Dose-Response:
An International Journal
January-March 2022:1–11

DOI: 10.1177/15593258221083486
journals.sagepub.com/home/dos
SAGE

Dexmedetomidine Protects Against Kidney Fibrosis in Diabetic Mice by Targeting miR-101-3p-Mediated EndMT

Li Song[1], Songlin Feng[2], Hao Yu[2], and Sen Shi[2,3,4]

Abstract

Objective: Our main purpose is to explore the effect and mechanism of Dexmedetomidine (DEX) in diabetic nephropathy fibrosis.

Methods: Diabetic model was established by intraperitoneal injection of streptozotocin (STZ) treated CD-1 mice and high glucose cultured human dermal microvascular endothelial cells (HMVECs). Immunofluorescence was used to detect renal endothelial-mesenchymal transition (EndMT); Hematoxylin and Eosin (HE) staining and Masson's Trichrome Staining (MTS) was used to analyze renal fibrosis; CCK-8 was used to evaluate cell viability; Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was used to assess the expression of miR-101-3p; Western blots were utilized to judge the protein expression levels of EndMT, extracellular matrix and TGF-β1/Smad3 signal pathway.

Results: In this study, we first found that the protective effect of DEX on DN was related to EndMT. DEX alleviated kidney fibrosis by inhibiting EndMT in diabetic CD-1 mice. DEX could also inhibit high glucose-induced HMVECs EndMT. Then, we confirmed that miR-101-3p was the regulatory target of DEX. The expression of miR-101-3p was decreased in diabetic CD-1 mice and high glucose-induced HMVECs. After DEX treatment, the miR-101-3p increased, and the inhibition of miR-101-3p could counteract the protective effect of DEX and aggravate the EndMT. Finally, we found that the TGF- β1/Smad3 signal pathway was involved in the protective effect of DEX on DN. DEX inhibited the activation of TGF-β1/Smad3 signal pathway. On the contrary, inhibiting miR-101-3p promoted the expression of TGF-β1/Smad3.

Conclusion: DEX protects kidney fibrosis in diabetic mice by targeting miR-101-3p-mediated EndMT.

Keywords

diabetic nephropathy, dexmedetomidine, kidney fibrosis, miR-101-3p, endothelial-mesenchymal transition, TGF-β/SMAD3 signaling pathway

Introduction

Diabetes is a metabolic disease characterized by persistent hyperglycemia, and the number of people with diabetes is expected to increase to 642 million by 2040.[1] Diabetic nephropathy (DN) is a progressive fibrosis nephropathy, and approximately one-third of diabetic patients will eventually develop DN and result in renal failure.[2] Our previous studies have shown that endothelial–mesenchymal transition (EndMT) occurs in DN and that inhibition of EndMT can alleviate the progression of fibrosis nephropathy.[3,4]

Some drugs have been found to inhibit renal fibrosis by inhibiting EndMT. For example, sodium glucose cotransporter 2 (SGLT2) inhibitors empagliflozin protects renal fibrosis by

[1]Department of Anesthesiology, The Affiliated Hospital of Southwest Medical University, Luzhou, China
[2]Department of Vascular Surgery, The Affiliated Hospital of Southwest Medical University, Luzhou, China
[3]Key Laboratory of Medical Electrophysiology, Ministry of Education, Collaborative Innovation Center of Prevention and Treatment of Cardiovascular Disease of Sichuan Province, Luzhou, China
[4]Cardiovascular and Metabolic Diseases Key Laboratory of Luzhou, Luzhou, China

Received 30 November 2021; accepted 8 February 2022

Corresponding Author:
Sen Shi, Department of Vascular Surgery, The Affiliated Hospital of Southwest Medical University, 23th taiping Road, Luzhou 646000, China.
Email: 50242042@qq.com

inhibiting glucose reabsorption and abnormal glycolysis in renal tubules.[5] Dexmedetomidine (DEX), one of the common drugs in anesthesiology, is a highly selective α2-adrenergic receptor agonist.[6] Previous studies have shown that DEX has a protective effect on DN, yet the mechanism is not clear.[7-9]

MicroRNA (miRNA) is a small non-coding RNA molecule of approximately 19–25 nucleotides, which regulates the gene expression at both the transcriptional and post-transcriptional levels by targeting the 3′ untranslated region of mRNA.[10] MiRNA is well known to regulate a range of diverse biological processes including EndMT.[11]

For example, Sun et al confirmed that the microRNA crosstalk of miR-133b and miR-199b attenuate TGF-β1-induced epithelial to mesenchymal transition and renal fibrosis by targeting SIRT1 in diabetic nephropathy.[12] Yang et al found that miR-101-3p was significantly down-regulated in circulating miRNA expression profiles in patients treated with DEX.[11] In addition, other studies suggested that miR-101-3p is related to the regulation of acute kidney injury–chronic kidney disease transition and renal fibrosis.[13,14] However, the role of DEX, miR-101-3p, and EndMT in diabetic renal fibrosis remains unclear. Therefore, we explored the possible mechanism of DEX in diabetic renal fibrosis and its potential therapeutic effect in a STZ-induced diabetic CD-1 mouse model, examining the mechanism of DEX in kidney fibrosis in association with miR-101-3p-mediated EndMT.

Materials and Methods

Animal Model and Treatment

All animal experimental procedures complied with the principles of the Ethics Committee of the Affiliated Hospital of Southwest Medical University. Eight-week-old male CD-1 mice (Dossy Laboratory Animal Co. Ltd., Chengdu, China) were used in this study. Before the experiment, the CD-1 mice were accommodated for one week. We used a previously described mice diabetic model of kidney fibrosis.[5] The model mice were administered with a single intraperitoneal injection of STZ (200 mg/kg); control mice were injected with citrate buffer. Two weeks after the STZ injection, mice with blood glucose levels >16 mmol/L were confirmed as valid diabetic mice and used for this study. By 20 weeks after the induction of diabetes, the diabetic mice were divided into a model group, Dex group, miR-101-3p inhibitor group, and scr-miR control group. The Dex group was given 1, 5, 10, or 100 μg/kg of Dex, respectively, and the control group was given an equal volume of normal saline. Then 4 weeks after injection of DEX and miR-101-3p inhibitor or negative controls, the mice were sacrificed. All mice were fasted for 12 h before anatomy and sampling, then the mice were anesthetized with 10% chloral hydrate, and taken their blood and kidneys for further study. The blood was placed at room temperature for 2 h, then centrifuged at 4°C, 3500 r/min for 15 min, and the upper serum was collected and stored at −20°C for spare use. Kidney tissues were stored at −80°C for histological, RNA, and protein analyses.

Cell Culture

Human dermal microvascular endothelial cells (HMVECs, Lonza, Basel, Switzerland) were grown in endothelial basal medium (EBM) (Lonza, Basel, Switzerland) containing 10% fetal bovine serum (FBS, Gibco), 100 units/ml penicillin (Sigma-Aldrich), and 100 μg/ml streptomycin (Sigma-Aldrich) in 5% CO2 humidified atmosphere at 37°C. When HMVECs reached 70% confluence, they were treated with 50 mM glucose (Sigma-Aldrich) for 48 h.

Cell Viability Assay

HMVECs cell viability was assessed using Cell Count Kit-8 (CCK-8, Biosharp, Hefei, China). In each group, HMVECs (100 μl) were seeded in a 96-well plate at a density of (1-2) $X10^4$ cells/ml overnight. The cells were first cultured with high glucose for 48 h, and then cultured in 100 μl high-sugar media containing 10% CCK-8 for another 1 h. The absorbance at 450 nm was measured using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

Transfection

The lentiviral vector, inhibitor, and the negative control of the mice miR-101-3p-inhibitor were all from Vigene Biosciences (Jinan, Shandong, China). Delivery of lentivirus in vivo was conducted as described earlier. The miR-101-3p inhibitor lentivirus was injected into mice by intravenous injection (1x109 TU/ml, 150 ml per mouse) 20 weeks after the induction of diabetes, and an empty lentivirus vector was used as a negative control. Mice were euthanized 4 weeks later. The expression of miR-101-3p was analyzed using quantitative real-time PCR (qPCR). For in vitro transfection studies, HMVECs were passaged in 6-well plates with a growth medium, and they were subsequently transfected with 100 nM miR-101-3p-inhibitor using lipofectamine 2000 transfection reagent (Jinan, Shandong, China), according to the instructions. Then, the transfected HMVECs were treated with glucose or DEX for 48 h.

Immunofluorescence

Immunofluorescence was conducted as previously described.[3] Briefly, kidney tissues were fixed and cut into frozen sections (5 μm), and these frozen sections were then dried and placed in acetone for 10 min at −30°C. Once the sections were dried, they were washed twice in phosphate-buffered saline (PBS) for 5 min and then blocked in 2% bovine serum albumin/PBS for 30 min at room temperature. Thereafter, the sections were incubated in primary antibody (1:400) including FSP-1 (cat:

ab197896; Abcam) and cd-31 (cat:ab9498; Abcam) overnight at 4°C. Next, the sections were incubated with the secondary antibodies (1:200, A0516; Beyotime, Shanghai, China) at room temperature for 1 h. Nuclear staining was carried out using DAPI (C1002; Beyotime) at room temperature for 5 min. The immunolabeled sections were analyzed with an Olympus fluorescence microscope (Olympus Corporation, Beijing, China) at an original magnification of 400X.

Histology

The mouse kidney tissues were fixed with 4% paraformaldehyde for 24 h, and paraffin-embedded renal tissues from mice were cut into 10 μm sections. Kidneys sections were stained using HE staining and MTS. The fibrotic area was observed by an optical microscope (Leica Imaging Systems, Cambridge, United Kingdom) at an original magnification of 200X. Masson's trichrome labeled sections were imaged and analyzed with ImageJ software, and fibrotic areas were quantified.

RNA Isolation and Quantitative Real-Time PCR

Total RNA was extracted from renal tissue or cells using Trizol reagent (Foregene, Chengdu, China). Reverse transcription was performed using the Premix RT EasyTM I (Foregene, Chengdu, China). The SYBR Green real-time qPCR Master Mix (Foregene, Chengdu, China) on a Bio-Rad CFX Connect Real-Time qPCR Detection system (Bio-Rad Laboratories, Inc.) was used to perform RT-qPCR following with the manufacturer's instructions. The comparative CT method was used to detect target gene expression in the test samples relative to control samples. The primers were synthesized by RIBOBIO (Guangzhou, China). U6 RNA level was used as a reference. The primers sequences were miR-101-3p: TACAGTACTGTGATAACTGAA (forward) and GCAGGGTCCGAGGTATTC (reverse); U6: CGCAAGGATGACACGCAAAT (forward) and GCAGGGTCCGAGGTATTC (forward), respectively.

Western Blotting

The proteins were extracted from renal tissues and cells using protein lysis buffer (Beyotime Biotechnology Co., Ltd., Shanghai, China). Protein concentrations were determined using a BCA protein assay kit (Beyotime Biotechnology Co., Ltd., Shanghai, China). The protein lysates were separated using 10% SDS gelelectrophoresis and then transferred to a PVDF membrane. After being blocked with 5% skimmed milk/TBST, the membranes were incubated with primary antibodies (1:1000) at 4°C overnight. The membranes were washed three

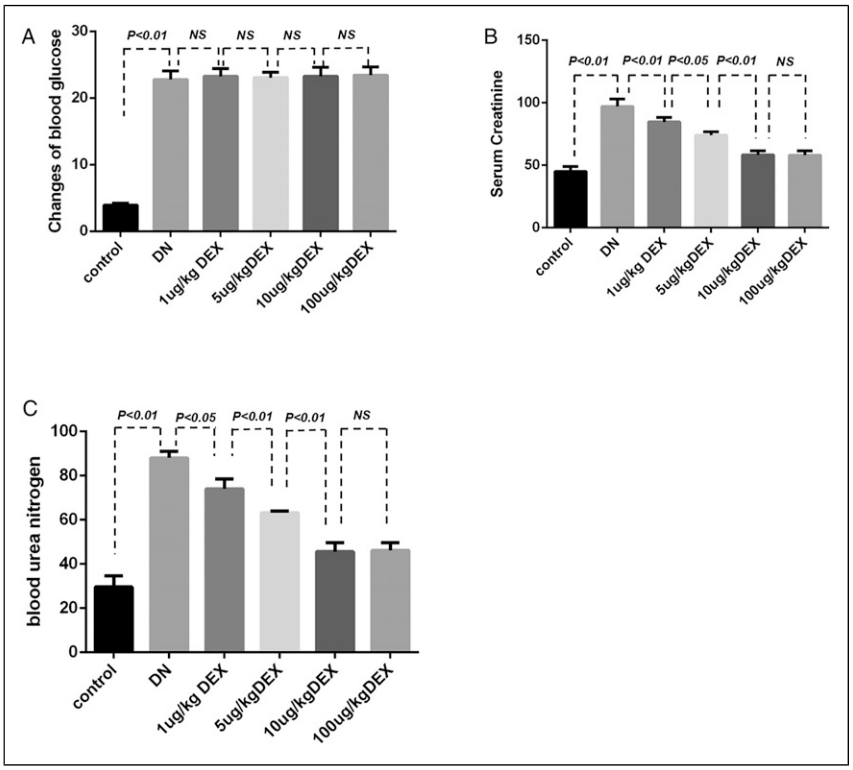

Figure 1. DEX reduced the levels of BUN and Cr in diabetic CD-1 mice. (A) Blood glucose level of diabetic CD-1 mice treated with DEX; **(B–C)** BUN and Cr level of diabetic CD-1 mice treated with DEX. The data are presented as mean ± SE in each group (n = 5) of three independent experiments.

times by TBST and incubated with secondary antibodies (1: 10000) for 1 h at room temperature. The rabbit polyclonal to cd-31 antibody (cat:ab9498; Abcam), rabbit polyclonal to alpha smooth muscle actin (cat: ab5694; Abcam), rabbit polyclonal anti-β-actin (cat: ab8226; Abcam), and rabbit monoclonal anti-fibroblast specific proteins (FSP-1, sometimes displayed as S100A4) antibody (cat:ab197896; Abcam) were all purchased from Abcam (Cambridge, UK). The IRDye 800 CW goat anti-rabbit IgG secondary antibody (cat:92632211; LI-COR) was purchased from LI-COR (Nebraska, USA).

Assessment of Blood Urea Nitrogen and Serum Creatinine

The levels of blood urea nitrogen (BUN) and serum creatinine (Cr) were detected using a full-automatic biochemical analyzer. The assay was conducted according to the manufacturer's protocol.

Statistical Analysis

Data were presented as mean ± SEM values and analyzed using SPSS16.0 software (SPSS Inc., Chicago, IL, U.S.A.). A one-way analysis of variance followed by the Bonferroni test for post hoc analysis was employed in multiple group comparisons, and $P < .05$ was considered statistically significant.

Results

DEX Reduced the Levels of BUN and Cr in Diabetic CD-1 Mice

As shown in Figure 1, DEX could significantly reduce the levels of BUN and Cr (Figure 1B-C), and this effect was most obvious at 10 μg/kg. When the DEX concentration increased to 100 μg/kg, the levels of BUN and Cr had no significant change compared with those at 10 μg/kg, and DEX did not affect the change of blood glucose level in diabetic CD-1 mice (Figure 1 A), indicating that the protective effect of DEX on diabetic CD-1 mice is not achieved by regulating blood glucose.

DEX Significantly Reduced the Degree of Renal Fibrosis in Diabetic CD-1 Mice

As shown in Figure 2, we used HE and MTS to evaluate the degree of renal fibrosis to find that severe fibrosis exhibited 20 weeks after the initiation of diabetic mice. HE results

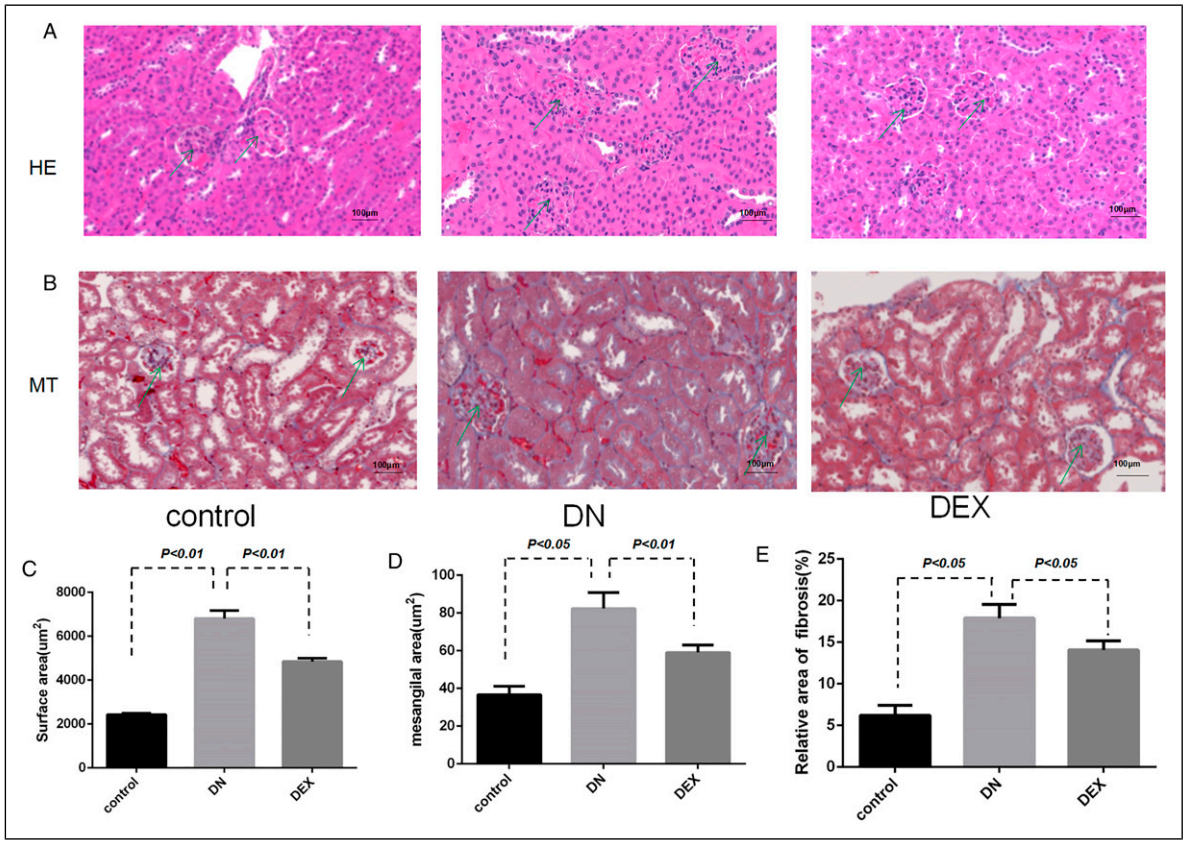

Figure 2. DEX significantly reduced the degree of renal fibrosis in diabetic CD-1 mice. (A–B) Representative images of hematoxylin and eosin, and Masson's trichrome staining used to evaluate fibrosis in the kidney after DEX treatment. Scale bar: 100 μm. **(C–E)** Morphometric analysis of kidney histology. The data are presented as mean ± SE in each group (n = 5) of three independent experiments.

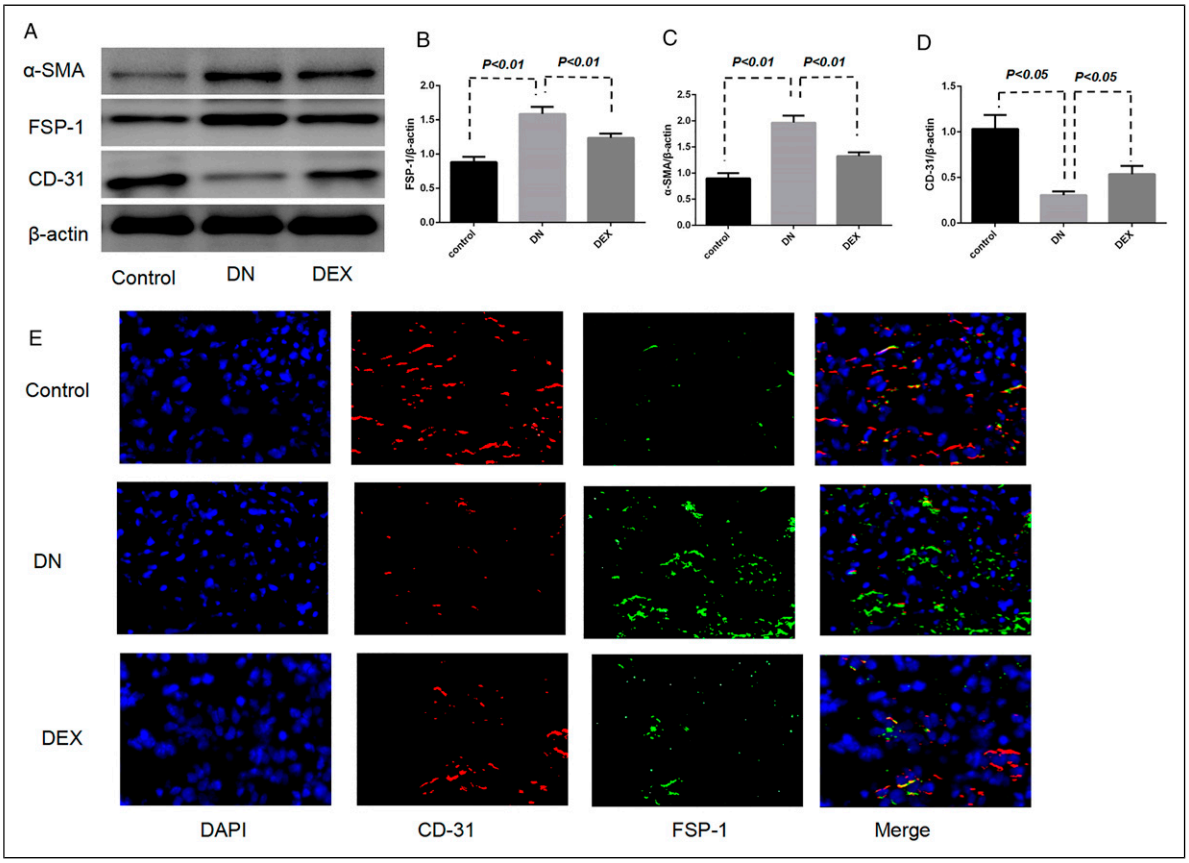

Figure 3. Alleviating effect of DEX on renal fibrosis was associated with inhibition of EndMT in diabetic mice. (A–D) Western blotting analysis of α-SMA FSP-1, cd-31, and β-actin in diabetic CD-1 mice treated with DEX; **(E)** Immunofluorescence analysis of cd-31 and FSP-1. The original magnification was ×400. Scale bar: 100 μm in each panel. The data are presented as mean ± SE in each group (n = 5) of three independent experiments.

showed glomerular hypertrophy and mesangial expansion in diabetic CD-1 mice (Figure 2A). MTS results showed that there was a large amount of collagen deposition in the kidney of diabetic mice (Figure 2B). After 4 weeks of DEX treatment, the above pathological changes were significantly improved, and renal fibrosis was inhibited. These changes in renal tissue structure also corresponded to the renal function changes in diabetic CD-1 mice in Figure 1. These results indicate that DEX can reduce the degree of renal fibrosis in diabetic CD-1 Mice

Alleviating Effect of DEX on Renal Fibrosis Was Associated With Inhibition of EndMT

As shown in Figure 3, to explore the relationship between DEX and EndMT, we used Western blotting and immunofluorescence to analyze the expression levels of CD-31, FSP-1, and α-SMA in the kidney of mice. Western Blotting results showed that the level of endothelial cell marker CD-31 in diabetic CD-1 mice was lower than that in control mice, while the level of mesothelial cell markers FSP-1 and α-SMA increased in diabetic CD-1 mice. 4 weeks after DEX treatment, the expression of CD-31 increased, whereas the expression of FSP-1 and α-SMA decreased (Figure 3A–D). The immunofluorescence results also supported the above experimental results (Figure 3E). These results indicate that EndMT occurs in the kidney of diabetic CD-1 mice, and that DEX can significantly inhibit this transition process.

Ameliorating Effect of Dex on Renal Fibrosis in Diabetic Mice Was Related to the EndMT Regulated by miR-101-3p

As shown in Figure 4, to further confirm whether the regulation of renal EndMT by DEX is achieved by miR-101-3p, we analyzed the expression levels of CD-31, FSP-1, and α-SMA by inhibiting the expression of miR-101-3p in DEX-treated diabetic CD-1 mice. Our qPCR results showed that the expression of miR-101-3p in diabetic CD-1 mice was inhibited and improved after DEX treatment (Figure 4A–B), and Western Blotting results showed that inhibiting the expression of miR-101-3p in DEX-treated diabetic mice could aggravate the EndMT in diabetic mice (Fig. 4C–F). These results suggest that the protective effect of DEX on renal fibrosis in diabetic mice is related to the inhibition of miR-101-3p on EndMT.

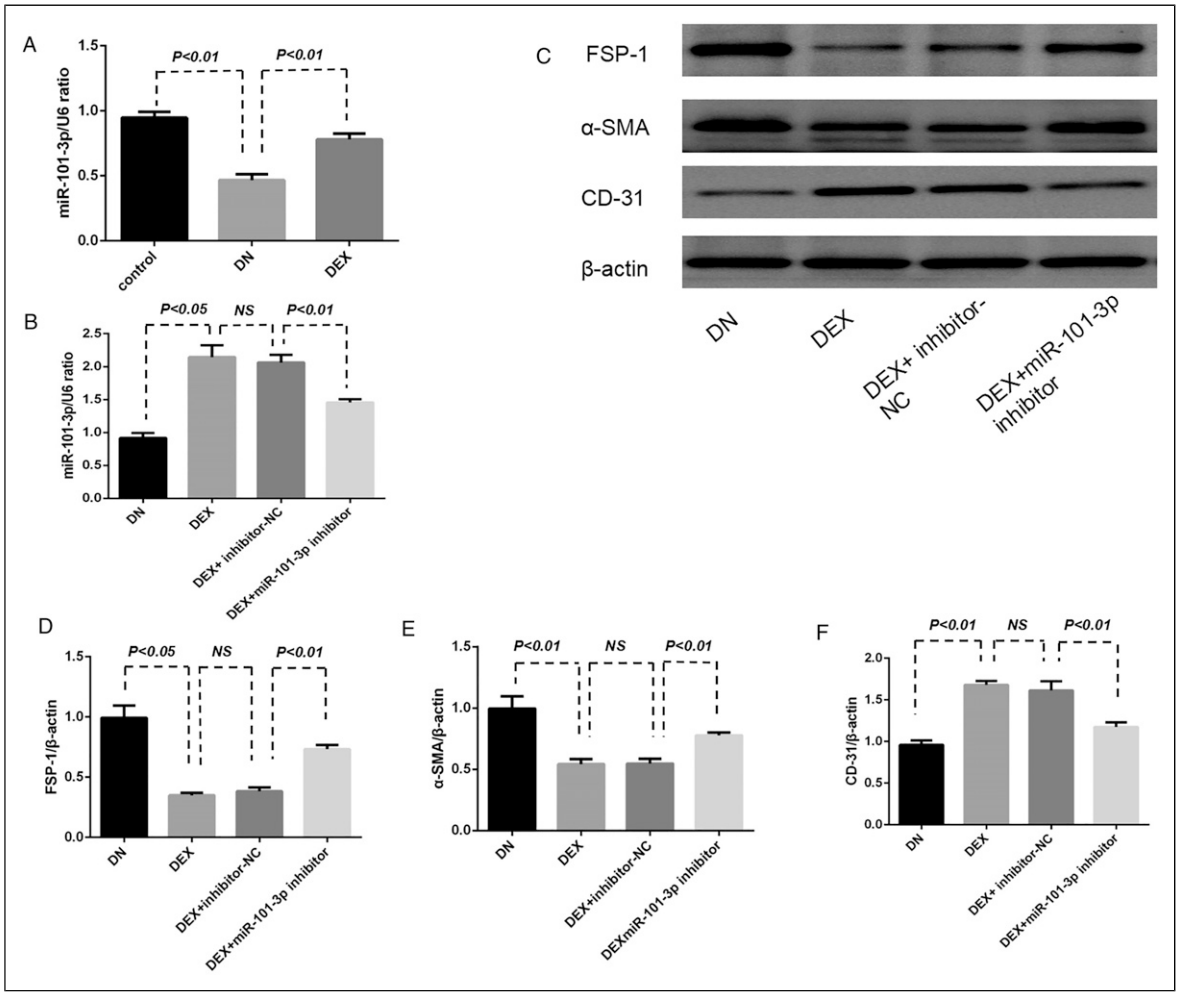

Figure 4. Ameliorating effect of DEX on renal fibrosis in diabetic mice was related to the EndMT regulated by miR-101-3p. (A–B) qPCR analysis of miR-101-3 p expression in vivo; **(C–F)** Western blotting analysis of α-SMA FSP-1, cd-31, and β-actin in vivo. The data are presented as mean ± SE in each group (n = 5) of three independent experiments.

DEX Inhibited High Glucose-Induced EndMT in HMVECs

As shown in Figure 5, to explore the effect of DEX on HMVECs cultured in high glucose, we used cck-8 to examine the effect of different concentrations of glucose on the activity of HMVECs. CCK-8 assay showed that the activity decreased significantly when cultured with 50 mM glucose, so the subsequent experiment was conducted with 50 mM glucose (Figure 5A). When different concentrations of DEX were used to culture HMVECs treated with high glucose, it was found that the cell viability most obviously increased at 10 μM (Figure 5B). Western Blotting showed that the protein level of endothelial cell marker CD-31 decreased, that the protein levels of interstitial cell markers FSP-1 and α-SMA increased in high-glucose condition, and that DEX could alleviate this process (Figure 5C–F). It was also found that DEX could inhibit the increase of Collagen I and Collagen III expression in EndMT (Figure 5G-(i)). These results confirm that DEX can inhibit the EndMT of HMVECs induced by high glucose.

miR-101-3p Knockdown Inhibited the Protective Effect of DEX on HMVECs

As shown in Figure 6, to further confirm the association between miR-101-3p and EndMT, we analyzed the level of high glucose-induced miR-101-3p and the change of EndMT after transfection of miRNA inhibitor in HMVECs. Our qPCR results showed that the expression of miR-101-3p was significantly decreased in high glucose condition, and that the level of miR-101-3p was increased after treatment with 10 μM DEX, while the inhibitor could restrain the enhancement of miR-101-3p (Figure 6A-B). Compared with DEX alone, the level of CD-31 decreased and the levels of FSP-1, α-SMA, Collagen I, and Collagen III increased after inhibiting miR-101-3p (Figure 5C-(i)).

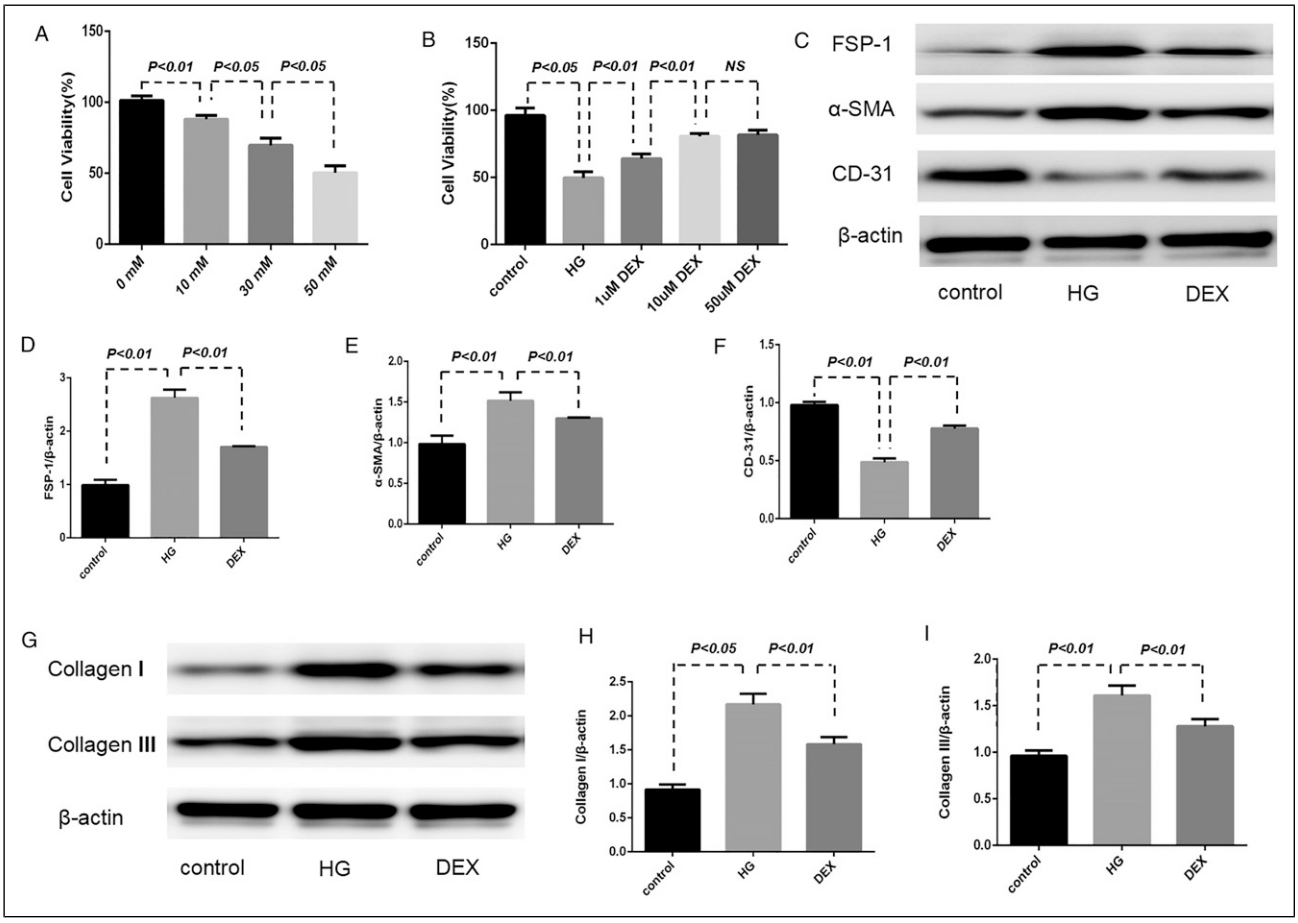

Figure 5. DEX inhibited high glucose-induced EndMT in HMVECs. (A–B) CCK-8 analysis of cell viability in HMVECs treated with high-glucose or DEX; **(C–F)** Western blotting analysis of α-SMA,FSP-1, cd-31, and β-actin in HMVECs treated with high-glucose or DEX; **(G–I)** Western blotting analysis of Collagen I, Collagen III, and β-actin in HMVECs treated with high glucose or DEX. The data are presented as mean ± SE in each group (n = 5) of three independent experiments.

These results suggest that inhibiting the expression of miR-101-3p can weaken the protective effect of DEX on HMVECs.

DEX Regulated the miR-101-3p/TGF-β1/Smad3 Signaling in High Glucose-Induced EndMT

Our previous studies have shown that the TGF-β/SMAD signaling pathway plays a protective role in diabetic renal fibrosis, and miR-101-3p has been reported to be able to target TGF-β Type 1 receptor and inhibit tubular EndMT.[14] As shown in Figure 7, to further investigate the mechanism of DEX in diabetic renal fibrosis, we analyzed TGF-β 1/Smad3 signaling pathway in vitro. Western Blotting results showed TGFβR1 and p-smad3 was significantly increased in HMVECs cultured in high glucose, while their expression was inhibited after DEX treatment; the inhibited expression of TGFβR1 and p-smad3 was reactivated by miR-101-3p knockdown. (Figure 7A-F). These results indicate that DEX can regulate the miR-101-3p/TGF-β1/Smad3 axis to inhibit high glucose-induced EndMT.

Discussion

DN is one of the major microvascular complications of diabetes (diabetic retinopathy, DN, and diabetic neuropathy). In the progression of diabetic renal fibrosis, microvascular endothelial dysfunction is the key and the first step. Hyperglycemia can damage MVECs through various mechanisms such as inflammation, oxidative stress, and apoptosis, and the pathological changes include glomerular hypertrophy, mesangial expansion, and extracellular matrix (ECM) accumulation. Inhibition of diabetic renal fibrosis is an important process in the treatment of DN, but there is no effective drug.[15-18] DEX as a highly selective α-2 adrenergic receptor agonist is an effective anesthetic adjuvant in clinical practice, which has sedative, analgesic, and anti-inflammatory effects. Hou et al has shown that DEX can protect neurons from high glucose by miR-125b-5p/VDR axis. Ran et al has found that

DEX attenuates diabetic retinopathy by inhibiting apoptosis. Our study analyzed the specific mechanism of DEX in the protection of DN.[19,20] Previous studies have shown that DEX alleviates oxidative stress induced by high glucose through RhoA/ROCK/Nox4 signaling pathway, and also alleviates early renal damage in diabetic rats. Our study further found that DEX has protective effects in the late stage of renal fibrosis, and significantly reduces serum creatinine and urinary nitrogen levels in diabetic mice.[21] We also confirmed that the protective effect of DEX on diabetic renal fibrosis is achieved through regulating the EndMT, and that this process involves miR-101-3p and TGF-β1/Smad3 signaling pathway. Figure 8

Our previous study found that EndMT was closely related to diabetic renal fibrosis, for example, DPP-4 could relieve renal fibrosis in diabetic mice by inhibiting EndMT.[22] EndMT is a complex biological process, which is characterized by the loss of intercellular connection of endothelial cells, the change of cell polarity, the decrease of the expression of endothelial markers such as CD-31 and vascular endothelial cadherin (VE-cadherin), the increase of the expression of mesenchymal markers such as α-SMA, and FSP-1, and the deposition of ECM.[23] In this study, the expression of CD-31 in diabetic CD-1 mice and high-glucose cultured HMVECs decreased, and the expression of a-SMA and FSP-1 increased significantly, indicating that the kidney and HMVECs have obvious EndMT, and that DEX inhibits the phenotypic transformation, and reduces the secretion of ECM.

As a no-coding RNA, miRNA can target many mRNAs to regulate DN. For example, miR-218 targets IKK-β to regulate podocyte inflammation, and miR-770-5p targets TIMP3 to regulate podocyte apoptosis.[24,25] Previous studies have shown that miR-101-3p can promote tumor proliferation and migration by regulating EndMT.Recent studies have found that miR-101-3p also plays an important role in renal diseases, for example, miR-101-3p in $CD4^+$ T cells can reduce the expression and release of IL-2, and acute renal injury.[26,27] In line

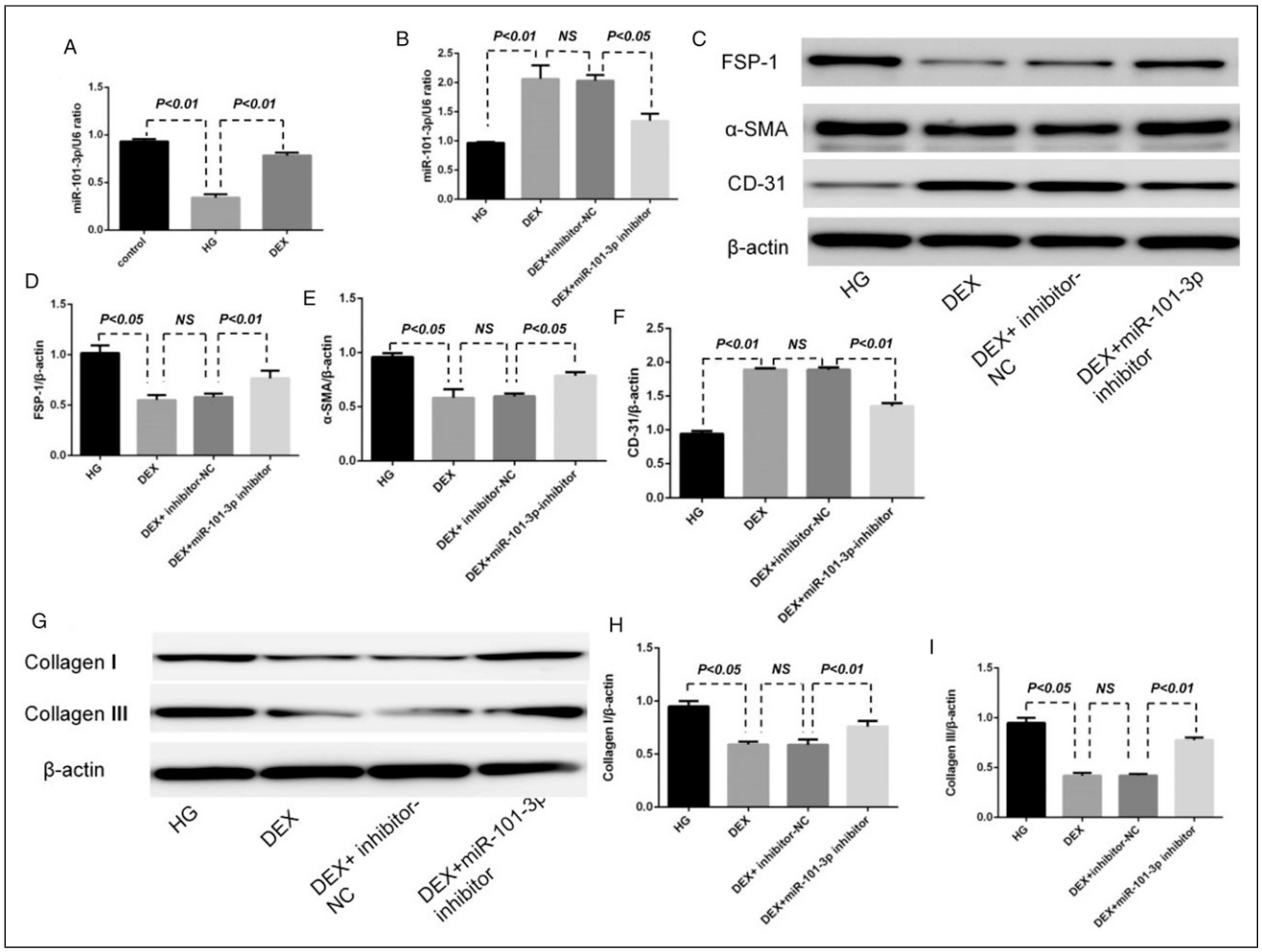

Figure 6. miR-101-3p Knockdown inhibited the protective effect of DEX on HMVECs. (A–B) qPCR analysis of miR-101-3 p expression in vitro; (C–F) Western blotting analysis of α-SMA FSP-1, cd-31, and β-actin in vitro; (G–I) Western blotting analysis of Collagen I, Collagen III, and β-actin in HMVECs after miR-101-3 p inhibitor transfection. The data are presented as mean ± SE in each group (n = 5) of three independent experiments.

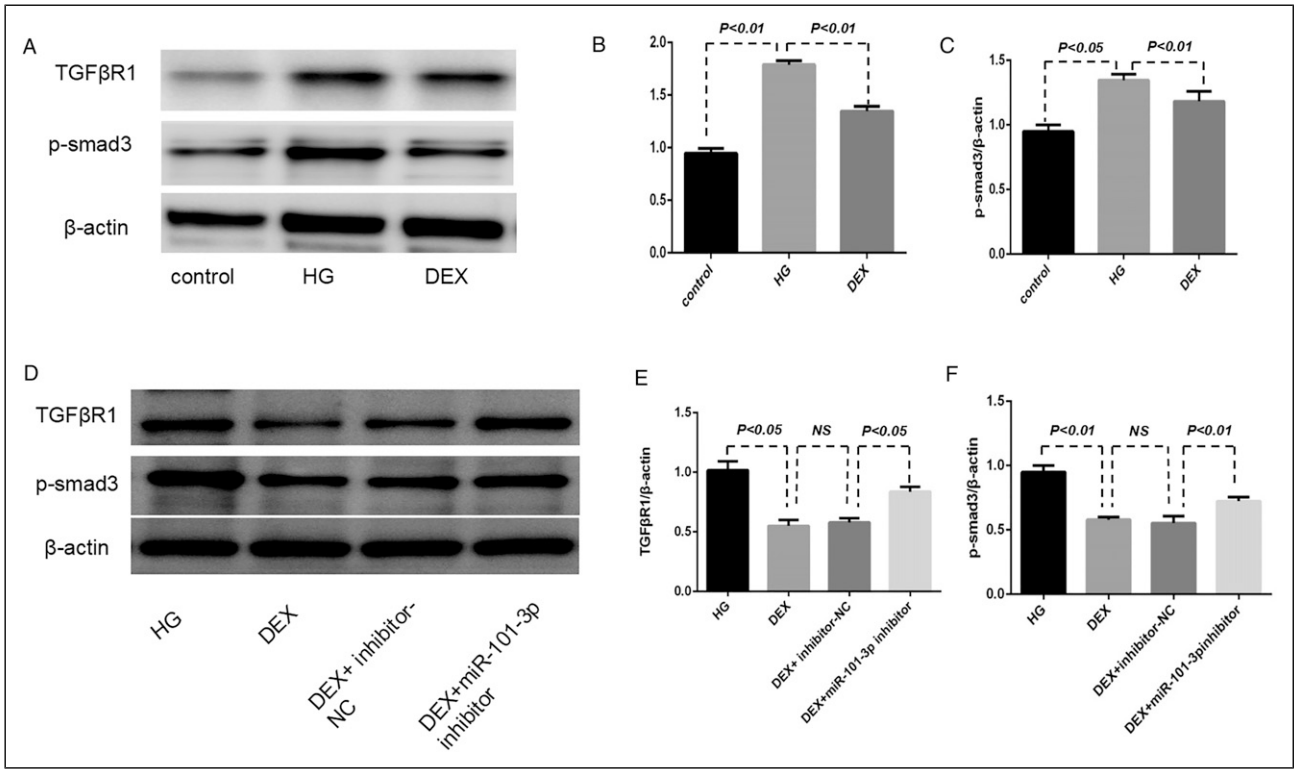

Figure 7. DEX regulated the miR-101-3p/TGF-β1/Smad3 Signaling in high glucose-induced EndMT. (A–C) Western blot analysis of TGFβR1, p-smad3 and β-actin in HMVECs treated with high glucose or DEX; **(D–F)** Western blotting analysis of TGFβR1, p-smad3 and β-actin in HMVECs after miR-101-3 p inhibitor transfection. The data are presented as mean ± SE in each group (n = 5) of three independent experiments.

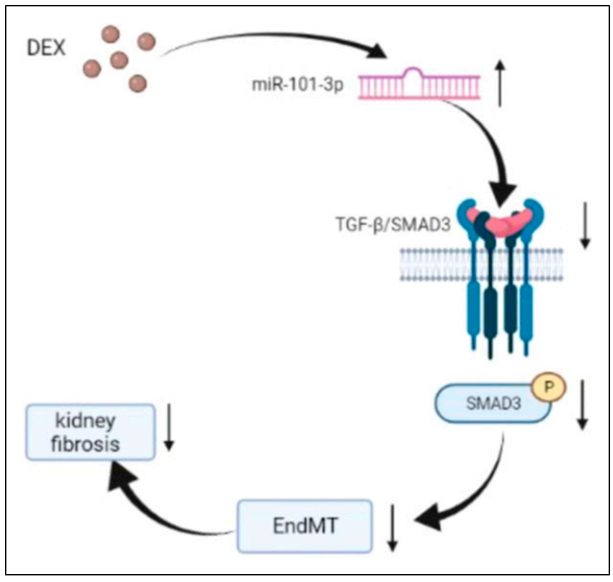

Figure 8. Possible mechanism of inhibition of DEX attenuated kidney fibrosis. DEX upregulate miR-101-3 p levels, inhibit the TGF-β/Smad3 pathway and down-regulate EndMT, leading to the suppression of kidney fibrosis.

with the above studies, our research confirmed that the expression of miR-101-3p decreased in kidneys or HMVECs with EndMT, and that knockout of miR-101-3p can aggravate EndMT and weaken the protective effect of DEX. Our previous studies have shown that the crosstalk of miR-29 s and let-7 s can alleviate renal fibrosis by regulating EndET, other studies have also pointed out that miR-101 interacts with miR-29 and let-7 in tumor proliferation and migration.[28,29] Whether miR-101-3p regulates miR-29 s and let-7 s needs further study.

The molecular mechanism of DN is complex and has not been fully elucidated. Recent studies have shown that the TGF-β/Smad signaling pathway plays an important role in DN.[30] TGF-β/Smad is a classic fibrosis regulatory pathway. Over-activated TGF-β1 binds to TGF-β1 receptors (TGFβR1) to form receptor-ligand complexes, promoting the phosphorylation of Smad3 and excessive deposition of ECM, and leading to exacerbation of the occurrence and development of DN.[31] In this study, we found the protective effect of DEX on DN involved the TGF-β1/Smad3 signaling pathway which was also regulated by miR-101-3p; DEX inhibited the activation of TGF-β1/Smad3 signal pathway by increasing the expression of miR-101-3p in the diabetic kidney. The above-

mentioned regulation of microRNA on the TGF-β1/Smad3 signal pathway corroborates findings from previous reports, for example, Kenichi Koga et al. found that miR-26a can inhibit the activation of TGF-β1 and alleviate DN.[32]

In summary, our findings show that DEX could inhibit EndMT and alleviated renal fibrosis by increasing the expression of miR-103-3p in diabetic patients, and that this regulation process was related to TGF-β1/Smad3 signal pathway. Taken together, our data show that DEX has a significant protective effect on diabetic nephropathy, suggesting that DEX may become a new drug treatment for diabetic nephropathy.

Author Contributions

Li Song and Songlin Feng performed the research and wrote the paper. Hao Yu, contributed to the animal experiments, and collected and analyzed data. Sen Shi designed the research project.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was generously supported by grants from The National Natural Science Foundation of China (Grant No 81500643).

Author's Note

Authors contributed equally

ORCID iD

Songlin Feng https://orcid.org/0000-0001-7171-0382

References

1. Cho NH, Shaw JE, Karuranga S, et al. IDF diabetes atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract*. 2018;138:271-281. doi:10.1016/j.diabres.2018.02.023.
2. Sutariya B, Saraf M. Betanin, isolated from fruits of opuntia elatior mill attenuates renal fibrosis in diabetic rats through regulating oxidative stress and TGF-β pathway. *J Ethnopharmacol*. 2017;198:432-443. doi:10.1016/j.jep.2016.12.048.
3. Shi S, Song L, Yu H, et al. Knockdown of LncRNA-H19 ameliorates kidney fibrosis in diabetic mice by suppressing miR-29a-mediated endMT. *Front Pharmacol*. 2020;11:586895. doi:10.3389/fphar.2020.586895.
4. Nitta K, Shi S, Nagai T, et al. Oral administration of N-acetyl-seryl-aspartyl-lysyl-proline ameliorates kidney disease in both type 1 and type 2 diabetic mice via a therapeutic regimen. *BioMed Res Int*. 2016;2016:9172157. doi:10.1155/2016/9172157.
5. Li J, Liu H, Takagi S, et al. Renal protective effects of empagliflozin via inhibition of EMT and aberrant glycolysis in proximal tubules. *JCI Insight*. 2020;5(6):e129034. doi:10.1172/jci.insight.129034.
6. Wang K, Wu M, Xu J, et al. Effects of dexmedetomidine on perioperative stress, inflammation, and immune function: Systematic review and meta-analysis. *Br J Anaesth*. 2019;123(6):777-794. doi:10.1016/j.bja.2019.07.027.
7. Bao N, Dai D. Dexmedetomidine protects against ischemia and reperfusion-induced kidney injury in rats. *Mediat Inflamm*. 2020;2020:2120971. doi:10.1155/2020/2120971.
8. Liu YT, Liu W, Wan ZH, Wang XQ, Gu FX. Protective effect of dexmedetomidine against renal injury in diabetic nephropathy rats through inhibiting NF-κB pathway. *Eur Rev Med Pharmacol Sci*. 2020;24(22):11865-11870. doi:10.26355/eurrev_202011_23844.
9. Tang C, Hu Y, Gao J, et al. Dexmedetomidine pretreatment attenuates myocardial ischemia reperfusion induced acute kidney injury and endoplasmic reticulum stress in human and rat. *Life Sci*. 2020;257:118004. doi:10.1016/j.lfs.2020.118004.
10. Lu TX, Rothenberg ME. MicroRNA. *J Allergy Clin Immunol*. 2018;141(4):1202-1207. doi:10.1016/j.jaci.2017.08.034.
11. Yang X, Chen H, Chen Y, et al. Circulating miRNA expression profiling and target prediction in patients receiving dexmedetomidine. *Cell Physiol Biochem*. 2018;50(2):552-568. doi:10.1159/000494168.
12. Sun Z, Ma Y, Chen F, et al. miR-133b and miR-199b knockdown attenuate TGF-β1-induced epithelial to mesenchymal transition and renal fibrosis by targeting SIRT1 in diabetic nephropathy. *Eur J Pharmacol*. 2018;837:96-104. doi:10.1016/j.ejphar.2018.08.022.
13. Zhao JY, Wang XL, Yang YC, Zhang B, Wu YB. Upregulated miR-101 inhibits acute kidney injury-chronic kidney disease transition by regulating epithelial-mesenchymal transition. *Hum Exp Toxicol*. 2020;39(12):1628-1638. doi:10.1177/0960327120937334.
14. Wang Q, Tao Y, Xie H, Liu C, Liu P. MicroRNA-101 inhibits renal tubular epithelial-to-mesenchymal transition by targeting TGF-β1 type I receptor. *Int J Mol Med*. 2021;47(6):119. doi:10.3892/ijmm.2021.4952.
15. Zhu M, Wang H, Chen J, Zhu H. Sinomenine improve diabetic nephropathy by inhibiting fibrosis and regulating the JAK2/STAT3/SOCS1 pathway in streptozotocin-induced diabetic rats. *Life Sci*. 2021;265:118855. doi:10.1016/j.lfs.2020.118855.
16. Wada J, Makino H. Inflammation and the pathogenesis of diabetic nephropathy. *Clinical Science*. 2013;124(3):139-152. doi:10.1042/CS20120198.
17. Hong SJ, Kim ST, Kim TJ, et al. Cellular and molecular changes associated with inhibitory effect of pioglitazone on neointimal growth in patients with type 2 diabetes after zotarolimus-eluting stent implantation. *Arterioscler Thromb Vasc Biol*. 2010;30(12):2655-2665. doi:10.1161/ATVBAHA.110.212670.

18. Xie X, Peng J, Huang K, et al. Polydatin ameliorates experimental diabetes-induced fibronectin through inhibiting the activation of NF-κB signaling pathway in rat glomerular mesangial cells. *Mol Cell Endocrinol*. 2012;362(1-2):183-193. doi:10.1016/j.mce.2012.06.008.
19. Hou X, Xu F, Zhang C, et al. Dexmedetomidine exerts neuroprotective effects during high glucose-induced neural injury by inhibiting miR-125b. *Biosci Rep*. 2020;40(6):BSR20200394. doi:10.1042/BSR20200394.
20. Liu R, Li X, Zhang X. Dexmedetomidine protects high-glucose induced apoptosis in human retinal pigment epithelial cells through inhibition on p75(NTR). *Biomed Pharmacother*. 2018; 106:466-471. doi:10.1016/j.biopha.2018.06.117.
21. Jihua C, Cai C, Xubin B, Yue Y. Effects of dexmedetomidine on the RhoA/ROCK/Nox4 signaling pathway in renal fibrosis of diabetic rats. *Open Med*. 2019;14:890-898. doi:10.1515/med-2019-0105.
22. Kanasaki K, Shi S, Kanasaki M, et al. Linagliptin-mediated DPP-4 inhibition ameliorates kidney fibrosis in streptozotocin-induced diabetic mice by inhibiting endothelial-to-mesenchymal transition in a therapeutic regimen. *Diabetes*. 2014;63(6):2120-2131. doi: 10.2337/db13-1029.
23. Gaikwad AV, Eapen MS, McAlinden KD, et al. Endothelial to mesenchymal transition (EndMT) and vascular remodeling in pulmonary hypertension and idiopathic pulmonary fibrosis. *Expert Rev Respir Med*. 2020;14(10):1027-1043. doi:10.1080/17476348.2020.1795832.
24. Li M, Guo Q, Cai H, Wang H, Ma Z, Zhang X. miR-218 regulates diabetic nephropathy via targeting IKK-β and modulating NK-κB-mediated inflammation. *J Cell Physiol*. 2020; 235(4):3362-3371. doi:10.1002/jcp.29224.
25. Wang L, Li H. MiR-770-5p facilitates podocyte apoptosis and inflammation in diabetic nephropathy by targeting TIMP3. *Biosci Rep*. 2020;40(4):BSR20193653. doi:10.1042/BSR20193653.
26. Liu J, Hua R, Gong Z, et al. Human amniotic epithelial cells inhibit CD4+ T cell activation in acute kidney injury patients by influencing the miR-101-c-Rel-IL-2 pathway. *Mol Immunol*. 2017;81:76-84. doi:10.1016/j.molimm.2016.11.019.
27. Cheng Y, Chang Q, Zheng B, Xu J, Li H, Wang R. LncRNA XIST promotes the epithelial to mesenchymal transition of retinoblastoma via sponging miR-101. *Eur J Pharmacol*. 2019; 843:210-216. doi:10.1016/j.ejphar.2018.11.028.
28. Buechner J, Tømte E, Haug BH, et al. Tumour-suppressor microRNAs let-7 and mir-101 target the proto-oncogene MYCN and inhibit cell proliferation in MYCN-amplified neuroblastoma. *Br J Cancer*. 2011;105(2):296-303. doi:10.1038/bjc.2011.220.
29. Tao J, Ji J, Li X, et al. Distinct anti-oncogenic effect of various microRNAs in different mouse models of liver cancer. *Oncotarget*. 2015;6(9):6977-6988. doi:10.18632/oncotarget.3166.
30. He X, Cheng R, Huang C, et al. A novel role of LRP5 in tubulointerstitial fibrosis through activating TGF-β/Smad signaling. *Signal Transduct Target Ther*. 2020;5(1):45. doi:10.1038/s41392-020-0142-x.
31. Ying Q, Wu G. Molecular mechanisms involved in podocyte EMT and concomitant diabetic kidney diseases: an update. *Ren Fail*. 2017;39(1):474-483. doi:10.1080/0886022X.2017.1313164.
32. Koga K, Yokoi H, Mori K, et al. MicroRNA-26a inhibits TGF-β-induced extracellular matrix protein expression in podocytes by targeting CTGF and is downregulated in diabetic nephropathy. *Diabetologia*. 2015;58(9):2169-2180. doi:10.1007/s00125-015-3642-4.