In vivo tracking on longer retention of transplanted myocardin gene-modified adipose-derived stem cells to improve erectile dysfunction in diabetic rats

Hai-Bo Zhang†, Feng-Zhi Chen†, Shu-Hua He†, Yan-Bing Liang, Zhi-Qiang Wang, Li Wang, Ze-Rong Chen, Wei Ding, Shan-Chao Zhao* and An-Yang Wei*†

Abstract

Background: Stem cell therapy has revealed a promising future for treating erectile dysfunction (ED), but the fate and curative mechanism of intracavernosal transplanted stem cells are under further exploration. This study aimed to demonstrate the effects of myocardin gene modification on improving erectile function and prolonging the retention of implanted adipose-derived stem cells (ASCs) using in vivo small animal imaging.

Methods: ASCs were isolated, cultured, and identified by flow cytometry and osteogenic and adipogenic induction. The effects of gene modification on cell proliferation, apoptosis, and contraction were determined by CCK-8, EdU, flow cytometry, and collagen gel lattice contraction assays as well as confocal microscopy. A total of 20 normal and 60 diabetes mellitus ED to (DMED) Sprague-Dawley rats were recruited to the 7 day and 21 day groups. Each group contained subgroups of 10 rats each: the negative control (NC), DMED + ASCs plus Ad-Luc-Myocardin, DMED + ASCs plus Ad-Luc, and DMED + phosphate buffer solution (PBS) groups. Erectile function was evaluated with the intracavernosal pressure/mean arterial pressure (△ICP/MAP) ratio. In vivo small animal imaging and an EdU cell tracking strategy were introduced to detect the transplanted ASCs, and IHC and WB were performed to assess smooth muscle cell protein levels.

Results: The ASCs expressed high CD29 and CD90 and scant CD45, while the multi-induction potential was verified by oil red O and alizarin red staining. Gene transfection of myocardin had no significant influence on ASC apoptosis but inhibited cell proliferation and promoted cell contraction. Myocardin combined with ASCs enhanced the therapeutic potential of ASCs for improving the △ICP/MAP ratio as well as α-SMA and calponin expression. In vivo imaging confirmed that ASCs resided within the cavernous body in 21 days, while only a few red EdU dots were detected.

Conclusions: Myocardin induced ASC differentiation towards smooth muscle-like cells and enhanced the therapeutic potential of ASCs for ameliorating ED in STZ-induced diabetic rats. Notably, in vivo small animal tracking was an effective strategy for monitoring the implanted stem cells, and this strategy might have advantages over traditional EdU assays.

Keywords: Erectile dysfunction (ED), Adipose-derived stem cells (ASCs), Myocardin, Cell tracking, Diabetes mellitus
**Background**

Erectile dysfunction (ED) affects an estimated 35–90% of men with diabetes mellitus (DM) [1], and current treatment strategies, such as phosphodiesterase 5 inhibitors (PDE5i) and vacuum constriction devices, reveal insufficient effects or limitations [2]. In recent years, animal experiments and clinical trials have shown promising therapeutic effects of stem cell transplantation on ED [3]. However, the literature has indicated a large difference in the retaining period of intracavernously injected cells using 5-ethynyl-2′-deoxyuridine (EdU) or other cell trackers in tissue sections [4], and this period differed from less than 5 days to more than 4 weeks [5, 6]. Thus, several issues should be addressed to clarify the cell fate of transplanted stem cells [7]. First, could the injected cells reside within the cavernous body? Second, should injections be repeated to replenish the cells? Finally, is there any difference between different methodologies? Recently, it was reported that repeat treatments did not provide any benefit for the recovery of erectile function and histomorphometric changes [8], and a single injection showed long-term improvements in ED [9]. Here, we introduce an in vivo strategy, in vivo small animal imaging, which is often applied for the detection of metastatic tumors [10], to track transplanted ASCs; we also compared this technique with the traditional EdU method.

Corpus cavernosum smooth muscle (CCSM) cells provide structural support, and their well-functioning dilation can control blood flow into the corpora, playing a vital role during penile erection [11]. Simultaneously, alleviation of CCSM apoptosis ameliorates ED in rats [12], suggesting the efficiency of this curative strategy for improving CCSM cell function. Among the regulators, myocardin is known to be required for the maintenance of functions in smooth muscle cells [13, 14], and it was demonstrated to enhance the therapeutic potential for myocardial infarction in mesenchymal stem cells in an animal model [15]. We have previously demonstrated the efficiency of myocardin gene therapy in ED in a rat model of bilateral cavernous nerve injury [16], but the effects of the gene combination with adipose-derived stem cells (ASCs) remain to be clarified. This study aims to prove the myocardin genetic modification of ASCs to improve erectile dysfunction in diabetic rats.

**Methods**

**Experimental design**

All experimental male Sprague–Dawley (SD) rats weighed 250–300 g were purchased and housed in the Experimental Animal Center of Nanfang Hospital, Southern Medical University of China. All experimental protocols were performed under the Institutional Animal Care and Use Committee-approved guidelines at our institution. The animals were designated into the 7 day and 21 day groups. Each group was divided into 4 subgroups: the negative control (NC), diabetic mellitus ED (DMED) + ASCs plus Ad-Luc-Myocardin, DMED + ASCs plus Ad-Luc, and DMED + PBS groups (n = 10 per group). The DMED rats were induced with streptozotocin and screened by the apomorphine-induced erection test to confirm ED before subsequent one-time intracavernosal injection of 50 μL PBS with 1 × 10^6 cells or PBS only. After 7 or 21 days, all rats were anesthetized for in vivo small animal imaging before intracavernosal pressure (ICP) and mean arterial pressure (MAP) tests. The penises of the rats in the 21 day group were harvested, weighed, and processed for further study.

**Apomorphine-induced erection test**

An apomorphine-induced erection test was performed as we previously described [17]. Rats were first moved to a tranquil and dimly lit laboratory and set in a separate transparent observation kit at least 10 min before any operation to allow them to adapt to the surroundings. Then, a single subcutaneous injection of 100 μg/kg apomorphine (APO, Sigma, USA) was given via the loose skin at the back of the neck. During the following 30 min, the status and frequency of penile erection in rats were observed by two trained technicians, and each instance of glans engorgement or the appearance of the penile shaft represented one erection. Finally, rats with no erection were defined as having ED and were included in the subsequent experiments.

**In vivo small animal imaging**

In vivo tracking of the transfected ASCs was performed using the IVIS Lumina II system as previously reported [10]. Briefly, all groups of animals were intraperitoneally injected with 150 mg/kg D-luciferin (Bioworld, Minneapolis, MN, USA) dissolved in DPBS (HyClone, GE, Boston, USA) at a concentration of 15 mg/ml for 5 min before anesthesia. Then, animals were placed in the camera apparatus, and local images were taken.

**Erectile function evaluation**

Erectile function was assessed with the △ICP/MAP ratio as previously described [16]. Rats were sterilized and anesthetized, and a low abdominal incision was made. The cavernous nerves were exposed for stimulation with a bipolar, stainless steel electrode. Subsequently, a 25-G needle containing 100 U/ml heparin solution was inserted into the right penile crus, which was connected to the transducer and amplifier of the MP150 biopac system (Biopac Systems Inc., CA, USA) and supporting software AcqKnowledge® V4.4. The stimulus parameters were as follows: amplitude (5 mA), frequency (20 Hz), pulse width (0.2 ms), and duration (60 s). The erectile
ing cells were double stained with Hoechst 33342 (blue) and EdU (red), while quiescent cells were double stained with Hoechst only. The total and EdU+ cells were counted in 3 independent fields under a ×400 microscope in every cultured well. Time growth curves were fabricated with GraphPad Prism 5 software.

Collagen gel lattice contraction assay
A modified in vitro cell contractility assay was carried out as we demonstrated before [16]. Briefly, ASCs with different treatments at a density of 2 × 10^5 cells/ml were mixed with solubilized type I collagen (Sigma, USA) to form a cell–collagen suspension at a final concentration of 1 mg/ml. A total of 200 μl of suspension was dropped in a 35-mm culture dish immediately and then incubated in a growth medium for another 5 days before the cell–collagen lattice was mechanically released from the underlying plastic substratum. Subsequently, the lattice was exposed to serum-free Dulbecco’s modified Eagle’s medium (DMEM), DMEM plus 10% fetal bovine serum (FBS), or DMEM plus 1 μM calcium ionophore (Ca-Ionophore, Sigma, USA). Serum-free DMEM was used as a negative control, while FBS was used as a positive control. The diameters before releasing the lattice and 10 min after the various exposures were recorded for relative percent contraction.

qRT-PCR
Total RNA was extracted from ASCs using RNAsiso plus reagent (Takara, Japan) according to the manufacturer’s instructions. qRT-PCR was performed using the LightCycler® 480 II (Roche, Basel, Switzerland) with the SYBR Green PCR kit (Takara, Japan). Gene β-actin was used as an internal control. The specificity of the amplification products was confirmed by melting curve analysis. Triplicate samples were analyzed in three independent experiments. Primer sequences are shown in Additional file 1: Table S1.

Western blotting
Protein samples were obtained from ASCs on ice and from penile tissues in liquid nitrogen with radioimmunoprecipitation assay buffer (Cell Signaling Technology, CST, MA, USA) containing protease and phosphatase inhibitors (Roche, Switzerland). The protein concentration was determined by the Bradford method using the BCA Protein Assay (Thermo Fisher Scientific Inc., MA, USA). Protein lysates were separated on an 8–10% sodium dodecyl sulfate-polyacrylamide gel and electrotransferred to a polyvinylidene difluoride membrane (Bio-Rad, CA, USA) that was blocked with 5% bovine serum albumin for 1 h at room temperature and probed overnight at 4 °C with primary antibodies, followed by incubation with horseradish peroxidase-conjugated secondary antibodies (Abcam, Cambridge, UK). Immunodetection was performed using enhanced chemiluminescence reagent (Bio-Rad, USA). Antibodies against myocardin, 1:400; collagen I, 1:1000
**Results**

**Adipogenic and osteogenic induction and immune phenotype of ASCs**

Primary ASCs were isolated and cultured. A typical long fusiform shape with a whirlpool-like growth on the passage 3 cell image is shown in Fig. 1A (a), as well as adipogenesis and osteogenesis images confirmed by oil red O (b) and alizarin red staining (c). Flow cytometry was performed with CD 29, CD 90, and CD 45 to detect the immune phenotype of the cultured ASCs. As shown in Fig. 1B, CD 29 and CD 90 were expressed in 99% of cells, while CD 45 was negatively expressed.

The proliferative capacity of ASCs was reduced with overexpression of myocardin

EdU showed that the proliferative cell rates were 35.93 ± 1.42% and 62.38 ± 2.53% in the Ad-myocardin and vector cells, respectively (Fig. 1C, D). Similarly, the CCK-8 assay was performed, and overexpression of myocardin resulted in reduced proliferative capacity within 48 h in ASCs transfected with Ad-myocardin compared with that in ASCs transfected with empty vector (Fig. 1E). Further qRT-PCR and immunoblotting analyses revealed downregulated mRNA and protein expression of the cell proliferation marker PCNA by myocardin (Fig. 1F, G).

**Histology**

Freshly dissected tissues were fixed and prepared for histological examinations. H&E and Masson’s trichrome staining as well as immunohistochemistry (IHC) were performed according to the manufacturer’s instructions. Sections were cut at 4 μm and incubated with myocardin (1:100, Abcam, UK) and α-SMA (1:50, Santa Cruz, USA). Digital images were acquired with an Olympus laser scanning confocal microscope (Olympus, Shinjuku Monolith, Japan), and the SM-to-collagen ratio of Masson’s trichrome staining was evaluated using Image-Pro Plus 6.0.

**Statistics**

Statistical analysis was performed with SPSS software version 21.0 for Windows. The results were recorded as the mean ± SEM. Groups were compared using Student’s T test or one-way ANOVA. The univariate general linear model with fixed factors of time and group was performed to test the CCK8 results. The statistical significance was determined at the 5% confidence level (p < 0.05).

**Gene modification of myocardin prolonged the retention of transplanted ASCs within the cavernous body**

In vivo small animal imaging and an EdU cell tracking strategy were performed to determine the retention of transplanted ASCs within the cavernous body in different animal groups. Before ASC injection, the EdU transfection efficiency was proven to be 81.53 ± 1.42%, which
is in support of subsequent detection (shown in Additional file 2: Figure S1). As shown in Fig. 4a, no fluorescence was detected in NC or DMED+PBS rats of both the 7 day and 21 day groups, which served as controls for luciferase gene transfection. The fluorescence intensities of the two 7 day experimental groups were significantly higher than those of the 21 day groups, revealing a time-dependent decrease in the retention of ASCs, and fluorescence in the DMED+Ad-Luc-Myocardin rats was notably stronger than that in the DMED+Ad-Luc rats in the 7 day and 21 day groups, respectively. Subsequently, frozen sections were made, and EdU+ cells were shown in the different groups (Fig. 4b–e), indicating the longer retention in the gene-modified stem cell groups, which is consistent with the results of in vivo imaging.

**Overexpression of myocardin promoted the therapeutic potential of ASCs in ED**

As shown in Fig. 4f and g, the 7 day and 21 day DMED+PBS rats showed a remarkable decline in maximum ICP compared with the NC+PBS rats. The ASC-treated rats showed a significant increase in maximum ICP in all subgroups. There was no difference in the MAP among all the groups. Statistically, the △ICP/MAP values in the two ASC groups were significantly higher than those in the DMED+PBS groups but were still much lower than those in the NC groups. There was no difference between the DMED+Ad-Luc-Myocardin and DMED+Ad-Luc groups at 7 days (Fig. 4f). However, in the 21 day groups, myocardin gene-modified ASCs significantly increased the △ICP/MAP ratio compared with the ASC plus vector group (Fig. 4g).
The transfection efficiency of myocardin was further confirmed using IHC and WB (Fig. 5A, B). The morphological changes and smooth muscle (SM)-to-collagen ratios of the different groups were detected with H&E and Masson’s trichrome staining, as shown in Fig. 5C and D. ASC treatment reduced the damage to morphological changes caused by diabetes mellitus and significantly increased the SM-to-collagen ratio compared with the DMED+PBS group, but the ratio was still lower than that in the NC group (Fig. 5E). In addition, gene modification remarkably increased the therapeutic effects of ASCs. As shown in Fig. 5F, the protein expression levels of myocardin, collagen I, cleaved-caspase 3, and the CCSM cell markers α-SMA and calponin were measured. Taken together, the results show that myocardin overexpression promotes the effect of ASCs on improving the CCSM cell number and functions and inhibits the pathological process of fibrosis and apoptosis.

**Discussion**
Over the past few years, efforts have been made to explore novel therapeutic strategies for erectile dysfunction (ED), including stem cell transplantation, gene therapy, and low energy shock wave therapy [20]. Among these strategies, stem cell therapy has demonstrated a
substantial curative effect in both animal studies and clinical trials [9, 21]. A meta-analysis by Ji-Hong Liu et al. summarized 10 animal studies containing 302 diabetic ED rats that received a single injection of stem cells and found main effects on increasing the intracavernosal pressure, cavernosal tissue smooth muscle/collagen ratio, and contents of nNOS, eNOS, and VEGF and inhibiting cell apoptosis [22]. However, how stem cells exert the aforementioned effects remains to be clarified. It was speculated that transplanted stem cells were capable of differentiating into corpus cavernosum smooth muscle cells (CCSMCs) or endothelial cells to repair damaged tissues. However, no direct evidence was found to support this transformation [23]. In recent years, researchers have tended to reveal the role of cytokines secreted by mesenchymal stem cells (MSCs) and have demonstrated therapeutic effects by the lysate or conditioned medium of MSCs. Notably, different cytokines were selected in the published studies, and the cytokine profile of the MSCs has not been revealed by medium- or high-throughput screening [24]. Furthermore, not limited to cytokines, other investigators and our team reported previously that exosomes derived from ASCs exerted a measure of benefit compared with ASCs alone [12, 19]. Thus, the mysteries of these miraculous stem cells have yet to be uncovered.

Among the published literature, diverse effects have been observed in different studies, especially on the transplanted stem cell retention period within the cavernous body. Dr. Jong-Ho Won et al. reported that injected bone marrow stem cells reside in the penis and slowly flow into the pelvic area in 3 months using MRI monitoring [25]. Furthermore, Yu-tian Dai et al. found that only a few implanted cells were detectable 4 weeks after injection using EdU cell tracking technology [6]. However, a similar study with similar stem cell tracker NanoShuttle magnetic nanoparticles suggested that no remaining stem cells were detected 9 days post-transplantation [5]. Therefore, it remains to be determined whether injected stem cells remain in the cavernous body or spread rapidly. In this study, we compared two methods for cell tracking using in vivo small animal imaging, which has frequently been applied for tumor measurement and metastatic detection, and tissue immunofluorescence of EdU, demonstrating the superiority of the in vivo strategy over pathological section detection, which required animal execution and cumbersome methods and was known to have the shortcoming of slicing randomness. Our data support evidence of the abundant retention of implanted cells for at least 21 days, which is consistent with other published studies using MRI [4, 25].

Another important issue is the duration of stem cell effects. When applied in clinical trials, this novel cell therapeutic regimen appeared to have diverse potencies with immediate and lasting effects. You Rene et al. suggested a total effective rate of 75% [3], while Haahr et al. reported that 8 of the 17 participants were free of immediate effects 6 months after the regimen [21]; Jong Yoon Bahk et al. demonstrated that 4 of 7 patients returned to the initial state after 9 months [26]. The benefits of stem
cell therapy include improvements in the International Index of Erectile Function-15 and Erection Hardness Scale questionnaires as well as peak systolic velocity and penile nitric oxide release test [3, 27]. Notably, almost all patients were in need of other assistive treatments to complete their regimens, such as a phosphodiesterase type 5 inhibitor (PDE5i) [28]. Therefore, to enhance the therapeutic effects of stem cells, two strategies have been implemented that target extending the retention of transplanted cells and improving certain curative capacity by gene modification in animal studies. It has been proven that chemical modification of hydrogel [29] or poly l-glutamic acid (PLGA) membranes [30] increases both the number of labeled stem cells and the

---

**Fig. 4** Myocardin prolonged the retention and enhanced the therapeutic potential of transplanted ASCs. **a** In vivo small animal imaging revealed the fluorescence intensities produced by luciferase incorporated into the adenoviruses, indicating the retention amount of ASCs 7 and 21 days post-transplantation. The EdU cell tracking strategy was introduced to detect the implanted ASCs, which were stained red with EdU at 7 (b, d) and 21 days (c, e). **f** Representative images of intracavernosal pressure (ICP) and mean arterial pressure (MAP) and the △ICP (maximum ICP - basal ICP)/MAP ratio are shown in the 7 day group as well as the 21 day group of rats (g). Animals tested n = 10. ***p < 0.001
intracavernosal pressure, but determining longer effects of these animal experiments and the safety of these synthetic compounds on the human body requires further observation.

In recent years, gene-modified stem cells have attracted unprecedented enthusiasm and were expected to play a stronger role in repairing damaged tissues compared with untreated stem cells [31]. In our study, myocardin, which has been confirmed to be essential for the development and function of smooth muscle cells [32, 33], was introduced to induce the differentiation of ASCs towards SML cells in vitro by interacting with SRF and inhibiting the general stem cell markers SOX2 and OCT4 [34], as well as enhancing therapeutic effects through both prolonging the retention of implanted ASCs and increasing the expression of smooth muscle contents. Notably, gene modification appeared safe and controllable, as it did not increase the cell apoptosis rate or mortality in the animal model. It is known that normal erectile function requires coordination among cavernous nerves, vessels, and endothelial and smooth muscle cells, and smooth muscle cells have been shown to be targets of other factors [35, 36]. Thus, in the current research, the focus was on improving smooth muscle function, although it has been demonstrated that stem cells have extensive effects on nerves [37] and endothelial cells [38]. However, the specific mechanism in which myocardin gene modification enhanced the therapeutic potential of ASCs was yet to be uncovered. In our previous study, myocardin was proved to maintain the contractile phenotype of CCSM cells, promote cell contractility, and suppress proliferative capacity in a rat model of bilateral

Fig. 5 Gene modification improved the effects of ASCs on ameliorating corpus cavernosum smooth muscle (CCSM) functions. IHC (A) and western blot (B) revealed the higher expression of myocardin in rats with gene-transfected ASCs. C A thinner smooth muscle layer and disordered cavernous sinuses were found in all diabetic rats, with more severe changes in the PBS group. D In the Masson’s trichrome staining images, the ASC and gene-modified ASC groups retained the SM-to-collagen ratio compared with the PBS group, which is shown in the statistical graph (E). F The protein expression levels of the smooth muscle markers α-SMA and calponin, fibrosis marker collagen I, anti-apoptosis factor Bcl-2, apoptosis molecule Bax, and cleaved-caspase3 were detected. Scale bar = 200 μm. Animals tested n = 10. *** p < 0.001
cavernous nerve injury [16]. In the current research, there were two possibilities in the contribution of myocardin to the improvement of erectile dysfunction in diabetic rats. The expression of myocardin was found to be upregulated in the DMED+Ad-Luc-Myocardin group and it was speculated that myocardin exerted similar regulatory effects in DMED rats. On the other hand, higher differentiation efficacy of ASCs induced by myocardin was detected and it might be of benefit for repairing the damaged tissues.

Our study, consistent with other similar experiments or clinical trials, revealed a promising future for stem cells in the treatment of ED. Moreover, we demonstrated the validity of gene-modified stem cells by myocardin and the superiority of an in vivo imaging strategy over traditional EdU tracking. However, limitations included the duration of adenovirus, leading to the longest observation of up to 3 weeks. However, the choice of adenovirus would meet the needs of high transfection efficiency and cotransfection with EdU in the current research. Future efforts could be made to monitor long-term effects using a lentivirus.

Conclusions
Myocardin induced ASC differentiation towards SML cells and enhanced the therapeutic potential of ASCs for ameliorating ED in diabetic rats. Notably, in vivo small animal tracking was an effective strategy for monitoring implanted stem cells, and this strategy might have advantages over traditional EdU assays.

Additional files

**Additional file 1: Table S1.** Primer sequences used in this study. The forward and reverse primer sequences of β-actin, PCNA, α-SMA, Calponin, myocardin, SRF, SOD2, and OCT4 are shown in Table S1. (DOCX 16 kb)

**Additional file 2: Figure S1.** EdU transfection efficiency was confirmed. Passage 2 ASCs were incubated with 10 μM EdU for 24 h before intracavernous injections and 81.53 ± 1.42% of cells were stained red under × 400 magnification. Scale bar = 200 μm. (TIF 608 kb)

Abbreviations
ED: Erectile dysfunction; ASCs: Adipose-derived stem cells; CCK-8: Cell Counting Kit; EdU: 5-Ethynyl-2′-deoxyuridine; DMED: Diabetes mellitus ED; NC: Negative control; Ad-Luc: Adenovirus-Luciferase; DMENA: Dulbecco’s modified Eagle’s medium; PBS: Phosphate buffer solution; DPBS: Dulbecco’s phosphate-buffered saline; IHC: Immunohistochemistry; WB: Western blot; SD: Sprague–Dawley; STZ: Streptozotocin; FBS: Fetal bovine serum; ICP: Intracavernosal pressure; MAP: Mean arterial pressure; APO: Apomorphine; CCSM: Corpus cavernosum smooth muscle; CCSMC: Corpus cavernosum smooth muscle cells; MSCs: Mesenchymal stem cells; PDES1: Phosphodiesterase type 5 inhibitor; PLGA: Poly l-glutamic acid; SML: Smooth muscle-like

Acknowledgements
This study was supported by grants from the National Natural Science Foundation of China (81705066, 81571433), the Natural Science Foundation of Guangdong Province, China (2017A030313453, 2018A030313638), and the President Foundation of Nanfang Hospital, Southern Medical University (2017B001, 2017Z001).

Authors’ contributions
HBZ, SHH, SCZ, and ZQW designed the study; HBZ and FZC performed the animal modeling and the transfection of genes and ASCs and wrote the manuscript; SHH and ZRC revised the paper; YBL and ZQW performed the molecular biology experiments and histology; LW analyzed the data; all of the authors approved and submitted the final version.

Funding
This study was supported by grants from the National Natural Science Foundation of China (81705066, 81571433), the Natural Science Foundation of Guangdong Province, China (2017A030313453, 2018A030313638), and the President Foundation of Nanfang Hospital, Southern Medical University (2017B001, 2017Z001).

Availability of data and materials
All data generated or analyzed during this study are included in the published article.

Ethics approval and consent to participate
All animal experimental protocols were performed in accordance with the Declaration of Helsinki and were approved by the Institutional Animal Care and Use Subcommittee of the Nanfang Hospital of Southern Medical University.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1Department of Urology, Nanfang Hospital, Southern Medical University, North of Guangzhou Avenue 1838#, Guangzhou, China. 2Department of Urology, Shenzhen People’s Hospital, The Second Clinical Medical College of Jinan University, The First Affiliated Hospital of Southern University of Science and Technology, Shenzhen, China. 3Department of Urology, The Fifth Affiliated Hospital of Guangdong Medical University, Guangzhou, China. 4Department of Urology, Tungwah Hospital, Dongguan, China. 5Department of Urology, The First Affiliated Hospital of Gуйyang University of Chinese Medicine, Gуйyang, China.

Received: 10 January 2019 Revised: 11 June 2019 Accepted: 3 July 2019 Published online: 16 July 2019

References
1. Ouyang B, Xie Y, Zhang C, Deng C, Lv L, Yao J, Zhang Y, Liu G, Deng J, Deng C. Extracellular vesicles from human urine-derived stem cells ameliorate erectile dysfunction in a diabetic rat model by delivering proangiogenic microRNA. Sex Med-Uk. 2019;7(2):241-50.
2. Jeon S, Zhu G, Bae W, Choi S, Jeong H, Cho H, Ha U, Hong S, Lee J, Kwon E, Kim H, Lee S, Kim H, Kim S. Engineered mesenchymal stem cells expressing harnidovirus cell-derived factor-1 improve erectile dysfunction in streptozotocin-induced diabetic rats. Int J Mol Sci. 2018;19(12):3730.
3. You R, Hamidou L, Birent B, Bitari D, Lecoviser P, Contremoulins I, Khodan M, Rodriguez A, Augustin D, Roudot-Thoraval F, de la Taille A, Rouard H. Safety of intracavernous bone marrow-mononuclear cells for postradical prostatectomy erectile dysfunction: an open dose-escalation pilot study. Eur Urol. 2016;69(6):988–91.
4. Kim JH, Lee HJ, Doo SH, Yang WJ, Choi D, Kim JH, Won JH, Song YS. Use of nanoparticles to monitor human mesenchymal stem cells transplanted into penile cavernous of rats with erectile dysfunction. Kor J Urol. 2015;56(4):280.
5. Lin H, Dhanani N, Tseng H, Souza GR, Wang G, Cao Y, Ko TC, Jiang H, Wang HBZ, SHH, SCZ, and AYW designed the study; HBZ and FZC performed the animal modeling and the transfection of genes and ASCs and wrote the manuscript; SHH and ZRC revised the paper; YBL and ZQW performed the molecular biology experiments and histology; LW analyzed the data; all of the authors approved and submitted the final version.
6. Qu X, Lin H, Wang Y, Yu W, Chen Y, Wang R, Dai Y. Intracavernous transplantation of bone marrow-derived mesenchymal stem cells restores erectile function of streptozotocin-induced diabetic rats. J Sex Med. 2011;8(2):37–36.
7. Pozzi E, Muneer A, Sangster P, Alnajjar HM, Salonia A, Bettocchi C, Castiglione F, Ralph DJ. Stem-cell regenerative medicine as applied to the penis. Curr Opin Urol. 2019;29(4):443-9.

8. You D, Jang MJ, Kim BH, Choi KR, Lee C, Song G, Shin HC, Jeong IG, Suh N, Kim YM, Ahn TR, Kim C. Bone marrow-derived mesenchymal stromal cell therapy in a rat model of cavernous nerve injury: preclinical study for approval. Cytotherapy. 2016;18(12):780–70.

9. Gu X, Hua S, Ethan M, Zhong L, Long T, Clouse C, Li W, Chen D, Chung H, Murphy S, Yoo J, Lin G, Lu T, Atala A, John J, Zhang Y. Long-term therapeutic effect of cell therapy on improvement in erectile function in a rat model with pelvic neurovascular injury. Bju Int. 2019;124(11):145-54.

10. Hua S, Lei L, Deng L, Weng X, Liu C, Qi X, Wang S, Zhang D, Zou X, Cao C, Liu L, Wu D. mir-139-5p inhibits aerobic glycolysis, cell proliferation, migration, and invasion in hepatocellular carcinoma via a reciprocal regulatory interaction with ETS1. Oncogene. 2018;37(12):1624–36.

11. Liu L, Li E, Li F, Luo L, Zhao S, Kang R, Luo J, Zhao Z. Effect of testosterone on the phenotypic modulation of corpus cavernosum smooth muscle cells in a castrated rat model. Urology. 2017;103:271–3.

12. Ouyang X, Han X, Chen Z, Fang J, Huang X, Wei H. MSC-derived exosomes ameliorate erectile dysfunction by alleviation of corpus cavernosum smooth muscle apoptosis in a rat model of cavernous nerve injury. Stem Cell Res Ther. 2018;9(1):246.

13. Huang J, Wang T, Wright AC, Yang J, Zhou S, Li L, Yang J, Small A, Parmacek MS. Myocardin is required for maintenance of vascular and visceral smooth muscle homeostasis during postnatal development. Proc Natl Acad Sci. 2015;112(4):4447–52.

14. Mughal W, Martens M, Field J, Chapman D, Huang J, Rattan S, Hai Y, Cheung KG, Kereluk S, West AR, Cole LK, Hatch GM, Diehl-Jones W, Keijzer R, Dolkinsky W, Dixon IM, Parmacek MS, Gordon JW. Myocardin regulates mitochondrial calcium homeostasis and prevents permeability transition. Cell Death Differ. 2018;25(10):1732–48.

15. Grauss RW, van Tuyn J, Steendijk P, Winter EM, Pijnappels DA, Hogers B, Miano JM, Zlokovic BV. SRF and myocardin regulate LRP-mediated amyloid-beta clearance in brain vascular cells. Nat Cell Biol. 2008;10(1):143–53.

16. Castiglione F, Ralph DJ. Stem-cell regenerative medicine as applied to the penis. Curr Opin Urol. 2019;29(4):443-9.

17. He S, Zhang T, Liu Y, Liu L, Zhang H, Chen F, Wei A. Myocardin restores erectile function in diabetic rats: phenotypic modulation of corpus cavernosum smooth muscle cells. Andrologia. 2015;47(3):303–11.

18. Orabi H, Lin G, Ferretti L, Lin CS, Lue TF. Scaffoldless tissue engineering of stem cell derived cavernous tissue for treatment of erectile dysfunction. J Sex Med. 2012;9(6):1522–34.

19. Chen F, Zhang H, Wang Z, Ding W, Zeng Q, Liu W, Huang C, He S, Wei A. Adipose-derived stem cell-derived exosomes ameliorate erectile dysfunction in a rat model of type 2 diabetes. J Sex Med. 2017;14(9):1084–94.

20. Zhu GQ, Jeon SH, Bae WJ, Choi SW, Jeong HC, Kim KS, Kim SJ, Cho HJ, Ha US, Hong SH, Lee JY, Kwon EB, Kim SW. Efficient promotion of autophagy and angiogenesis using mesenchymal stem cell therapy enhanced by the low-energy shock waves in the treatment of erectile dysfunction. Stem Cells Int. 2018;2018:1–14.

21. Haahr MK, Jensen CH, Toyserkani NM, Andersen DC, Damkier P, Sørensen LI, Caspersen P, Salonia A. Myocardin restores erectile function in diabetic rats: a meta-analysis. Cardiovasc Res. 2016;103(8):2665–70.

22. Levy JA, Marchand M, Iorio L, Cassini W, Zakhalsky MP. Determining the feasibility of managing erectile dysfunction in humans with placental-derived stem cells. J Am. Osteopat. Assoc. 2016;116(1):e1.

23. Capogrosso P, Montorsi F, Salonia A. Phase I and phase II clinical trials for the treatment of male sexual dysfunction—a systematic review of the literature. Expert Opin Investig Drugs. 2018;27(7):583–93.

24. Kim IG, Piao S, Lee JY, Hong SH, Hwang T, Kim SW, Kim CS, Ra JC, Noh J, Lee JY. Effect of an adipose-derived stem cell and nerve growth factor- incorporated hydrogel on recovery of erectile function in a rat model of cavernous nerve injury. Tissue Eng A. 2013;19(1):244–34.

25. Piao S, Kim IG, Lee JY, Hong SH, Kim SW, Hwang TK, Oh SH, Lee JH, Ra JC, Lee JY. Therapeutic effect of adipose-derived stem cells and BDNF-immobilized PLGA membrane in a rat model of cavernous nerve injury. J Sex Med. 2012;9(8):1968–79.

26. Bahk JY, Jung JH, Han H, Min SK, Lee YS. Treatment of diabetic impotence with umbilical cord blood stem cell intracavernosal transplant: preliminary report of 7 cases. Exp Clin Transplant. 2010;8(2):150–60.

Publisher’s Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.