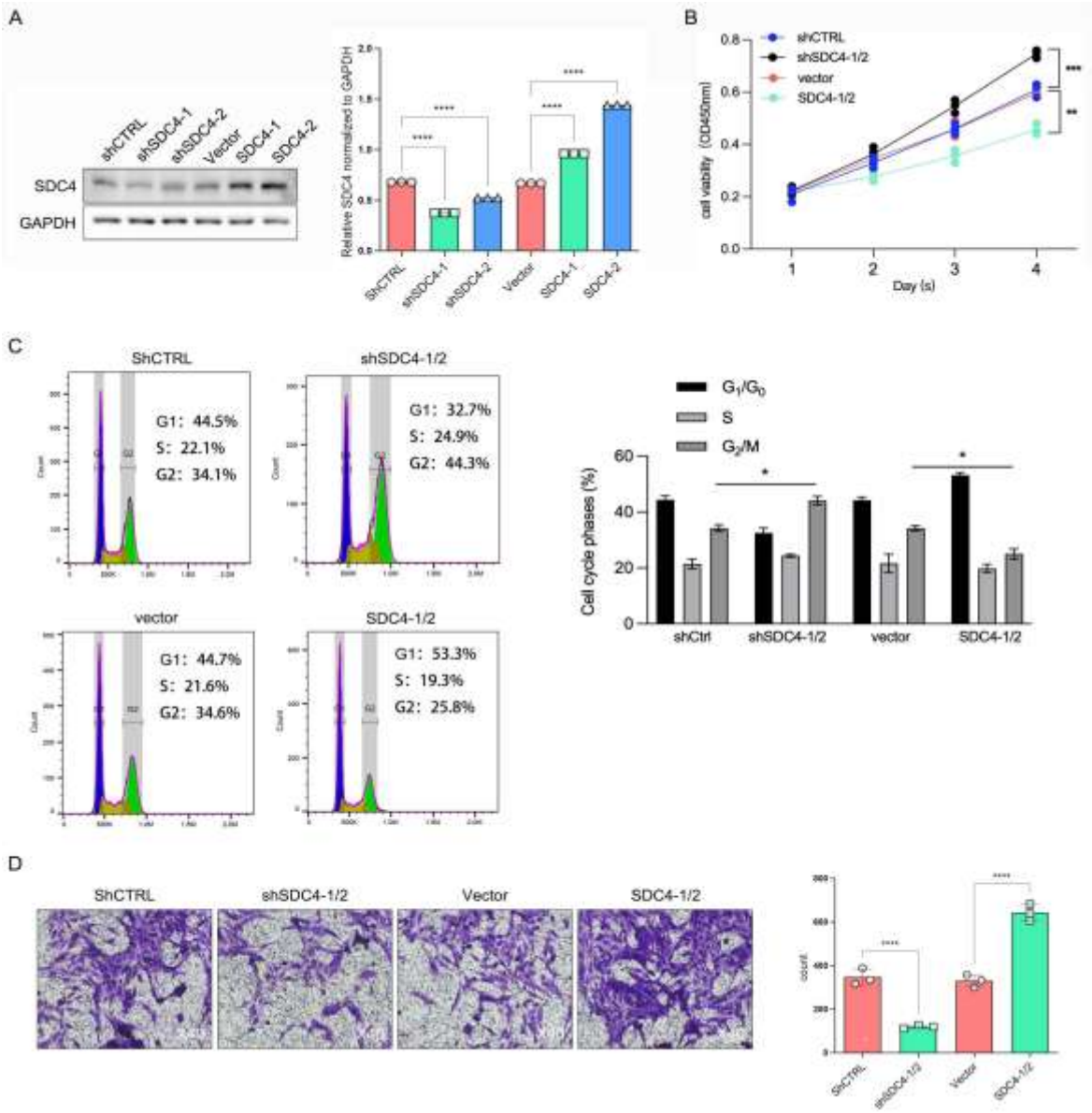

Figure 4. SDC4 Regulates MSC Proliferation, Cell Cycle, and Migration

A. After stably knocking down or overexpressing SDC4, two clones were picked and SDC4 protein level was measured by performing western blot. ****P < 0.001(ShCTRL vs ShSDC4-1; ShCTRL vs ShSDC4-2; Vector vs SDC4-1; Vector vs SDC4-2).

B. Cell viability was measured by performing CCK-8 assay. **P < 0.01(Vector vs SDC4-1/2); ***P < 0.005(ShCTRL vs ShSDC4-1/2).

C. Cell cycle distribution was measured by performing PI staining and flow cytometry assay. *P<0.05 (ShCTRL vs ShSDC4-1/2; Vector vs SDC4-1/2).

D. Cell migration was evaluated by performing transwell assay. ****P < 0.001 (ShCTRL vs ShSDC4-1/2; Vector vs SDC4-1/2).

Based on the research result reported by Giuliani et al. (18) that SDC4 can be used as a serological indicator for vascular endothelial inflammation in diabetic patients, we hypothesized that the protein level changes induced by TGF-β1 might be related to vascular endothelial inflammation. To verify this hypothesis, the expression levels of angiogenesis markers bFGF, VEGF, and HGF in the cell supernatant after TGF-β1 intervention were detected using ELISA. As shown in Figure 5A, TGF-β1 treatment significantly reduced the concentrations of these factors in the supernatant, and the addition of the TGF-β1 inhibitor SB431542 could reverse this downward trend. Further experiments demonstrated that overexpression of SDC4 inhibited the expression of these angiogenesis markers, while interfering with SDC4 promoted their expression levels (Figure 5B).

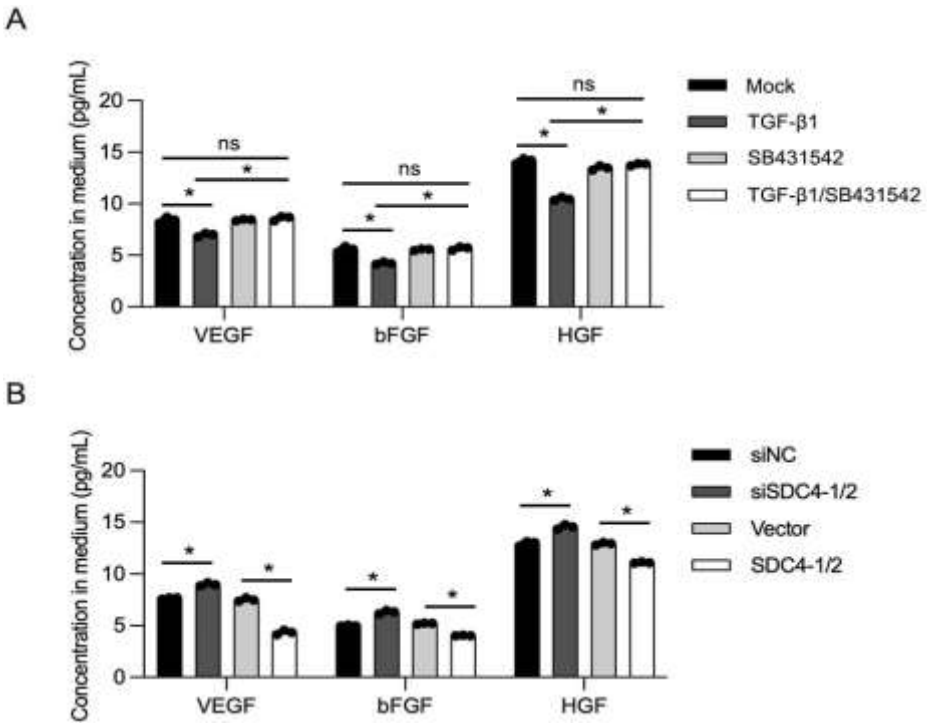

Figure 5. TGF-β1 Regulates Angiogenic Factors Through SDC4 in Endothelial Inflammation

A. TGF-β1 Suppresses Pro-Angiogenic Factors, Which Is Rescued by SB431542. TGF-β1 treatment (10 ng/mL) significantly reduces VEGF, bFGF, and HGF secretion levels in endothelial cells compared to the control. Co-treatment with SB431542 (1 μM) restores angiogenic factor levels to baseline. *P<0.05 (Mock vs TGF-β1; TGF-β1 vs TGF-β1/SB431542).

B. Overexpression of SDC4 (Ad-SDC4) decreases VEGF, bFGF, and HGF levels compared to empty vector controls. SDC4 Knockdown (siSDC4) further increases angiogenic factors, with statistical significance. *P<0.05 (siNC vs siSDC4-1/2; Vector vs SDC4-1/2).

P5: SDC4 Suppresses β-Catenin and the Wnt/β-Catenin Signaling Pathway

To assess whether TGF-β1 regulates the Wnt/β-catenin signaling pathway by activating SDC4, we examined the effect of TGF-β1 on the transcriptional activity of β-catenin. As shown in Figure 6A (left), in bone marrow mesenchymal

stem cells, TGF-β1 treatment significantly inhibited the activation ability of Wnt3a on the TOPFlash reporter gene. In contrast, after siRNA knockdown of SDC4, the activity of the TOPFlash reporter gene induced by Wnt3a was significantly enhanced (Figure 6A, right). To further verify whether the inhibition of TGF-β1 on the transcriptional activity of β-catenin can be reversed by knockdown of SDC4, we transfected siSDC4 into TGF-β1-treated cells, and found that SDC4 knockout significantly increased the activity of the TOPFlash reporter gene (Figure 6B), indicating that TGF-β1 inhibits the β-catenin signaling pathway by upregulating SDC4 expression. Western blot results showed that TGF-β1 treatment significantly reduced the protein level of β-catenin, while knockdown of SDC4 increased its protein expression (Figure 6C). In summary, this study confirmed that TGF-β1 inhibits the β-catenin protein level by upregulating SDC4 expression.

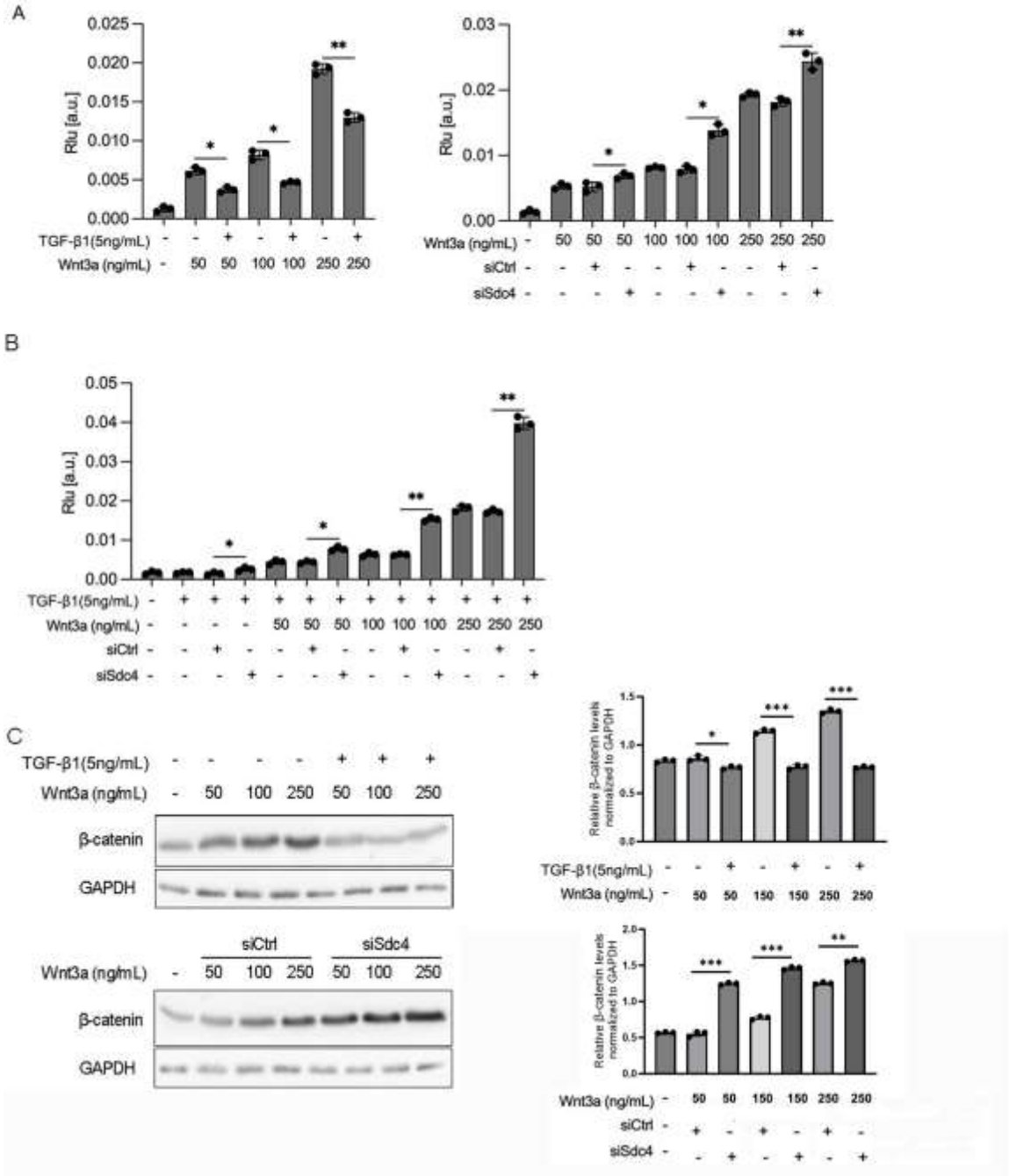

Figure 6. TGF-β1 Inhibits Wnt/β-catenin Signaling via Upregulation of SDC4 in BMSCs

A. TGF-β1 Suppresses Wnt3a-Induced TOPFlash Activity, Which Is Reversed by SDC4 Knockdown; Left panel: TGF-β1 treatment (5 ng/mL) significantly inhibits Wnt3a-induced TOPFlash reporter activity in BMSCs compared to control , *P<0.05, **P<0.01 (At the same concentration of Wnt-3α, Mock vs TGF-β1) Right panel: SDC4 knockdown (siSdc4) potently enhances TOPFlash activity in the presence of Wnt3a. *P<0.05, **P<0.01 (At the same concentration of Wnt-3α, siCtrl vs siSdc4).

B. SDC4 Knockdown Rescues TGF-β1-Mediated Inhibition of β-catenin Transcriptional Activity. *P<0.05, **P<0.01 (At the same concentration of Wnt-3α, TGF-β1/siCtrl vs TGF-β1/siSdc4)

C. Western blot analysis was performed to present TGF-β1 treatment significantly reduced β-catenin protein levels, while siSdc4 knockdown significantly increased β-catenin protein levels.*P<0.05, ***P<0.001 (At the same concentration of Wnt-3α, Mock vs TGF-β1); **P<0.01, ***P<0.001 (At the same concentration of Wnt-3α, siCtrl vs siSdc4).

Discussion

In this study, we demonstrated a clear correlation between TGF-β1 and SDC4 in diabetes. TGF-β1 can upregulate SDC4 protein expression in mesenchymal stem cells by activating Smad3. In turn, SDC4 suppresses inflammation and oxidative stress in vascular endothelium by inhibiting β-catenin and the Wnt/β-catenin signaling pathway, thereby mitigating vascular endothelial damage. The above results indicate that under the regulation of TGF-β1, mesenchymal stem cells may help improve the abnormal angiogenesis caused by diabetic endothelial dysfunction. Combined with our findings there may be a bidirectional regulatory relationship between TGF-β1 and SDC4. This study mainly focuses on the upstream regulatory effect of TGF-β1 on SDC4, while whether SDC4 can feedback regulate the expression or activity of TGF-β1 still needs further exploration, which is also one of the key points of our subsequent research.

The precise mechanisms by which SDC4 modulates MSC proliferation and cell cycle progression warrant further discussion. The observed G2/M phase arrest upon SDC4 knockdown suggests a potential disruption in the regulation of cyclin-dependent kinase (CDK) activity and checkpoint control. It is plausible that SDC4 influences the expression or activity of key cell cycle regulators, such as cyclin B1 and CDK1, which are critical for the G2/M transition. Alternatively, SDC4 may modulate the expression of CDK inhibitors (e.g., p21 or p27), thereby restraining cell cycle progression. Furthermore, given the well-established role of SDC4 in integrin signaling and focal adhesion assembly, its impact on proliferation and migration could be mediated through downstream pathways such as ERK/MAPK or PI3K/Akt, which are central to survival, growth, and motility signals. The pro-migratory effect of SDC4 is consistent with its known function in facilitating cytoskeletal reorganization and focal adhesion turnover, processes essential for efficient cell migration. Future studies will specifically investigate the involvement of these candidate pathways in SDC4-mediated MSC functions.

Previous studies have confirmed that diabetes can affect angiogenesis through various pathways such as oxidative stress, inflammation, and epigenetic modifications (3). In this study, the inhibitory effect of SDC4 on the expression of angiogenic factors VEGF, bFGF, and HGF was not consistent with the initial hypothesis, but its inhibitory effect on β-catenin and the Wnt/β-catenin signaling pathway was in line with expectations, suggesting that SDC4 may play a multifaceted and complex role in a high-sugar environment. It is known that SDC4 is highly

expressed in endothelial cells and its expression is regulated by inflammation (18). When the inflammatory level increases, the expression of SDC4 rises, thereby inhibiting angiogenesis; however, this inhibitory effect is not long-lasting. Upon reaching a specific threshold, SDC4 suppresses the β-catenin and Wnt/β-catenin pathways, consequently reducing the expression of inflammatory factors including IL-6, IL-1β, and VCAM-1 (13). Thereby alleviating the inflammatory response and reducing persistent vascular endothelial damage.This mechanism belongs to the self-protection of vascular endothelial cells. Under this effect, SDC4 acts as a regulatory molecule, continuously preventing inflammatory damage. Upon recruitment to sites of vascular injury, mesenchymal stem cells secrete SDC4 in a TGF-β1/Smad3-dependent manner. This SDC4-mediated response suppresses β-catenin and Wnt/β-catenin signaling even in the absence of sustained inflammation, thereby promoting the angiogenesis of damaged vascular endothelium.

The apparent dual role of SDC4—suppressing both pro-inflammatory cytokines and pro-angiogenic factors—highlights the pleiotropic nature of syndecan family members in regulating tissue homeostasis. This complexity is consistent with established literature showing that proteoglycans like SDC4 can exert context-dependent effects on angiogenesis by modulating the availability and signaling of various growth factors and cytokines within the pericellular environment (22, 23). In the pathological context of diabetes, characterized by excessive inflammatory signaling and aberrant angiogenesis, the primary function of MSC-derived SDC4 may be to restore balance by first dampening the inflammatory cascade. Its inhibitory effect on factors like VEGF and bFGF could represent a secondary mechanism to prevent pathological, inflammation-driven angiogenesis, thereby creating a permissive environment for subsequent orderly vascular repair. This sequence of events aligns with the concept that effective vascular regeneration requires the coordinated resolution of inflammation before the initiation of proper tissue rebuilding.

Our findings reveal a seemingly paradoxical role of SDC4: it suppresses key pro-angiogenic factors (VEGF, bFGF, HGF) yet ultimately promotes vascular endothelial generation. This apparent contradiction underscores the pleiotropic and context-dependent nature of SDC4 in vascular biology. We propose a model to reconcile these observations: in the pathological milieu of diabetes, characterized by excessive inflammation and oxidative stress, the primary and immediate beneficial function of MSC-derived SDC4 is to restore endothelial homeostasis. By inhibiting the Wnt/β-catenin pathway, SDC4 effectively dampens the inflammatory cascade and reduces oxidative stress, which are fundamental barriers to effective vascular repair (15-17). The concomitant suppression of certain angiogenic factors may represent a crucial mechanism to prevent aberrant, inflammation-driven angiogenesis, thereby facilitating a transition from a pro-inflammatory to a pro-repair microenvironment. This sequence of events—resolving inflammation prior to initiating tissue rebuilding—is essential for orderly vascular regeneration. Consequently, the net effect of SDC4, by creating a healthier endothelial microenvironment conducive to proper signaling, is the promotion of functional vascular endothelial generation. This context-dependent duality aligns with established literature on proteoglycans, which can exert complex, fine-tuning effects on growth factor availability and signaling within the pericellular space (22, 23).

A limitation of this study is that the proposed TGF-β1/SDC4 signaling axis was investigated primarily through in vitromodels and clinical serum analysis. While these approaches provide

compelling mechanistic insights, future studies utilizing in vivodiabetic animal models are essential to validate our findings and confirm the translational relevance of this pathway for treating diabetic vascular complications.At the same time, the translational potential of our findings should be considered in the context of the models used. Our mechanistic discoveries are primarily based on rat BMSCs, whereas the ultimate clinical application targets human biology. Although the TGF-β/Smad3 and Wnt/β-catenin pathways are fundamentally conserved, potential species-specific differences in regulatory details cannot be ruled out. Therefore, a critical next step will be to replicate key experiments in human mesenchymal stem cells to validate this axis and assess its therapeutic potential for human diabetic vascular complications.

MSC-derived SDC4 ameliorates hyperglycemia-induced vascular endothelial impairment in diabetes by not only facilitating endothelial generation via VEGFA-cadherin signaling but also mitigating inflammation and oxidative stress through cytokine regulation and Wnt/β-catenin suppression, thereby promoting tissue recovery.

Acknowledgments: The authors thank the patients and their families. The authors also thank Dr. Zhao for his editorial assistance in preparing this manuscript.

Funding: No funding was received.

Data Availability Statement: The data generated in this study are available upon request from the corresponding author.

Consent for Publication: All authors reviewed and approved the final manuscript. Shanshan Jin is responsible for the overall content as the guarantor.

Competing Interests: The authors declare no potential conflicts of interest.

Author's Contributions: Dezhen Liu: Conceptualization; methodology; investigation; formal analysis; visualization; Writing original draft; writing review and editing.

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