ACTA ABP BIOCHIMICA POLONICA

Vol. 70, No 4/2023
791–797
https://doi.org/10.18388/abp.2020_6315

Regular paper

Simvastatin attenuates diabetes mellitus erectile dysfunction in rats by miR-9-5p-regulated PDCD4

YiMing Weng[1#], YuanShen Mao[3#], YanQiu Wang[1], YuFan Jiao[1], Jun Xiang[2] and Wei Le[2]

[1]Department of Reproductive Center, Tongji Hospital, Tongji University School of Medicine, Shanghai, 200065, China; [2]Department of Urology, Tongji Hospital, Tongji University School of Medicine, Shanghai, 200092, China; [3]Department of Urology, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, 201999, China

DMED is a common complication of diabetes, for which new treatment methods are urgently required. Focused on DMED, the pharmacological mechanism of simvastatin (Sim) was probed. A model of DMED was made in rats with streptozotocin and orally medicated with Sim. Lentiviral vectors that interfere with miR-9-5p or PDCD4 were injected, and the erectile function, histopathology of cavernous tissue, and α-SMA expression were evaluated. Cavernous smooth muscle cells (CMSCs) obtained from DMED rats were treated with Sim and transfected with the plasmid vector that interferes with miR-9-5p or PDCD4 to observe cell viability and apoptosis. The binding relationship between miR-9-5p and PDCD4 was checked. After 8-week treatment with Sim, erectile function was improved and the corpus cavernosum injury was alleviated. Upregulating miR-9-5p or downregulating PDCD4 further improved erectile function and cavernous injury in rats. miR-9-5p targeted regulation of PDCD4. *In vitro* cell experiment results showed that Sim induced proliferation and reduced apoptosis of CSMCs by enhancing miR-9-5p-targeted regulating PDCD4 *in vitro*. Sim attenuates DMED in rats *via* miR-9-5p/PDCD4.

Keywords: Simvastatin, miR-9-5p, PDCD4, Diabetes, Erectile dysfunction

Received: 08 August, 2023; revised: 20 May, 2023; accepted: 25 June, 2023; available on-line: 06 November, 2023

e-mail: catottisina@hotmail.com (JX); wallyxiang@126.com (WL)
#These authors contributed equally to this work
Acknowledgements of Fianancial Support: This work was funded by the Shanghai Municipal Health Commission's Research Project (Grant Number 202040078). The present study was sponsored by National Natural Science Foundation of China (82001610).
Abbreviations: CMSCs, Cavernous smooth muscle cells; DMED, diabetes mellitus erectile dysfunction; ED, erectile dysfunction; Sim, simvastatin

INTRODUCTION

Erectile dysfunction (ED) is defined as erection failure during sexual intercourse and is a common complication of diabetes (Che *et al*., 2020). With lifestyle changes and population ages, the incidence rate of ED is increasing (He *et al*., 2019). The incidence rate of ED in the normal population is 0.1–18%, while that in diabetic patients is nearly 3 times higher (Seftel, 2005). ED can affect physical, emotional, social, sexual, and interpersonal relationships, for which drug therapy is the main treatment, including PDE5 inhibitors, androgen therapy, and vasoactive drugs (Lau *et al*., 2007). Compared with other treatments, such as intracavernous injection or intraurethral injection of alprostadil, drug treatment is less invasive (Talib *et al*., 2021). However, the effect of oral medication on ED is significantly lower (Martínez-Salamanca*et al*., 2014). Therefore, there is an urgent need to develop new drugs for the treatment of diabetes mellitus erectile dysfunction (DMED).

Statins are 3-hydroxy-3-methylglutaryl CoA reductase inhibitors, which are widely used to reduce cholesterol levels in patients with lipid metabolism disorders. Simvastatin (Sim) is one of the most widely used lipophilic statins (Aschenbrenner *et al*., 2021) that has therapeutic value in human diseases, such as fracture healing (Hajializade *et al*., 2020), tumorigenesis (Kopacz *et al*., 2020), hydrocephalus (Chen *et al*., 2017), Parkinson's disease (Carroll *et al*., 2017). Sim can alleviate DMED by enhancing autophagy (Ding *et al*., 2020), but other potential mechanisms of Sim action have not been fully studied.

MicroRNA (miRNA) involves in a variety of disease-related signaling pathways after transcription (Rupaimoole *et al*., 2017). miR-126-engineered MDSCs can reconstruct blood vessels and repair rat cavernous injury, to reduce ED caused by rat cavernous injury (Zou *et al*., 2021) and miR-205 is involved in the pathogenesis of DMED (Wen *et al*., 2019). miR-9-5p has been widely studied in tumors, such as liver cancer (Wang *et al*., 2020), prostate cancer (Wang *et al*., 2021), and gastric cancer (Ba *et al*., 2021). However, the role of miR-9-5p in DMED is not yet clear.

Our research aims to explore the potential mechanism of DMED by targeting miR-9-5p/PDCD4 and provide a new treatment for DMED.

MATERIALS AND METHODS

DMED rat model

The animal treatment complied with the "Experimental Animal Care and Guide" and was approved by the Experimental Animal Ethics Committee of Tongji Hospital, Tongji University School of Medicine, ShangHai (NO.T20116A301). Healthy male SD rats, aged 8 weeks old, were standard fed in a non-pathogenic environment, and blood glucose was tested a week later. After 24 h of fasting, 64 rats were injected with streptozotocin (STZ)-citrate buffer at 60 mg/kg, and the other 6 rats were with citric acid buffer. After that, rats were given normal feeding. At 24 h, 72 h, and 1-week post-injection, blood glucose was assessed and >16.67 mmol/L indicated the induction of diabetes. After 8 w of normal feeding, rats were subcutaneously injected with Apomorphine (APO) at 100 μg/kg in the neck and placed in the dark for 30 min during which the number of penis erections was re-

corded. A total of 54 DMED rats (rats without erection) were established, and the success rate was 84.4%.

Animal treatment

After modeling, rats were given oral medication with Sim (dissolved in DMSO and physiological saline, 2 mg/kg) or saline, once a day, for 8 days. Meanwhile, 48 DMED rats were intravenously injected with 7.6×10^7 IFU lentivirus (10 mg/kg), carrying miR-9-5p agomir, agomir NC, miR-9-5p antagomir, antagomir NC, si-PDCD4, si-NC, miR-9-5p agomir + oe-PDCD4, and miR-9-5p agomir + oe-NC, respectively. After the last injection, rats were fed ad libitum for 3 days, and body weight and blood glucose were measured. The rats were euthanized to harvest corpus cavernosum which was preserved in liquid nitrogen.

Construction and injection of lentivirus

Plasmid DNA (12.5 μg) and liposomes (9 μL) were diluted in 250 μL of serum-free RPMI1640 medium (R8758, Gibco, CA, USA), respectively, and mixed to form a plasmid-liposome complex which was then added into 293T cells (ATCC, VA, USA). After that, cells were incubated for 6–8 hours, and the whole culture medium was centrifuged at 716 g for 20 min to obtain lentivirus-enriched supernatant. Next, the supernatant was filtered through a 0.45 μm filter, stored at –80°C, and injected into the tail vein of DMED rats.

Evaluation of erectile function in vivo

Rats were anesthetized by intraperitoneal injection of 2% pentobarbital sodium (3 mg/kg). After the separation of the corpus cavernosum, the prostate was exposed and then the left common carotid artery was separated. Intracavernous pressure (ICP) and mean arterial pressure (MAP) to 50-s electrical stimulation were monitored (20 Hz, 5 V, 0.2 ms). The electrode stimulation interval lasted 15 min.

H&E staining

Penile tissue was routinely made into paraffin slices (about 4 μm) for staining with hematoxylin for 5 min and with eosin for 2 min. Afterward, the slices were dehydrated with ethanol, cleared with xylene, and sealed with neutral glue for microscopic observation.

Immunofluorescence

Sections were dewaxed, dehydrated in gradient ethanol, treated overnight with sodium citrate buffer solution (pH 6.0), permeabilized with 0.5% Triton X-100 and incubated at 5% bovine serum albumin. The slices were then treated with primary antibody α-SMA (1:200, millipore sigma) and the secondary antibody combined with Alexa fluor 488 or Alexa fluor 555 (1:200). Next, counter-staining with 4,6-diamidino-2-phenylindole was performed to capture the images under a fluorescence microscope (Olympus, ix83-fv3000, Tokyo, Japan) followed by quantification using ImageJ (v1.8.0, NIH, MD, USA).

TUNEL staining

Apoptosis in the paraffin section was detected by Apop Tag Plus In situ apoptosis Detection kit (Oncor Inc, Gaithersburg) and analyzed under an optical microscope (Nikon, Japan) (Hirfanoglu *et al*., 2019).

Cell culture

The euthanized DMED rats were sterilized in 75% alcohol, the penis was dissected along the inferior edge of the pubis, and the glans of the penis were collected. The albuginea vessels and urethra were stripped, and the corpus cavernosum was cut into 0.5–1 mm^3 pieces and cultured for 5–7 days in a medium containing 20% fetal bovine serum. After 2-3 passages, cavernous smooth muscle cells (CMSCs) were treated with Sim (2 μM) for 24 h.

Cell transfection

CMSCs were transfected with miR-9-5p mimic, mimic NC, miR-9-5p inhibitor, inhibitor NC, sh-NC, sh-PDCD4, miR-9-5p mimic + pcDNA-PDCD4 and miR-9-5p mimic + pcDNA-NC using Lipofectamine 2000 (11668019, Invitrogen). The above plasmid was constructed by Sangon (Shanghai, China) with pEGFP-4.1N (Invitrogen) as the vector.

Cell viability assay

CMSCs proliferation was assessed at 24, 48, and 72 h using cell counting kit-8 (CK04, Dojindo, Kumamoto, Japan) by evaluating the optical density (OD) at 450 nm on a microplate reader.

Flow cytometry

CMSCs apoptosis was measured by annexin V-FITC apoptosis Kit (Abcam, Cambridge, UK) and detected on a flow cytometer (br168323; Luminex, Austin, TX, USA). Data were analyzed by Kaluza C analysis software (Beckman Coulter, CA, USA).

RNA extraction and analysis

Total RNA was extracted with RNeasy Mini Kit (Qiagen, CA, USA), and cDNA was generated with PrimeScript RT reagent Kit (RR047A, Takara, Japan) or miRNA first-strand cDNA synthesis Kit (Sangon). qPCR was performed using SYBR Premix Ex Taq™ II (Perfect Real Time) kit (DRR081, Takara, Japan) and ABI7500 Real-Time PCR system (ABI, Foster City, USA). Glycer-

Table 1. Primer sequences

Genes	Sequences (5′– 3′)
miR-9-5p	F: GTGCAGGGTCCGAGGT
	R: GCGCTCTTTGGTTATCTAGC
PDCD4	F: ATGTGGAGGAGGTGGATGTG
	R: TGGTGTTAAAGTCTTCTCAAATGC
PCNA	F: GCCATATTGGAGATGCTGT
	R: TGAGTGTCACCGTTGAAGA
Bax	F: GATCGAGCAGGGCGAATG
	R: CATCTCAGCTGCCACTCG
U6	F: CTCGCTTCGGCAGCACA
	R: AACGCTTCACGAATTTGCGT
GAPDH	F: CGGAGTCAACGGATTTGGTCGTAT
	R: AGCCTTCTCCATGGTGGTGAAGAC

Note: miR-9-5p, microRNA-9-5p; PDCD4, Programmed cell death 4; PCNA, Proliferating cell nuclear antigen; Bax, Bcl-2-associated X; GAPDH, glyceraldehyde 3-phosphate dehydrogenase

aldehyde-3-phosphate dehydrogenase (GAPDH) and U6 were considered internal controls. The primer sequences are shown in Table 1, miRNA negative primers and U6 upstream primers were provided by miRNA first-strand cDNA synthesis (Tailing Reaction) kit. Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

Western Blot

Proteins were extracted using RIPA lysis buffer (P0013C, Beyotime, China) and analyzed BCA protein detection kit (20201ES76, Yeasen, Shanghai, China). Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred to polyvinylidene fluoride membranes. After blocking with 5% skim milk, membranes were incubated with primary antibodies PDCD4 (1:1000, 9535, Cell Signaling Technology) and GAPDH (1:1000, ab8245, Abcam) and with goat anti-rabbit IgG (ab205718, Abcam) to analyze gray values using ImageJ 1.48u (NIH).

Dual-Luciferase Reporter Experiment

The 3'UTR fragment of PDCD4 and its mutation containing potential miR-9-5p binding site were inserted into the PGLO vector, namely PGLO-PDCD4 wild type (WT) and PGLO-PDCD4 mutant (MUT). The vectors were co-transfected with miR-9-5p mimic and mimic NC into 293T cells, respectively, thereby analyzing luciferase activity using a dual luciferase reporter gene detection system (e1910, Promega, WI, USA).

Data analysis

All data were statistically analyzed by SPSS 21.0 statistical software. Reported as mean ± standard deviation, the measurement data were compared by independent sample *t*-test if following normal distribution, otherwise by one-way ANOVA and Tukey's multiple comparison post hoc analysis. With $P<0.05$, the difference was considered to be statistically significant.

RESULTS

Sim can improve ED in DMED rats

Before intraperitoneal injection of STZ, rats showed no significant difference in body weight, blood glucose, or erectile function. Except for 8 rats that died due to STZ, the other 64 diabetic rats and 6 normal rats were subjected to the APO test. Among them, 1-3 erections happened in 10 diabetic rats in 30 min, and no erection in the other 54 diabetic rats. To verify the improvement effect of Sim on DMED rats, DMED rats took Sim orally. Diabetic rats had reduced body weight and increased blood glucose, which could be ameliorated by Sim (Fig. 1A, B). The ICP/MAP values were decreased in diabetic rats and could be restored by Sim (Fig. 1C). Then, we observed the pathological condition of the cavernous by HE staining, which showed that the capillaries of the cavernous sinus contained erythrocytes, which were intertwined in a network. A large number of capillaries were seen in the corpus cavernosum tissue of sham-operated rats while less in diabetic rats; after Sim treatment, the corpus cavernosum injury was relieved (Fig. 1D). Immunofluorescence analysis in the penile cavernous demonstrated that α-SMA expression was decreased in DMED rats; Sim increased α-SMA expression (Fig. 1E, F). In addition, RNA and protein expression analysis revealed that miR-9-5p expression was decreased and PDCD4 expression was increased in DMED rats; after Sim treatment, their expression trends were recovered (Fig. 1G).

miR-9-5p alleviates corpus cavernosum damage in DMED rats

For defining the impacts of miR-9-5p in DMED rats, Sim-treated DMED rats were injected with the lentiviral vector into the tail vein to interfere with miR-9-5p expression (Fig. 2A). Due to miR-9-5p overexpression,

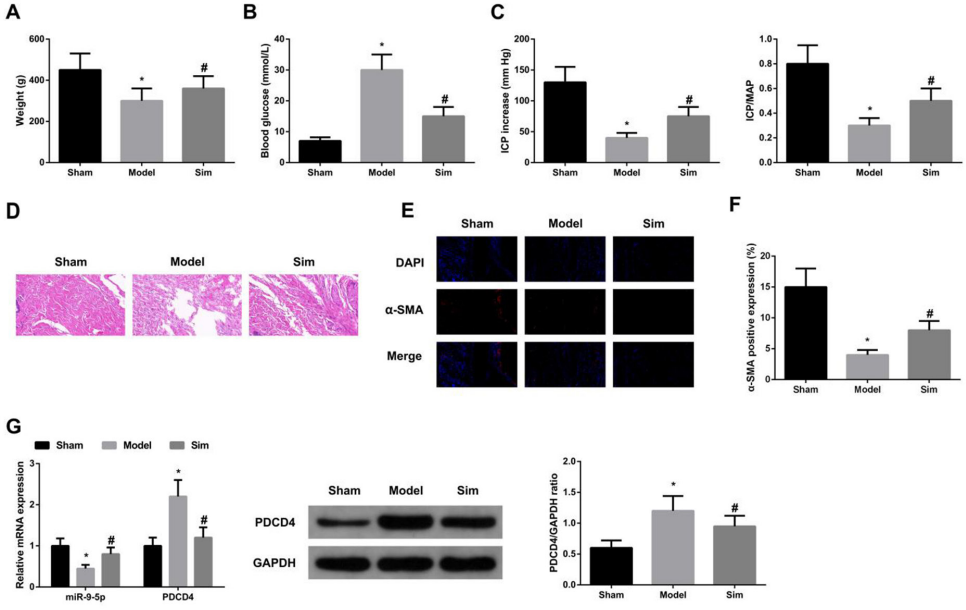

Figure 1. Sim can improve ED in DMED rats
(**A–B**) Changes in body weight and blood glucose of rats; (**C**) Erectile function assessment *in vivo*; (**D**) HE staining; (**E–F**) immunohistochemical staining for α-SMA; (**G**) miR-9-5p and PDCD4 expression; values are expressed as mean ± standard deviation; **P*<0.05 *vs*. Sham group; #*P*<0.05 *vs*. Model group.

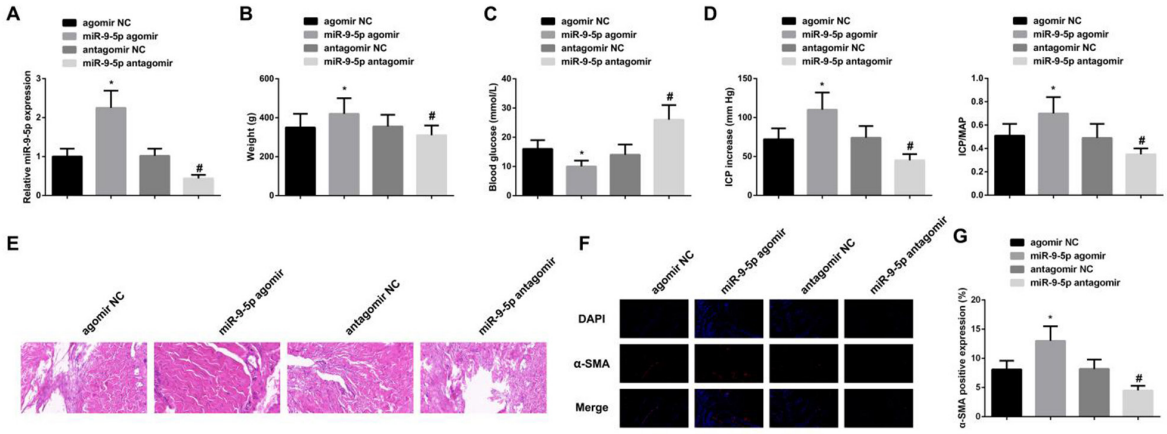

Figure 2. miR-9-5p reduces corpus cavernosum damage in DMED rats.
(**A**) miR-9-5p expression; (**B–C**) Changes in body weight and blood glucose of rats; (**D**) Erectile function assessment *in vivo*; (**E**) HE staining; (**F–G**) immunohistochemical staining for α-SMA; values are expressed as mean ± standard deviation; **P*<0.05 *vs*. agomir NC group; #*P*<0.05 *vs*. antagomir NC group.

rats gained weight, blood glucose decreased, and ICP/MAP values elevated (Fig. 2B-D), the corpus cavernosum damage was alleviated (Fig. 2E), and α-SMA expression was raised (Fig. 2F, G). While miR-9-5p inhibition counter-acted the ameliorating effect of Sim (Fig. 2B-G).

miR-9-5p targets PDCD4

The specific binding site between PDCD4 and miR-9-5p was identified through the bioinformatic website RNA22 (Fig. 3A). The binding of miR-9-5p to PDCD4 was verified by dual-luciferase reporter gene assay based on the experimental result that miR-9-5p mimic induced the impairment of PGLO-PDCD4-WT luciferase activity (Fig. 3B). Analysis of PDCD4 expression indicated that with the change in miR-9-5p expression, PDCD4 expression was altered in an opposite way (Fig. 3C).

Depleting PDCD4 attenuates ED in DMED rats

In Sim-treated DMED rats, a lentiviral vector carrying si-PDCD4 or miR-9-5p agomir + oe-PDCD4 was injected into the tail vein (Fig. 4A). It was verified that si-PDCD4 had the ameliorating effects as miR-9-5p agomir, however, oe-PDCD4 mitigated the protective role of miR-9-5p agomir in Sim-treated DMED rats (Fig. 4B-G).

Sim protects CSMCs *in vitro* by enhancing miR-9-5p and suppressing PDCD4 expression

The regulatory role of Sim through the miR-9-5p/PDCD4 axis was tested using CSMCs. CSMCs were treated with Sim and then transfected. Quantitative PCR detection (Fig. 5A) manifested that Sim treatment elevated miR-9-5p and restrained PDCD4 expression in CSMCs; miR-9-5p mimic and miR-9-5p inhibitor transfection could strengthen and impair Sim-mediated effects; sh-PDCD4 transfection promoted the inhibitory effect of Sim on PDCD4 expression; pcDNA-PDCD4 enhanced the level of PDCD4 that had been suppressed by miR-9-5p mimic. The regulatory effect of Sim on the biological characteristics of CSMCs was investigated, showing that Sim-mediated induction of proliferation and reduction of apoptosis could be further bolstered by overexpressing miR-9-5p or suppressing PDCD4 while impaired by inhibiting miR-9-5p; PDCD4 expression in-

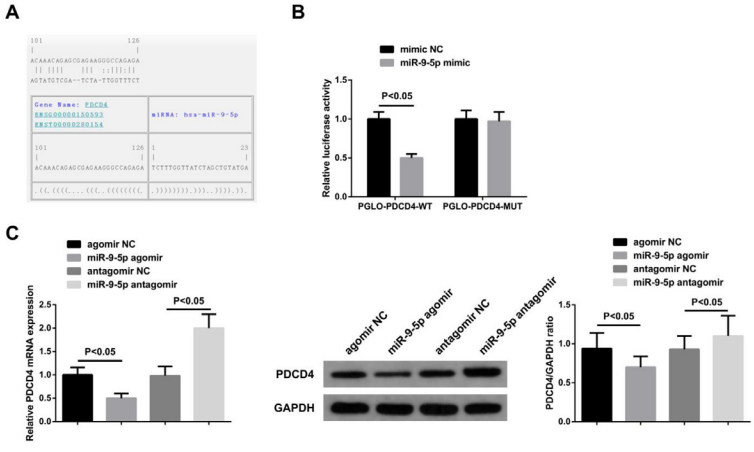

Figure 3. miR-9-5p targets PDCD4
(**A**) binding site of miR-9-5p and PDCD4; (**B**) the targeting relationship between miR-9-5p and PDCD4; (**C**) PDCD4 expression; values are expressed as mean ± standard deviation.

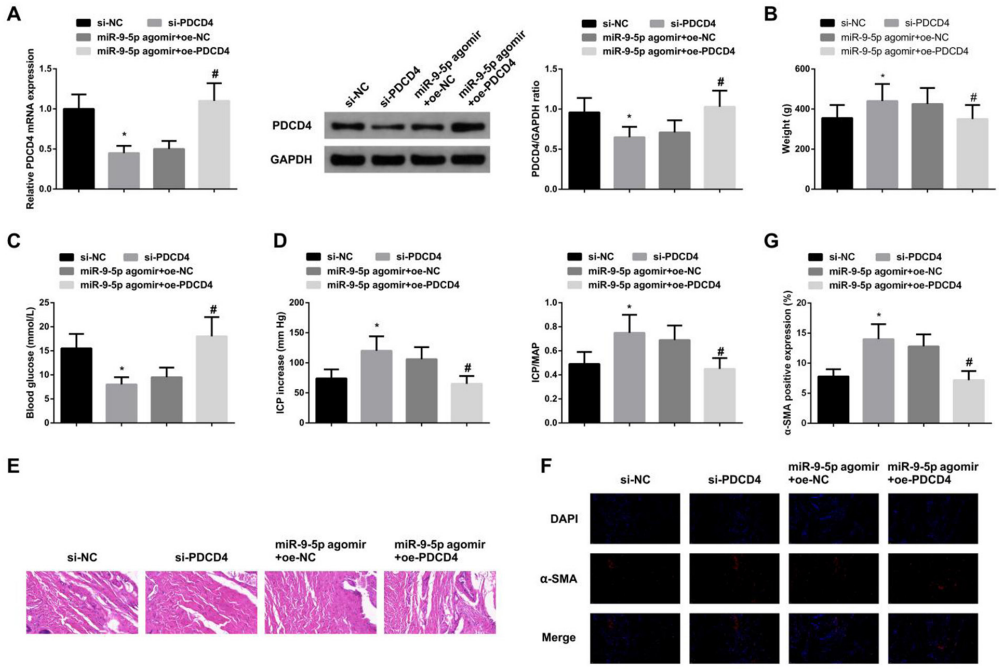

Figure 4. Depleting PDCD4 ameliorates ED in DMED rats
(**A**) PDCD4 expression; (**B–C**) Changes in body weight and blood glucose of rats; (**D**) Erectile function assessment *in vivo*; (**E**) HE staining; (**F–G**) immunohistochemical staining for α-SMA; values are expressed as mean ± standard deviation; **P*<0.05 *vs*. si-NC group; #*P*<0.05 *vs*. miR-9-5p agomir + oe-NC group.

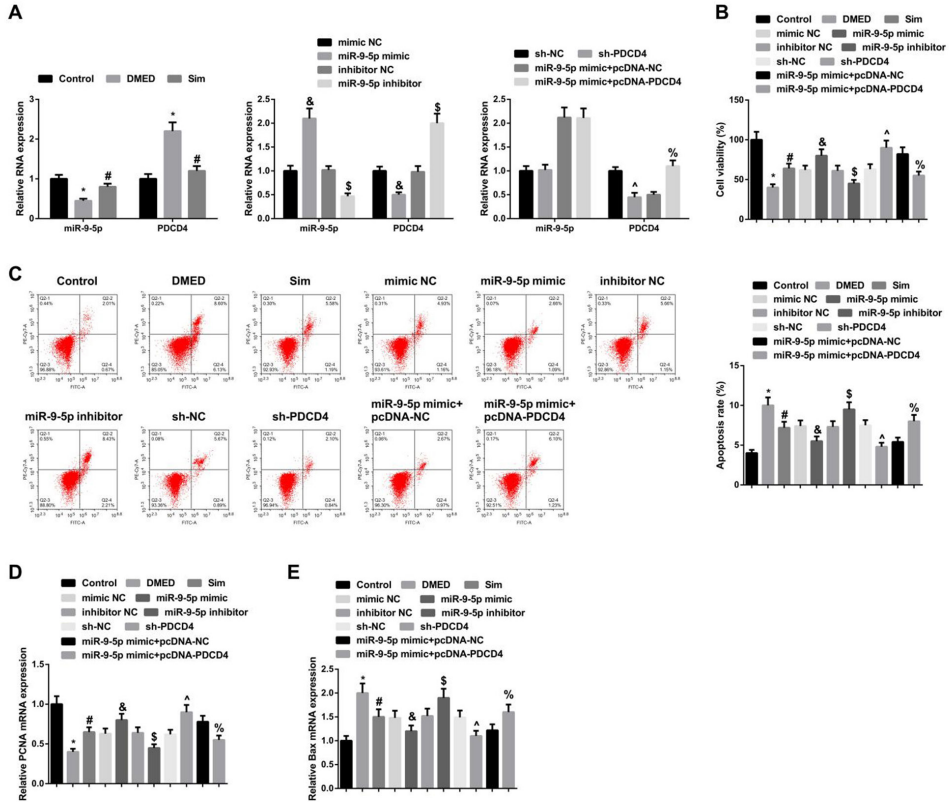

Figure 5. Sim protects CSMCs *in vitro* by enhancing miR-9-5p and suppressing PDCD4 expression
(**A**) miR-9-5p and PDCD4 expression; (**B**) cell viability; (**C**) cell apoptosis; (**D–E**) PCNA and Bax mRNA expression; values are expressed as mean ± standard deviation; **P*<0.05 *vs*. Control group; #*P*<0.05 *vs*. DMED group; &*P*<0.05 *vs*. mimic NC group; $*P*<0.05 *vs*. inhibitor NC group; ^*P*<0.05 *vs*. sh-NC group; %*P*<0.05 *vs*. miR-9-5p mimic + pcDNA-NC group.

duction served a blocker for miR-9-5p-mediated protection against anti-proliferation and apoptosis (Fig. 5B, C). As expected, mRNA expression of PCNA and Bax also confirmed the above results (Fig. 5D, E).

DISCUSSION

The number of diabetic patients is increasing every year and DMED affects more than half of men with diabetes (Huo *et al*., 2020). The pathogenesis of DMED involves a contraction-relaxation imbalance of CSMCs. It has been reported that CSMCs can change their phenotype from a contractile state to a diastolic state under hyperglycemic conditions (Wei *et al*., 2012). At present, oral drugs are often used in the clinical treatment of ED. Sim, a commonly used cholesterol-lowering drug, has been found to improve ED in patients over the age of 40 (Trivedi *et al*., 2014). Particularly, our study suggested the therapeutic action of Sim in DMED by miR-9-5p/PDCD4 axis and further provided evidence to verify the therapeutic potential of Sim for DMED.

Bilateral cavernous nerve injury is a cause of ED, leading to profound changes in the penis and damage to CSMCs (Hannan *et al*., 2014). It has been reported that a sufficient amount of relaxed smooth muscle is required for penile erection, and the lack of smooth muscle can lead to venous occlusive dysfunction that exacerbates the symptoms of ED (Rogers *et al*., 2003). α-SMA, an important marker of smooth muscle, is downregulated during ED (Cho *et al*., 2015). It is well known that Sim treatment upregulates α-SMA and enhances erectile function. Erectile function is generally assessed by measuring ICP/MAP and low ICP/MAP is detectable in ED rats (Wang *et al*., 2019). We constructed an STZ-induced DMED rat model and evaluated it by APO. As the results presented, STZ treatment reduced body weight, ICP/MAP value, and α-SMA expression, increased blood glucose and corpus cavernosum damage, while Sim treatment attenuated the damage of STZ to DMED rats. Furthermore, we determined that Sim exerted its effects by targeting the regulation of the miR-9-5p/PDCD4 axis. To our knowledge, this is the first report that indicates that Sim regulates DMED by regulating miRNA expression.

Some miRNAs are abnormally expressed in the corpus cavernosum of ED rats, including miR-1, miR-200a, miR-203, and miR-206 (Pan *et al*., 2014). In addition, miR-101a, miR-138, miR-338, and miR-142 are dysregulated in a rat model of bilateral cavernous nerve crush (Liu *et al*., 2018). The abnormal expression of miR-9-5p is obvious in diabetes and controlling miR-9-5p confers a protection against pyroptosis in the illness (Roshanravan *et al*., 2020). In addition, miR-9-5p has also been surveyed to affect the biological behavior of smooth muscle cells (Wang *et al*., 2021). Our paper described for the first time that miR-9-5p was downregulated in DMED rats, and upregulating miR-9-5p could further enhance the ameliorating role of Sim in ED rats and CSMCs while downregulating miR-9-5p resulted in the opposite consequences.

PDCD4 was selected as a downstream target gene of miR-9-5p. PDCD4 is a pro-apoptotic gene that regulates many important cellular processes (Shuvalova *et al*., 2021) and involves in the progression of ED (Huo *et al*., 2020). Our research presented that PDCD4 expression was upregulated in DMED rats, and silencing PDCD4 protected DMED rats and CMSCs, but overexpressing PDCD4 mitigated the protection induced by miR-9-5p.

However, the study had limitations. First of all, the sample size of the study was small, and we hope to verify our research results with a larger sample size in the future. Secondly, we only explored the regulatory axis of Sim/miR-9-5p/PDCD4, and other possible regulatory mechanisms of Sim in DMED were not explored. For example, studies have shown that Sim can improve the endothelial function of retinal capillary endothelial cells under high glucose conditions by increasing the expression level of eNOS mRNA and NO production (Tun *et al*., 2017). NOS is a kind of NO-generating enzyme with three isoforms: nNOS, iNOS, and eNOS (Suksawat *et al*., 2018). Recently, an increasing number of studies have demonstrated that NO is a critical neurotransmitter in the corpus cavernosum for the mediation of penile erection (Coletto *et al*., 2018; Li *et al*., 2017). Evidence from previous studies indicates that OS-induced NO- mediated endothelial dysfunction is a key process in DMED (Castela *et al*., 2016). Therefore, we hope to further explore NOS-related pathways in future studies and improve the molecular mechanism of simvastatin alleviating DMED.

CONCLUSION

Our study demonstrated that DMED was associated with apoptosis and Sim attenuated DMED by enhancing miR-9-5p-targeted regulation of PDCD4 expression to ameliorate cavernosal injury and apoptosis in CSMCs. Our results provide a reference for Sim as a therapeutic drug for DMED.

Declarations

Acknowledgments. Not applicable.

Competing interests. The authors have no conflicts of interest to declare.

REFERENCES

Aschenbrenner B, Negro G, Savic D, Sorokin M, Buzdin A, Ganswindt U, Cemazar M, Sersa G, Skvortsov S, Skvortsova I (2021) Simvastatin is effective in killing the radioresistant breast carcinoma cells. *Radiol Oncol* **55**: 305–316. https://doi.org/10.2478/raon-2021-0020

Ba MC, Ba Z, Gong YF, Lin KP, Wu YB, Tu YN (2021) Knockdown of lncRNA ZNRD1-AS1 suppresses gastric cancer cell proliferation and metastasis by targeting the miR-9-5p/HSP90AA1 axis. *Aging (Albany NY)* **13**: 17285–17301. https://doi.org/10.18632/aging.203209

Carroll CB, Wyse RKH (2017) Simvastatin as a potential disease-modifying therapy for patients with Parkinson's disease: rationale for clinical trial, and current progress. *J Parkinsons Dis* **7**: 545–568. https://doi.org/10.3233/jpd-171203

Castela Á, Costa C (2016) Molecular mechanisms associated with diabetic endothelial-erectile dysfunction. *Nat Rev Urol* **13**: 266–274. https://doi.org/10.1038/nrurol.2016.23

Che D, Fang Z, Yan L, Du J, Li F, Xie J, Feng J, Yin P, Qi W, Yang Z, Ma J, Yang X, Gao G, Zhou T (2020) Elevated pigment epithelium-derived factor induces diabetic erectile dysfunction *via* interruption of the Akt/Hsp90β/eNOS complex. *Diabetologia* **63**: 1857–1871. https://doi.org/10.1007/s00125-020-05147-y

Chen Q, Shi X, Tan Q, Feng Z, Wang Y, Yuan Q, Tao Y, Zhang J, Tan L, Zhu G, Feng H, Chen Z (2017) Simvastatin promotes hematoma absorption and reduces hydrocephalus following intraventricular hemorrhage in part by upregulating CD36. *Transl Stroke Res* **8**: 362–373. https://doi.org/10.1007/s12975-017-0521-y

Cho MC, Park K, Kim SW, Paick JS (2015) Restoration of erectile function by suppression of corporal apoptosis, fibrosis and corporal veno-occlusive dysfunction with rho-kinase inhibitors in a rat model of cavernous nerve injury. *J Urol,* **193**: 1716–1723. https://doi.org/10.1016/j.juro.2014.10.099

Coletto E, Dolan JS, Pritchard S, Gant A, Hikima A, Jackson MJ, Benham CD, Chaudhuri KR, Rose S, Jenner P, Iravani MM (2019) Contractile dysfunction and nitrergic dysregulation in small intestine

of a primate model of Parkinson's disease. *NPJ Parkinsons Dis* **5**: 10. https://doi.org/10.1038/s41531-019-0081-9

Ding F, Shan C, Li H, Zhang Y, Guo C, Zhou Z, Zheng J, Shen W, Dai Q, Ouyang Q, Wu X (2020) Simvastatin alleviated diabetes mellitus-induced erectile dysfunction in rats by enhancing AMPK pathway-induced autophagy. *Andrology* **8**: 780–792. https://doi.org/10.1111/andr.12758

Hajializade M, Moghtadaei M, Mirzaei A, Abdollahi Kordkandi S, Babaheidarian P, Pazoki-Toroudi H, Yeganeh A (2020) Significant effect of simvastatin and/or ezetimibe-loaded nanofibers on the healing of femoral defect: An experimental study. *Mater Sci Eng C Mater Biol Appl* **111**: 110861. https://doi.org/10.1016/j.msec.2020.110861

Hannan JL, Kutlu O, Stopak BL, Liu X, Castiglione F, Hedlund P, Burnett AL, Bivalacqua TJ (2014) Valproic acid prevents penile fibrosis and erectile dysfunction in cavernous nerve-injured rats. *J Sex Med* **11**: 1442–1451. https://doi.org/10.1111/jsm.12522

He J, Li X, Dai HH, Wang JS, Li HS, Zhang XJ, Wang P, Zhang D, Zuo LY, Xie N, Li Y (2019) The safety and efficacy of PDE5-inhibitors-vardenafil on treating diabetes mellitus erectile dysfunction: A protocol for systematic review and meta analysis. *Medicine (Baltimore)* **98**: e18361. https://doi.org/10.1097/md.0000000000018361

Hirfanoglu I, Turkyilmaz C, Turkyilmaz Z, Onal E, Soylemezoglu F, Karabulut R, Atalay Y (2019) Neuroprotective effect of L-arginine in a neonatal rat model of hypoxic-ischemia. *Int J Neurosci* **129**: 1139–1144. https://doi.org/10.1080/00207454.2019.1636794

Huo W, Li H, Zhang Y, Li H (2020) Epigenetic silencing of microRNA-874-3p implicates in erectile dysfunction in diabetic rats by activating the Nupr1/Chop-mediated pathway. *Faseb J* **34**: 1695–1709. https://doi.org/10.1096/fj.201902086R

Huo W, Li Y, Zhang Y, Li H (2020) Mesenchymal stem cells-derived exosomal microRNA-21-5p downregulates PDCD4 and ameliorates erectile dysfunction in a rat model of diabetes mellitus. *Faseb J* **34**: 13345–13360. https://doi.org/10.1096/fj.202000102RR

Kopacz A, Werner E, Grochot-Przęczek A, Klóska D, Hajduk K, Neumayer C, Józkowicz A, Piechota-Polanczyk A (2020) Simvastatin attenuates abdominal aortic aneurysm formation favoured by lack of Nrf2 transcriptional activity. *Oxid Med Cell Longev* **2020**: 6340190. https://doi.org/10.1155/2020/6340190

Kopacz A, Werner E, Grochot-Przęczek A, Klóska D, Hajduk K, Neumayer C, Józkowicz A, Piechota-Polanczyk A (2007) Gene therapy and erectile dysfunction: the current status. *Asian J Androl* **9**: 8–15. https://doi.org/10.1111/j.1745-7262.2007.00224.x

Li R, Cui K, Liu K, Li H, Zhang Y, Liu X, Chen R, Li M, Wang T, Wang S, Liu J, Rao K (2017) Metabolic syndrome in rats is associated with erectile dysfunction by impairing PI3K/Akt/eNOS activity. *Sci Rep* **7**: 13464. https://doi.org/10.1038/s41598-017-12907-1

Liu C, Cao Y, Ko TC, Chen M, Zhou X, Wang R (2018) The changes of MicroRNA expression in the corpus cavernosum of a rat model with cavernous nerve injury. *J Sex Med* **15**: 958–965. https://doi.org/10.1016/j.jsxm.2018.05.006

Martínez-Salamanca JI, La Fuente JM, Cardoso J, Fernández A, Cuevas P, Wright HM, Angulo J (2014) Nebivolol potentiates the efficacy of PDE5 inhibitors to relax corpus cavernosum and penile arteries from diabetic patients by enhancing the NO/cGMP pathway. *J Sex Med* **11**: 1182–1192. https://doi.org/10.1111/jsm.12477

Pan F, Xu J, Zhang Q, Qiu X, Yu W, Xia J, Chen T, Pan L, Chen Y, Dai Y (2014) Identification and characterization of the MicroRNA profile in aging rats with erectile dysfunction. *J Sex Med* **11**: 1646–1656. https://doi.org/10.1111/jsm.12500

Rogers RS, Graziottin TM, Lin CS, Kan YW, Lue TF (2003) Intracavernosal vascular endothelial growth factor (VEGF) injection and adeno-associated virus-mediated VEGF gene therapy prevent and reverse venogenic erectile dysfunction in rats. *Int J Impot Res* **15**: 26–37. https://doi.org/10.1038/sj.ijir.3900943

Roshanravan N, Alamdari NM, Jafarabadi MA, Mohammadi A, Shabestari BR, Nasirzadeh N, Asghari S, Mansoori B, Akbarzadeh M, Ghavami A, Ghaffari S, Ostadrahimi A (2020) Effects of oral butyrate and inulin supplementation on inflammation-induced pyroptosis pathway in type 2 diabetes: A randomized, double-blind, placebo-controlled trial. *Cytokine* **131**: 155101. https://doi.org/10.1016/j.cyto.2020.155101

Rupaimoole R, Slack FJ (2017) MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. *Nat Rev Drug Discov* **16**: 203–222. https://doi.org/10.1038/nrd.2016.246

Seftel AD (2005) Erectile dysfunction is strongly linked with decreased libido in diabetic men. *J Urol* **174**: 657–658

Shuvalova E, Egorova T, Ivanov A, Shuvalov A, Biziaev N, Mukba S, Pustogarov N, Terenin I, Alkalaeva E (2021) Discovery of a novel role of tumor suppressor PDCD4 in stimulation of translation termination. *J Biol Chem* **297**: 101269. https://doi.org/10.1016/j.jbc.2021.101269

Suksawat M, Techasen A, Namwat N, Boonsong T, Titapun A, Ungarreevittaya P, Yongvanit P, Loilome W (2018) Inhibition of endothelial nitric oxide synthase in cholangiocarcinoma cell lines – a new strategy for therapy. *FEBS Open Bio* **8**: 513–522. https://doi.org/10.1002/2211-5463.12388

Talib R, Alnadhari I, Canguven O, Yassin A, Shamsodini A, Alrumaihi K, Al-Ansari A (2021) HbA1c over 8.5% is not predictive of increased infection rate following penile prosthesis implant surgery in diabetic patients with erectile dysfunction. *Andrologia* **53**: e14132. https://doi.org/10.1111/and.14132

Trivedi D, Wellsted DM, Collard JB, Kirby M (2014) Simvastatin improves the sexual health-related quality of life in men aged 40 years and over with erectile dysfunction: additional data from the erectile dysfunction and statin trial. *BMC Urol* **14**: 24. https://doi.org/10.1186/1471-2490-14-24

Tun T, Kang YS (2017) Effects of simvastatin on CAT-1-mediated arginine transport and NO level under high glucose conditions in conditionally immortalized rat inner blood-retinal barrier cell lines (TR-iBRB) *Microvasc Res* **111**: 60–66. https://doi.org/10.1016/j.mvr.2017.01.002

Wang HS, Ruan Y, Banie L, Cui K, Kang N, Peng D, Liu T, Wang T, Wang B, Wang G, Shindel AW, Lin G, Lue TF (2019) Delayed low-intensity extracorporeal shock wave therapy ameliorates impaired penile hemodynamics in rats subjected to pelvic neurovascular injury. *J Sex Med* **16**: 17–26. https://doi.org/10.1016/j.jsxm.2018.11.003

Wang J, Tan Q, Wang W, Yu J (2020) Mechanism of the regulatory effect of overexpression of circMTO1 on proliferation and apoptosis of hepatoma cells *via* miR-9-5p/NOX4 axis. *Cancer Manag Res* **12**: 3915–3925. https://doi.org/10.2147/cmar.s240719

Wang X, Cai J, Zhao L, Zhang D, Xu G, Hu J, Zhang T, Jin M (2021) NUMB suppression by miR-9-5P enhances CD44(+) prostate cancer stem cell growth and metastasis. *Sci Rep* **11**: 11210. https://doi.org/10.1038/s41598-021-90700-x

Wang X, Xu R, Chi D, Dai C, Sheng M (2021) Role of NEAT1/MiR-9-5p/SLC26A2 pathway on human airway smooth muscle cell. *Yonsei Med J* **62**: 858–867. https://doi.org/10.3349/ymj.2021.62.9.858

Wei AY, He SH, Zhao JF, liu Y, Liu Y, Hu YW, Zhang T, Wu ZY (2012) Characterization of corpus cavernosum smooth muscle cell phenotype in diabetic rats with erectile dysfunction. *Int J Impot Res* **24**: 196–201. https://doi.org/10.1038/ijir.2012.16

Wen Y, Liu G, Zhang Y, Li H (2019) MicroRNA-205 is associated with diabetes mellitus-induced erectile dysfunction *via* down-regulating the androgen receptor. *J Cell Mol Med* **23**: 3257–3270. https://doi.org/10.1111/jcmm.14212

Zou Z, Chai M, Guo F, Fu X, Lan Y, Cao S, Liu J, Tian L, An G (2021) MicroRNA-126 engineered muscle-derived stem cells attenuates cavernosa injury-induced erectile dysfunction in rats. *Aging (Albany NY)* **13**: 14399–14415. https://doi.org/10.18632/aging.203057