Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

MicroRNA-503-5p protects streptozotocin-induced erectile dysfunction in diabetic rats by downregulating SYDE2

Gangqin Li, Lin Jiang, Kuan Bai, Guangxing Tan*

Department of Andrology, Wuxi Hospital of Traditional Chinese Medicine, Wuxi City, Jiangsu Province, 214071, China

Article Info

Article history:

Received: January 08, 2024
Accepted: February 22, 2024
Published: March 31, 2024

Use your device to scan and read the article online

Abstract

Plentiful studies have clarified miRNAs take on a key role in the sexual dysfunction of diabetic rats. This study aimed to figure out microRNA (miR)-503-5p/SYDE2 axis' latent mechanisms in streptozotocin-induced diabetic rat sexual dysfunction. A model of erectile dysfunction (ED) in diabetic rats was established by injecting streptozotocin. MiR-503-5p and SYDE2 in ED rats were altered by injection of miR-503-5p mimic or si/oe-SYDE2. The targeting link between miR-503-5p and SYDE2 was testified. ICP/MAP value was tested by pressure sensor; Penile capillary abundance was assessed; Penile cGMP and AGEs were detected; penile smooth muscle cell apoptosis was assessed; MiR-503-5p and SYDE2 were tested. In streptozotocin-induced ED rats, miR-503-5p was reduced and SYDE2 was elevated. Elevating miR-503-5p or silencing of SYDE2 can enhance penile erection rate, ICP/MAP value, capillary abundance, and cGMP but reduce AGEs and penile smooth muscle cell apoptosis rate in ED rats. Strengthening SYDE2 with elevating miR-503-5p turned around the accelerating effect of elevated miR-503-5p on penile erection in ED rats. SYDE2 was a downstream target gene of miR-503-5p. MiR-503-5p protects streptozotocin-induced sexual dysfunction in diabetic rats by targeting SYDE2.

Keywords: MiR-503-5p; SYDE2; Diabetes; Erectile dysfunction.

1. Introduction

The incidence of diabetes worldwide is increasing year by year. Erectile dysfunction (ED) in men is a familiar diabetic complication characterized by the inability to achieve or maintain a prolonged erection, resulting in men not getting satisfactory results from sexual intercourse [1]. ED typically becomes more severe with elevated duration of diabetes onset, with the prevalence of ED being approximately 15 percent among diabetic patients under age of 30 and 55 percent after age of 60 [2]. The pathogenesis of diabetic ED involves multiple factors, such as weakened central nervous system stimulation, changes in penile endothelial function, etc. [3]. Among them, endothelial function changes are considered a crucial feature of diabetic ED. Hyperglycemia stimulates the formation of advanced glycation end products (AGEs) and oxidative stress, mediates smooth muscle cell death and disrupts vascular homeostasis in penile functional responses [4, 5]. This induces dysfunction of smooth muscle contraction and reduces corpus cavernosum relaxation, hyperemia, and erectile function [6]. The present cure for diabetic ED is mainly controlled by oral phosphodiesterase type 5 (PDE5) inhibitors [7]. However, it has many drawbacks with safety implications [8]. Therefore, it is imperative to seek a safe and efficient treatment for diabetic ED.

MicroRNAs (miRNAs) with their unique expression patterns can be applied as disease biomarkers [9, 10]. It turns out miRNAs take on a key role in diabetes or ED [11, 12]. MiR-503-5p, a tumor suppressor gene, is reduced in colorectal and cervical cancer [13, 14]. A recent study reports miR-503-5p is clearly associated with diabetes and arterial stenosis, and could affect vascular smooth muscle cell proliferation [15]. Therefore, it was speculated that miR-503-5p might control ED by affecting the viability of penile smooth muscle cells.

This research studied miR-503-5p and its target SYDE2 in diabetic ED and identified a positive role of this axis in disease treatment.

2. Materials and methods

2.1. Ethics Statement

Approval of the protocol was by the Wuxi Hospital of Traditional Chinese Medicine Animal Care and Use Committee, and all animals were performed in the light of the Guide for the Care and Use of Laboratory Animals Wuxi Hospital of Traditional Chinese Medicine to minimize animal distress.

* Corresponding author.
E-mail address: tgx01guangxing@outlook.com (G. Tan).
Doi: http://dx.doi.org/10.14715/cmb/2024.70.3.7

2.2. Establishment of animal models

The ED model was established with reference to the former method [16], and eight-week-old male Sprague-Dawley rats of specific pathogen-free grade (Shanghai SLAC Laboratory Animal Co., Ltd., Shanghai) were chosen and maintained at 24 ± 2°C with humidity of 60±5% and 12-h light per day. All rats had free access to clean drinking water and maintenance feed (H10010, Huafukang Biotechnology Co., Ltd., Beijing). 10 mg/mL streptozotocin (STZ), prepared with sodium citrate, was injected into the left lower abdominal cavity at 60 mg/kg once a week (w). After 4 w, the injection was reduced to once every 2 w for 12 w. After each injection, blood was collected from the caudal vein to measure blood glucose (BG). Erection (congestion, growth, and exposure) was observed when 100 μg/kg apomorphine (saline + 0.5% vitamin C) was injected into the skin of the cervix of rats. Rats with BG >16.7 mol/L and no erectile function were considered diabetic ED rats. Conversely, rats with BG values >16.7 mol/L but no change in erectile function were considered normal diabetic rats.

2.3. Grouping of rats

Eighty rats were equally assigned into 8 groups, 10 in each group: the Control, the ED, the ED+miR-503-5p mimic, the ED+NC mimic, the ED + pPLK-shRNA-NC, the ED + pPLK -shRNA-SYDE2, the ED+miR-503-5p mimic + pLenti-SYDE, the ED+miR-503-5p+oe-NC. To elevate or reduce miR-503-5p and SYDE2, ED rats were injected with miR-503-5p/NC mimic (0.5 mg/mL, 2 mL) or shRNA lentiviral plasmid vector targeting SYDE2 and elevated lentiviral plasmid vector and corresponding negative control (pPLK-sh-SYDE2, pPLK-sh-NC, pLenti-SYDE2, pLenti-NC, 2×10^8 TU/ml) into the tail vein for 1 week. After 4 w, the rats were euthanized and samples were collected for subsequent experiments. The rats in the control and the ED were injected with the same dose of normal saline. MiR-503-5p mimic and its NC were bought from RiboBio (Guangzhou, China). SYDE2-targeting siRNA and elevation plasmids and NCs were gained from Genepharma (Shanghai, China).

2.4. Intracavernous pressure (ICP)/mean arterial pressure (MAP)

ICP and MAP were evaluated as set forth [17]. The skin of the rat cervix was cut open and the carotid artery was inserted. Then, the pelvic ganglia and cavernous nerves of the penis were exposed and stimulated with a bipolar hook electrode (5.0 mV, 20 Hz, 5 ms, for 1 min). 23 G syringe needle containing 250 U/mL heparin was inserted into the calf of the penis. Pressure sensors were used to measure MAP and ICP values, and ICP/MAP values were calculated to assess erectile function.

2.5. Hematoxylin-eosin (HE) staining

Fixation with 4% paraformaldehyde and dehydration of penile tissue with graded concentrations of ethanol were implemented. Washing of tissues with xylene, waxing, embedding in paraffin, and slicing (approximately 4 μm thickness) of tissues were put into effect. Dewaxing of the sections with ethanol and preparation into tissue sections for HE staining were conducted. Staining of sections with hematoxylin and eosin separately, dehydration with ethanol, clearance with xylene, and seal with neutral glue were implemented. The tissue morphology of the penis was observed under an optical microscope, and 20 fields of view were casually chosen to detect the cavernous sinus capillaries covering red blood cells. Calculation of the capillary density (capillaries/mm^2) was implemented.

2.6. Enzyme-linked immunosorbent assay (ELISA)

Cyclic guanosine monophosphate (cGMP) and AGEs in penile tissue were measured with ELISA kits (Assay Designs, Ann Arbor, MI, USA). The optical density value was read at 450 nm on a microplate reader (Thermo Fisher Scientific, USA).

2.7. Isolation of corpus cavernosum smooth muscle cells (CCSMC)

Isolation of CCSMCs was from rat penis as set forth [PMID: 29603682]. The isolated CCSMCs were cultivated in DMEM supplemented with 10% FBS. Differential adhesion was used to eliminate other cells and only CCSMCs were present. The positive rate of α-SMA was tested by flow cytometry to identify CCSMCs.

2.8. Flow cytometry detection of apoptosis

Rat CCSMCs (10^6 cells/mL) were mixed with 500 μL of binding buffer, treated with 5 μL Annexin-V-FITC and 5 μL PI (Sigma-Aldrich, MO, USA), and examined within 1 h by a FACSCalibur flow cytometer (BD Biosciences, NJ, USA).

2.9. RT-qPCR

After isolation of total RNA using TRIzol reagent, synthesis of cDNA was implemented by applying a reverse transcription kit (Sangon, China) or microRNA reverse transcription kit (RiboBio). Quantitative PCR was performed by applying qPCR Master Mix (Promega, USA). Relative mRNA and miRNA were calculated in the light of the $2^{-\Delta\Delta Ct}$ method. The primer sequence was clarified in Table 1.

2.10. Western blot

Briefly, lysis of total protein was with lysis buffer covering protease inhibitors. Separation of tissue protein samples on 10% SDS-PAGE and complete transfer to PVDF membranes (Millipore, USA) were conducted. After incubation with primary antibodies, incubation of membranes was with goat anti-rabbit Immunoglobulin G H&L (Abeam, USA). After washing the membrane, the signal was tested applying a chemiluminescence system and analyzed employing FluorChem FC3 software (Pro-

Table 1. RT-qPCR primer sequence.

	Primer sequence (5'- 3')
GAPDH	F: 5'-ATGGGGAAGGTGAAGGTCG-3'
	R: 5'-TTACTCCTTGGAGGCCATGTG-3'
U6	F: 5'-CTCGCTTCGGCAGCACATATACT-3'
	R: 5'-ACGCTTCACGAATTTGCGTGTC-3'
MiR-503-5p	F: 5'- TGTACCACCTTGTCGG-3'
	R: 5'- TGCTGTTGCCATGAGAT-3'
SYDE2	F: 5'- ACAGCTACCAGATGGCCCT -3'
	R: 5'- CAGCCTCAGAGTTCCCACA -3'

Note: F, forward; R, reverse.

teinSimple, USA). Primary antibodies applied in this study included GAPDH (sc-32233, 1: 1000, Santa Cruz Biotechnology), SYDE2 (NBP1-94015, 1: 1000, Novus Biologicals).

2.11. Immunofluorescence

Replenishment of 3% H_2O_2 to block endogenous enzymes was conducted. Boiling penile tissue sections in antigen retrieval buffer, cooling, and repeating the heating-cooling operation twice were implemented. Blocking sections were with 5% BSA to block nonspecific antibody binding. After removing excess liquid, incubation of sections was with antibody SYDE2 (NBP1-94015, 1: 1000, Novus Biologicals), and Alexa Fluor 594-conjugated secondary antibody. Incubation of cells was with 300 nM DAPI (Sigma-Aldrich, St. Louis, MO, USA) for nuclear counterstaining. Fluorescence images were acquired under a Nikon confocal microscope (Eclipse TE2000U).

2.12. Luciferase activity assay

Cloning wild-type and mutant SYDE2 (WT/MUT-SYDE2) was into pmirGLO dual-luciferase vector (GenePharma) to construct dual-luciferase reporter plasmids. Applying Lipofectamine 2000, co-transfection of HEK293T cells was with wild-type/mutant pmirGLO-SYDE2 and miR-503-5p mimic (or NC). Luciferase activity was analyzed after transfection by applying a dual luciferase reporting kit (Promega, USA).

2.13. Data statistics

Manifestation of the data was as mean ± standard deviation (SD). Student's t-test was applied to determine differences between two groups. Prism Software 8.0 (GraphPad Software, US) was employed to analyze data. * $P < 0.05$ emphasized obvious statistical meaning. The significance among multiple groups was calculated by applying one-way analysis of variance, and the variance was corrected using the Tukey test. * $P < 0.05$.

3. Results

3.1. MiR-503-5p is reduced in diabetic ED

To figure out miR-503-5p's role in diabetic ED, it induced diabetic ED by injecting streptozotocin. As clarified in Fig. 1A, the erection rate was reduced in the ED vs. the Control. Meanwhile, the ICP/MAP ratio of the ED declined vs. the Control (Fig. 1B). It came out the penile capillary density of the rats in the ED was distinctly declined vs. the Control (Fig. 1C). The cGMP and AGEs have been shown to be associated with the pathogenesis of ED [18, 19]. It turned out in the comparison with the control, cGMP in the ED was decreased but AGEs were elevated (Fig. 1D). To determine CCSMC apoptosis, CCSMCs were separated from the corpus cavernosum and the isolated cells were identified as positive for α-SMC (Fig. 1E). Moreover, it came out that apoptosis rate of CCSMCs in the ED was elevated vs. the Control (Fig. 1F). This indicated it was successfully established a diabetic ED model by injecting streptozotocin. Subsequently, it was detected that miR-503-5p, as clarified in Fig. 1G, miR-503-5p in the ED rats was declined vs. rats in the Control. These results suggest miR-503-5p is reduced in streptozotocin-induced diabetic ED.

3.2. Elevating miR-503-5p mitigates ED in rats

To figure out miR-503-5p's role in sexual dysfunction in diabetic rats, it was first examined miR-503-5p. miR-503-5p was apparently elevated after injection of miR-503-5p mimic (Fig. 2A). Subsequently, it was examined the effect of elevating miR-503-5p on ED in rats. After treatment with miR-503-5p mimic, the erection rate of ED rats was clearly increased and the ICP/MAP ratio was effectively restored (Fig. 2B-C). The results clarified (Fig. 2D), the penile capillary density of ED rats was distinctly elevated after miR-503-5p mimic treatment. Meanwhile, elevating miR-503-5p memorably enhanced cGMP but declined AGEs in the penis of ED rats (Fig. 2E). Moreover, elevating miR-503-5p also reduced the apoptosis rate of CCSMCs in ED rats (Fig. 2F). Taken together, these results suggest elevating miR-503-5p can effectively treat ED in diabetic rats.

3.3. SYDE2 is elevated in ED and a target gene of miR-503-5p

Bioinformatics website http://starbase.sysu.edu.cn/ discovered SYDE2 and miR-503-5p's latent binding sites (Fig. 3A). For further confirming the direct targeting link between SYDE2 and miR-503-5p, it was conducted the luciferase activity assay. WT SYDE2 clearly reduced the dual luciferase activity in the miR-503-5p mimic, while mutant SYDE2 had no clear effect on the luciferase activity in the miR-503-5p mimic (Fig. 3B). Moreover, it was found that SYDE2 was clearly increased in the ED vs. the Control, while treatment with miR-503-5p mimic significantly decreased SYDE2 (Fig. 3C-D). These results sug-

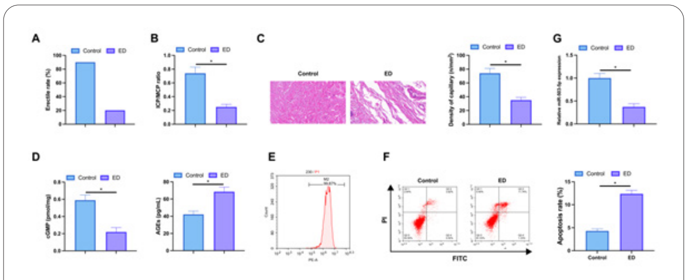

Fig. 1. MiR-503-5p is reduced in diabetic ED. A: Erection rate; B: ICP/MAP ratio; C: Representative images of penile HE staining and capillary density; D: ELISA detection of cGMP and AGEs in the penile tissues; E: The positive rate of α-SMA in the isolated cells identified by flow cytometry; F: CCSMC apoptosis rate tested by flow cytometry; G: RT-qPCR detection of miR-503-5p; Apart from E, A-G, in the rats of the Control and the ED; n = 10.

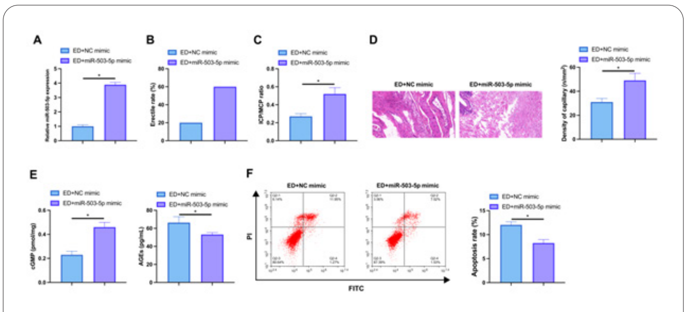

Fig. 2. Elevating miR-503-5p mitigates ED in rats. A: RT-qPCR detection of miR-503-5p; B: The erection rate; C: ICP/MAP ratios; D: Representative images and capillaries' density of penile HE staining; E: ELISA detection of cGMP and AGEs in penile tissue; F: Flow cytometry detection of apoptosis rate of CCSMCs; A-F, in the rats of the ED+NC mimic and the ED+miR-503-5p mimic, n = 10.

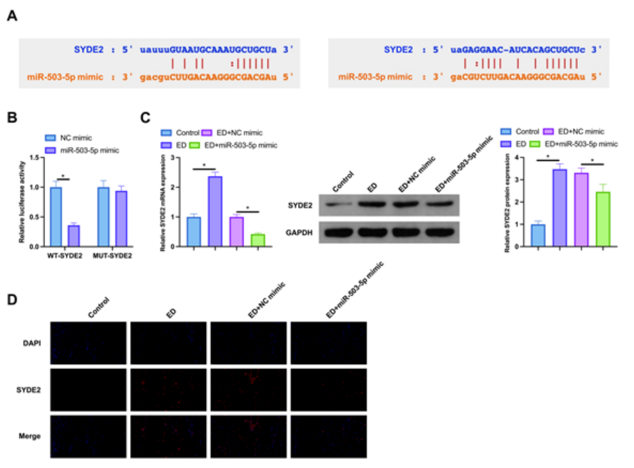

Fig. 3. SYDE2 is elevated in ED and a target gene of miR-503-5p. A: http://starbase.sysu.edu.cn/ prediction of the binding site of miR-503-5p and SYDE2; B: The luciferase activity assay to verify the targeting relationship between miR-503-5p and SYDE2; C: RT-qPCR and western blot detection of SYDE2 in rats; D: Immunofluorescence detection of SYDE2 in the penis of rats; C-D, in the Control, the ED, the ED+NC mimic and the ED+miR-503-5p mimic. Fig. B, n = 3; Fig. C, n = 10.

gest SYDE2 is elevated in diabetic ED and is targeted by miR-503-5p.

3.4. Silencing SYDE2 helps to improve ED in diabetic rats

To further demonstrate the role of SYDE2 in diabetic ED, it was silenced SYDE2 in ED rats (Fig. 4A-B). In Fig. 4C, after silencing SYDE2, the erection rate in ED rats was clearly elevated. Furthermore, silencing SYDE2 clearly increased the ICP/MAP ratio in ED rats (Fig. 4D). Moreover, after silencing SYDE2, the capillary density in the penis of ED rats was significantly increased (Fig. 4E). And silencing of SYDE2 resulted in a significant elevation in cGMP and an apparent reduction in AGEs in penile tissue (Fig. 4F). Moreover, silencing SYDE2 also reduced the apoptosis rate of CCSMCs in ED rats (Fig. 4G). This suggests silencing SYDE2 can effectively improve ED in diabetic rats.

3.5. MiR-503-5p/SYDE2 axis plays a key role in diabetic ED

Subsequently, it was explored whether SYDE2 participates in the regulation of miR-503-5p in diabetic ED. It was elevated SYDE2 with strengthening miR-503-5p in ED rats. As clarified in Fig. 5A-B, SYDE2 was memorably elevated in ED rats after injection of miR-503-5p mimic and oe-SYDE2 mixture. Moreover, vs. the ED+miR-503-5p mimic + pLenti-NC, the erection rate, ratio of ICP/MAP and capillary density of the rats in the ED+miR-503-5p mimic + pLenti-SYDE were significantly reduced, and cGMP in the penis tissue was clearly declined and AGEs was apparently elevated, in addition, the apoptosis rate of CCSMCs was significantly increased (Fig. 5C-G). These results suggest miR-503-5p influences the development of ED in diabetes by regulating SYDE2.

4. Discussion

ED often takes place as a complication of atherosclerosis, diabetes and other diseases [20, 21]. Therefore, in this

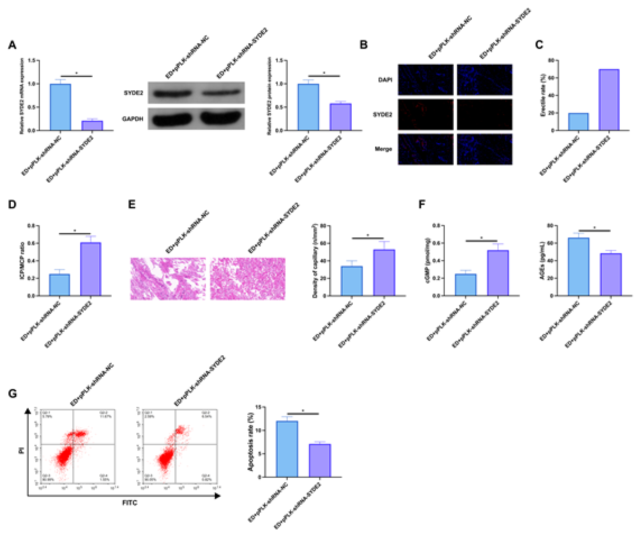

Fig. 4. Silencing SYDE2 helps to improve ED in diabetic rats. A: RT-qPCR and western blot detection of SYDE2; B: Immunofluorescence detection of SYDE2; C: Erection rate; D: ICP/MAP ratio; E: Representative images of penile HE staining and capillary density; F: ELISA detection of cGMP and AGEs in penile tissues; G: Flow cytometry detection of apoptosis rate of CCSMCs; n = 10. A-G, in the rats of the ED + pPLK-shRNA-NC and the ED+pPLK-shRNA-SYDE2.

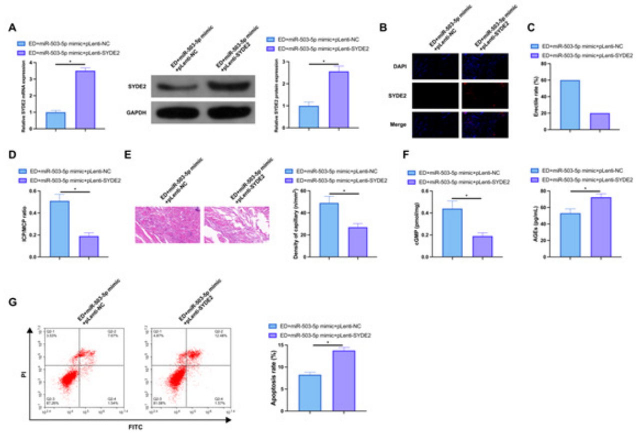

Fig. 5. MiR-503-5p/SYDE2 axis plays a key role in diabetic ED. A: RT-qPCR and western blot detection of SYDE2; B: Immunofluorescence detection of SYDE2; C: Erection rate; D: ICP/ MAP ratio; E: Representative images of penile HE staining and capillary density; F: ELISA detection of cGMP and AGEs in penile tissues; G: Apoptosis rate of CCSMCs detected by flow cytometry; n = 10. A-G, in the rats of the ED+miR-503-5p mimic + pLenti-NC and the ED+miR-503-5p mimic + pLenti-SYDE.

study, streptozotocin was applied to induce and establish a diabetic ED rat model in order to explore potential molecular targets for the treatment of ED. The results clarified that miR-503-5p was reduced in diabetic ED, and elevating miR-503-5p could improve diabetic ED by depressing SYDE2.

The mechanism of action of miRNAs in ED in diabetes is not fully understood. Previous studies have exhibited that miR-205 is associated with the pathogenesis of diabetic ED by downregulating androgen receptor expression [22]. Furthermore, Li *et al.* report that miR-328 could ameliorate diabetic ED by regulating cGMP and AGEs in penile tissue [11]. This is consistent with the results. The cGMP can induce relaxation of cavernous smooth muscle cells and motivate erection by activating protein kinase G and reducing intracellular calcium levels [23]. Elevating miR-530-5p or silencing SYDE2 could accelerate cGMP in rat penis tissue, which may help improve ED in diabetic rats. More and more studies have illustrated diabetes accelerates the production of AGEs, and represses NO synthesis and smooth muscle cell proliferation, which may be attributed to the increase in BG levels [3, 24]. The upregulation of AGEs is linked with declined erectile function in ED rats, and repressing their formation may become an advanced approach for the treatment of diabetic ED [25]. Meanwhile, up-regulation of AGEs and down-regulation of NO-cGMP are often accompanied by ED models [19]. miR-503-5p/SYDE2 axis affected AGEs in the penis of diabetic ED rats, which may contribute to the improvement of erectile function.

An erection requires a certain number of relaxed smooth muscle cells, and the lack of smooth muscle cells can occlude veins and exacerbate ED symptoms [26]. A previous study clarifies that miR-145 motivates smooth muscle cell numbers in aged ED rats by regulating Kruppel-like factor 4 and transforming growth factor beta receptor 2 [27]. This study found elevating miR-503-5p/silencing of SYDE2 could significantly reduce the apoptosis rate of CCSMCs of diabetic ED rats, which may contribute to the promotion of penile erection. However, the specific molecular mechanism of miR-503-5p involved in regulating CCSMC apoptosis remains to be further studied. Moreover, increasing the capillary density of the corpus cavernosum can alleviate ED symptoms [16]. In this study, modulation of the miR-503-5p/SYDE2 axis could clearly elevate the capillary density in the penis of diabetic ED rats.

In conclusion, the data indicated enhancement of miR-503-5p could increase penile erection rate, ICP/MAP value, capillary abundance, and cGMP but reduce AGEs and CCSMC apoptosis rate in ED rats. This helps improve ED in rats. Moreover, these effects of miR-503-5p were mainly achieved by regulating SYDE2.

Acknowledgments
Not applicable.

Funding
Not applicable.

Availability of data and materials
The data are available from the corresponding author upon request.

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

References

1. Compostella L, Compostella C, Truong LV, et al., History of erectile dysfunction as a predictor of poor physical performance after an acute myocardial infarction. European journal of preventive cardiology, 2017. 24(5): p460-467.
2. Matsui H, Sopko NA, Hannan JL, et al., Pathophysiology of erectile dysfunction. Current drug targets, 2015. 16(5): p. 411-419.
3. B Musicki , J L Hannan, G Lagoda, et al., Mechanistic link between erectile dysfunction and systemic endothelial dysfunction in type 2 diabetic rats. Andrology, 2016. 4(5): p977-983.
4. Ahmed, N. Advanced glycation endproducts--role in pathology of diabetic complications. Diabetes research and clinical practice, 2005. 67(1): p3-21.
5. Angela C, Pedro G, Valentina F D, et al., Role of oxidative stress-induced systemic and cavernosal molecular alterations in the progression of diabetic erectile dysfunction. Journal of diabetes, 2015. 7(3): p393-401.
6. Mobley D, M Khera, and N Baum, Recent advances in the treatment of erectile dysfunction. Postgraduate medical journal, 2017. 93(1105): p679-685.
7. David H W L, Sashi K, Faiz H M, et al., The management of phosphodiesterase-5 (PDE5) inhibitor failure. Current vascular pharmacology, 2006. 4(2): p89-93.
8. Stacy L, Yasin F, Mats L, et al., Use of Phosphodiesterase Type 5 Inhibitors for Erectile Dysfunction and Risk of Malignant Melanoma. JAMA, 2015. 313(24): p2449-2455.
9. Xin L, Martin E, Ulf S, et al., Systems biology-based investigation of cooperating microRNAs as monotherapy or adjuvant therapy in cancer. Nucleic acids research, 2019. 47(15): p7753-7766.
10. Moritz M, Vera P, Richard R, et al., MicroRNAs in Inflammatory Heart Diseases and Sepsis-Induced Cardiac Dysfunction: A Potential Scope for the Future? Cells, 2019. 8(11):p1352.
11. Dong-Shui L, Liang F, Long-Hua L, et al., The Effect of microRNA-328 antagomir on erectile dysfunction in streptozotocin-induced diabetic rats. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, 2017. 92: p888-895.
12. Francesco P, Giulia M, Angelica G, et al., Extracellular vesicle-shuttled miRNAs: a critical appraisal of their potential as nano-diagnostics and nano-therapeutics in type 2 diabetes mellitus and its cardiovascular complications. Theranostics, 2021. 11(3): p1031-1045.
13. Wei R, Yu-Hua Z, Xiao-Jie M, et al., The Effect of miR-503-5p on the Proliferation, Invasion, Migration and Epithelial Interstitium of Cervical Cancer HeLa Cells via Targeting E2 F3. Sichuan da xue xue bao. Yi xue ban = Journal of Sichuan University. Medical science edition, 2020. 51(2): p178-184.
14. Linlin W, Chaonan S, Yaotian Z, et al., miR-503-5p inhibits colon cancer tumorigenesis, angiogenesis, and lymphangiogenesis by directly downregulating VEGF-A. Gene therapy, 2020.
15. Zhiyuan Y, Hong W, Junjun L, et al., MicroRNA-503-5p improves carotid artery stenosis by inhibiting the proliferation of vascular smooth muscle cells. Experimental and therapeutic medicine, 2020. 20(5): p85.
16. Yun Z, Wei H, Yan W, et al., Silencing Nogo-B receptor inhibits penile corpus cavernosum vascular smooth muscle cell apoptosis of rats with diabetic erectile dysfunction by down-regulating ICAM-1. PloS one, 2019. 14(8): pe0220715.
17. XiYou W, CuiLong L, ShaoDan L, et al., Hypoxia precondition promotes adipose-derived mesenchymal stem cells based repair of diabetic erectile dysfunction via augmenting angiogenesis and neuroprotection. PloS one, 2015. 10(3): pe0118951.
18. Javier A, Rocío G -C, Pedro C, et al., Diabetes exacerbates the functional deficiency of NO/cGMP pathway associated with erectile dysfunction in human corpus cavernosum and penile arteries. The journal of sexual medicine, 2010. 7: p758-768.
19. Jian Z, Ai-Min L, Bao-Xing L, et al., Effect of icarisid II on diabetic rats with erectile dysfunction and its potential mechanism via assessment of AGEs, autophagy, mTOR and the NO-cGMP pathway. Asian journal of andrology, 2013. 15(1): p143-148.
20. A Aversa, E Greco, R Bruzziches, et al., Relationship between chronic tadalafil administration and improvement of endothelial function in men with erectile dysfunction: a pilot study. International journal of impotence research, 2007. 19(2): p200-207.
21. Ji-Kan R, Hai-Rong J, Guo N Y, et al., Erectile dysfunction pre-

cedes other systemic vascular diseases due to incompetent cavernous endothelial cell-cell junctions. The Journal of urology, 2013. 190(2): p779-789.
22. Yan W, Guohui L, Yun Z, et al., MicroRNA-205 is associated with diabetes mellitus-induced erectile dysfunction via down-regulating the androgen receptor. Journal of cellular and molecular medicine, 2019. 23(5): p3257-3270.
23. Prieto D. Physiological regulation of penile arteries and veins. International journal of impotence research, 2008. 20(1): p17-29.
24. Thorve VS, Kshirsagar AD, Vyawahare NS, et al., Diabetes-induced erectile dysfunction: epidemiology, pathophysiology and management. Journal of diabetes and its complications, 2011. 25(2): p129-136.
25. Barbosa J, S Oliveira, and L Seara. The role of advanced glycation end-products (AGEs) in the development of vascular diabetic complications. Arquivos brasileiros de endocrinologia e metabologia, 2008. 52(6): p940-950.
26. R S Rogers , T M Graziottin, C-S Lin, et al., Intracavernosal vascular endothelial growth factor (VEGF) injection and adeno-associated virus-mediated VEGF gene therapy prevent and reverse venogenic erectile dysfunction in rats. International journal of impotence research, 2003. 15(1): p26-37.
27. Qiwei L, Yubin C, Haojian L, et al., MicroRNA-145 engineered bone marrow-derived mesenchymal stem cells alleviated erectile dysfunction in aged rats. Stem cell research & therapy, 2019. 10(1): p398.