

Stem cell therapy for erectile dysfunction

Etsu Suzuki, Hiroaki Nishimatsu, Yukio Homma

Etsu Suzuki, Institute of Medical Science, St. Marianna University School of Medicine, Miyamae-ku, Kawasaki 216-8512, Japan

Hiroaki Nishimatsu, Yukio Homma, The Department of Urology, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo 113-8655, Japan

Author contributions: Suzuki E, Nishimatsu H and Homma Y contributed to this paper.

Correspondence to: Etsu Suzuki, MD, PhD, Institute of Medical Science, St. Marianna University School of Medicine, 2-16-1 Sugao, Miyamae-ku, Kawasaki 216-8512, Japan. esuzuki-ky@umin.ac.jp

Telephone: +81-044-9778361 Fax: +81-044-9778361

Received: April 1, 2014 Revised: May 3, 2014

Accepted: October 1, 2014

Published online: November 24, 2014

Abstract

Erectile dysfunction (ED) is an important health problem that has commonly been clinically treated using phosphodiesterase type 5 inhibitors (PDE5Is). However, PDE5Is are less effective when the structure of the cavernous body has been severely injured, and thus regeneration is required. Stem cell therapy has been investigated as a possible means for regenerating the injured cavernous body. Stem cells are classified into embryonic stem cells and adult stem cells (ASCs), and the intracavernous injection of ASCs has been explored as a therapy in animal ED models. Bone marrow-derived mesenchymal stem cells and adipose tissue-derived stem cells are major sources of ASCs used for the treatment of ED, and accumulated evidence now suggests that ASCs are useful in the restoration of erectile function and the regeneration of the cavernous body. However, the mechanisms by which ASCs recover erectile function remain controversial. Some studies indicated that ASCs were differentiated into the vascular endothelial cells, vascular smooth muscle cells, and nerve cells that originally resided in the cavernous body, whereas other studies have suggested that ASCs improved erectile function *via* the secretion of anti-apoptotic and/or proangiogenic cytokines rather

than differentiation into other cell types. In this paper, we reviewed the characteristics of stem cells used for the treatment of ED, and the possible mechanisms by which these cells exert their effects. We also discussed the problems to be solved before implementation in the clinical setting.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Erectile dysfunction; Stem cell therapy; Bone marrow-derived mesenchymal stem cells; Adipose tissue-derived stem cells; Endothelial progenitor cells; Adrenomedullin; Angiopoietin-1

Core tip: Adult stem cells (ASCs) have been used for the treatment of erectile dysfunction. Although previous studies reported that ASCs differentiated into cells that originally resided in the cavernous body, recent studies indicate that the major, if not all, effects of ASCs on erectile function are achieved through the secretion of paracrine factors rather than their direct differentiation into the cells in the cavernous body. Among various cytokines that ASCs produce, we have recently identified adrenomedullin as a candidate peptide that is implicated in the restoration of erectile function. We introduced these data in this review.

Suzuki E, Nishimatsu H, Homma Y. Stem cell therapy for erectile dysfunction. *World J Clin Urol* 2014; 3(3): 272-282 Available from: URL: <http://www.wjgnet.com/2219-2816/full/v3/i3/272.htm> DOI: <http://dx.doi.org/10.5410/wjcu.v3.i3.272>

INTRODUCTION

Erectile dysfunction (ED) is a worldwide health problem. Although psychogenic factors are a major cause of ED, other factors such as age, diabetes, total prostatectomy and radiation in the pelvis also contribute to the occurrence of ED. These factors cause structural changes as well as functional abnormalities in the cavernous body,

and therefore selective phosphodiesterase type 5 inhibitors (PDE5Is) are not so effective in the treatment of these diseases. For the recovery of erectile function in these patients, the regeneration of the cavernous body is necessary. In this regard, much attention has recently been placed on gene therapy and stem cell therapy.

Stem cells are defined as being capable of self-renewal and of differentiation into a variety of phenotypes^[1]. There are two categories of stem cells: embryonic stem cells (ESCs) and adult stem cells (ASCs). ESCs were originally isolated from the inner cell mass of blastocysts^[2]. ESCs are pluripotent stem cells that can give rise to the three germ layers. However, harvesting ESCs requires the destruction of human embryos and has therefore raised ethical concerns. To overcome this limitation, induced pluripotent stem (iPS) cells have been produced. Adult fibroblasts were reprogrammed by introducing four factors, Oct3/4, Sox2, c-Myc and Klf4 under ESCs culture conditions^[3,4]. iPS cells are pluripotent stem cells with very similar characteristics to ESCs. Furthermore, many groups have now succeeded in reprogramming somatic cells to create iPS cells by overexpression of variable sets of several transcription factors in cells without employing viruses or vectors^[5-7]. Therefore, iPS cells are a promising option for regenerative medicine in the near future. A further option for avoiding the ethical problems of ESCs is the use of ASCs, which are basically multipotent stem cells that reside in various tissues, including the brain, skeletal muscle, bone marrow, adipose tissue, and dental pulp^[8-11]. Besides having the potential to differentiate into various cell types, ASCs produce a broad range of cytokines that exert their effects in a paracrine and/or autocrine manner. Among ASCs, bone marrow-derived mesenchymal stem cells (BMMSCs) are the most commonly studied. BMMSCs reportedly have a potential to differentiate into various cell types including bone, cartilage, cardiac muscle, skeletal muscle, vascular endothelial cells (VECs) and vascular smooth muscle cells (VSMCs)^[12,13]. Recently, adipose tissue-derived stem cells (ADSCs) have gained much attention because of the simplicity of harvesting hundreds of grams of subcutaneous adipose tissue without using invasive procedures, whereas painful bone marrow aspiration is required to collect just grams of bone marrow. Similar to BMMSCs, ADSCs have the potential to differentiate into various cell types^[14]. ADSCs strongly resemble BMMSCs in that they share similar expression patterns of cell surface markers and similar gene expression profiles^[15,16].

TREATMENT OF ED USING ASCs

BMMSCs

Many studies have demonstrated the efficacy of BMMSCs in the treatment of ED, and their efficacy seems to be reliable^[17-27]. However, the mechanism by which BMMSCs restore erectile function remains controversial. BMMSCs were originally believed to home in on damaged tissues efficiently and differentiate into various cells at the target

site. Genetically manipulated BMMSCs were also used expecting that these cells remain in the tissues and express a specific gene over a long period. Bivalacqua *et al.*^[17] infected BMMSCs with an adenovirus expressing endothelial nitric oxide synthase (eNOS) and injected these cells into the penis of aged rats. The eNOS-modified BMMSCs restored erectile function, with the recovery being associated with increased eNOS protein expression, NOS activity, and cyclic guanosine monophosphate levels. Furthermore, the authors demonstrated that the injected BMMSCs survived for at least 21 d in the cavernous body and expressed markers for VECs and VSMCs. Song *et al.*^[18] used immortalized human BMMSCs and injected them into rat penises, where the cells expressed markers for VECs and VSMCs in the cavernous body, although the authors did not examine whether erectile function was restored or not. Qiu *et al.*^[21] injected BMMSCs into the penis of streptozotocin (STZ)-induced diabetic rats and found that the BMMSCs injection restored erectile function. The authors also demonstrated that injected cells remained in the cavernous body for at least 4 wk and some expressed markers for VECs and VSMCs. Although the injection of BMMSCs restored erectile function in these studies, there are several problems. First, these studies did not clearly calculate the percentage of injected BMMSCs that remained in the cavernous body and obtained markers for VECs and VSMCs, or the percentage mentioned in these studies was not sufficient to explain the recovery of erectile function. Second, there was a possibility that the injected BMMSCs fused with the residing cells in the cavernous body and acquired the phenotype of the residing cells. Terada *et al.*^[28] used BMMSCs obtained from female transgenic mice expressing green fluorescent protein (GFP) and puromycin resistance gene. The BMMSCs were cocultured with a male embryonic stem cell line and then puromycin was added to remove the embryonic stem cells. The remaining cells were GFP positive and puromycin resistant, and morphologically similar to embryonic stem cells. These cells could be induced to differentiate into cardiac myocytes and neuronal cells, suggesting that embryonic stem cell-like pluripotent stem cells were established from BMMSCs. However, following DNA ploidy (the number of DNA copies) analysis using fluorescence-activated cell sorting, the cells were found to be tetraploid (4n) or hexaploid (6n), suggesting that they had developed from spontaneous fusion between the BMMSCs and the embryonic stem cells. The possibility of cell fusion *in vivo* was also reported. Alvarez-Dolado *et al.*^[29] used transgenic mice that contain the *lacZ* reporter gene downstream of a stop codon flanked by loxP sites (floxed). Therefore, the *lacZ* reporter gene was only expressed when the loxP-flanked stop codon was excised by Cre recombinase. The authors lethally irradiated these mice and intraperitoneally injected BMMSCs from mice that ubiquitously express Cre recombinase and GFP. If cells from the donor and recipient fused, the Cre enzyme would excise the Lox P-flanked stop codon, thereby allowing expression of

the *lacZ* gene. Results revealed that β -gal+ (fused) and GFP+ cells were found in the brain, heart, and liver of recipients, 2 and 4 mo post-transplantation^[29]. Thus, BMMSCs potentially fuse with other cell types *in vivo* and it appears that BMMSCs are differentiated into other cell types because of this phenomenon. In contrast, the paracrine effects of BMMSCs have been reported. Kendirci *et al.*^[20] isolated BMMSCs positive for p75 low-affinity nerve growth factor receptor using magnetic-activated cell sorting, and injected these cells into the penis of rats following bilateral cavernous nerve crush injury. The injection of these cells restored erectile function^[20]. The engraftment of these cells in the cavernous tissue occurred very rarely, and the engrafted cells appeared fibroblastic. Furthermore, these cells secreted fibroblast growth factor 2 (FGF2), which suggested that FGF2 might protect the cavernous nerve after crush injury. More direct evidence of the paracrine effects of BMMSCs was reported by Yeghiazarians *et al.*^[30] who injected BMMSCs extracts into infarcted hearts and found that the procedure effectively improved cardiac function, suggesting that BMMSCs *per se* were not required for their tissue protective effects. Although this scenario is attractive, no cytokines that are implicated in the recovery of erectile function have been specifically identified.

ADSCs

The efficacy of ADSCs in the treatment of ED seems to be reliable from the results of many previous reports^[31-45]. However, the mechanisms by which ADSCs restore erectile function remain controversial. Ning *et al.*^[46] reported that ADSCs could differentiate into VECs. ADSCs injected into the penis obtained a marker for VECs. FGF2 appeared to be necessary for the differentiation of ADSCs into VECs *in vitro*, although the functional role of FGF2 in the differentiation of ADSCs into VECs *in vivo* was not studied^[46]. Ryu *et al.*^[40] demonstrated that the injection of ADSCs into the penis of STZ-induced diabetic mice restored erectile function. They also found that some injected ADSCs became CD31 positive, suggesting that these cells differentiated into VECs^[40]. However, ADSCs injected into the penis disappeared within 14 d. Kim *et al.*^[43] applied human ADSCs and nerve growth factor-incorporated hyaluronic acid-based hydrogel to the cavernous nerve of rats following bilateral cavernous nerve crush injury. The authors demonstrated that this treatment restored erectile function. They also showed that some ADSCs were engrafted into the cavernous nerve 4 wk after treatment, suggesting that ADSCs could differentiate into nerve tissue^[43]. Although these studies showed that ADSCs have the ability to differentiate into the cells located in the cavernous body, the possibility of cell-cell fusion was not excluded in these studies. Alternatively, paracrine effects of ADSCs have been suggested. Several reports suggested the possibility of paracrine effects of ADSCs because ADSCs did not remain in the cavernous body for a long period^[32,33]. Albersen *et al.*^[31] injected both ADSCs and the lysate of AD-

SCs into the penis of rats that were subjected to bilateral cavernous nerve crush injury. They demonstrated that the injection of ADSCs lysate significantly restored erectile function^[31]. These results suggested that most, if not all, of the effects of ADSCs were mediated through their production of cytokines and/or immune modulators, although the authors did not identify any molecules that are functionally implicated in the restoration of erectile function. Zhang *et al.*^[47] reported that ADSCs produced chemokine (C-X-C motif) ligand 5 (CXCL5) and that CXCL5 was implicated in the neurotrophic effects of ADSCs *in vitro*, although they did not confirm this finding *in vivo*. We recently reported that adrenomedullin (AM) is implicated in ADSCs-induced restoration of erectile function in diabetic rats^[36]. AM was originally isolated from human pheochromocytoma tissue, and has potent vasorelaxant and diuretic effects^[48]. In addition, AM is also produced by VECs, VSMCs and macrophages^[49-51] and has the ability to stimulate angiogenesis^[52-54]. When rat ADSCs were cultured in a medium containing growth factors for VECs, ADSCs produced significant amounts of AM (Figure 1A). The injection of ADSCs into the penis of diabetic rats restored erectile function (Figure 1B), the morphology of the cavernous body (Figure 1C), and the expression of vascular endothelial (VE)-cadherin, a marker for VECs. However, when the expression of AM was knocked down using a small interfering RNA for AM, the favorable effects of ADSCs disappeared (Figure 1B and C). Furthermore, when AM was overexpressed in the penis using an adenovirus expressing AM, erectile function and the morphology of the cavernous body were restored in diabetic rats (Figure 1B and C). We also demonstrated that ADSCs produce angiopoietin-1 (Ang-1) and Ang-1 secreted from ADSCs are implicated in ADSCs-induced suppression of neointimal formation and stimulation of reendothelialization in a wire injury model of the rat femoral artery^[55]. Furthermore, we reported that overexpression of both AM and Ang-1 using adenoviruses expressing those proteins restored erectile function in diabetic rats to the same level as that observed in age-matched positive control rats (Figure 2)^[44]. Therefore, it seems obvious that ADSCs produce various cytokines that potentially restore erectile function. The limitation is that ADSCs do not remain in the cavernous body for a long period, usually disappearing within a month^[32,33,36]. Where do they go? Do they die or migrate to other tissues? Several interesting papers have been published regarding these points. Lin *et al.*^[56] injected rat ADSCs and traced their locations after 2 and 7 d. ADSCs remained not only in the penis but also in the major pelvic ganglia (MPG), spleen and bone marrow. ADSCs preferentially remained in the bone marrow and the number of ADSCs remaining in the bone marrow was much larger than that remaining in the penis. Because ADSCs secrete various cytokines, this result suggests that ADSCs remaining outside the penis may affect the restoration of erectile function, although the survival of ADSCs for long periods was not examined in the study. Several

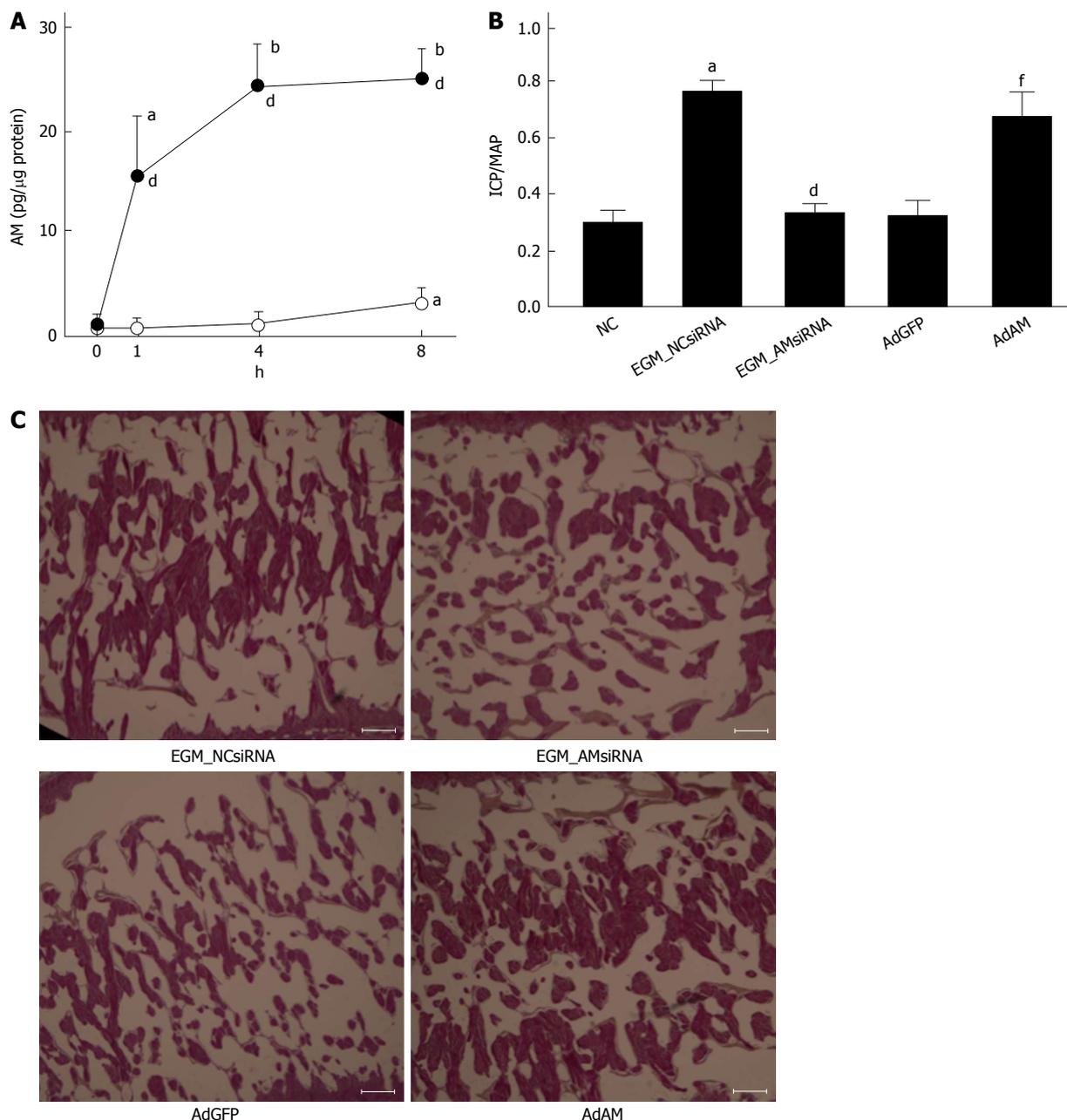


Figure 1 Production of adrenomedullin by adipose tissue-derived stem cells, and effects of knockdown and overexpression of adrenomedullin on the function and histology of the penis. **A:** Adipose tissue-derived stem cells produce adrenomedullin especially when they were cultured in a medium containing growth factors for vascular endothelial cells (VECs); adipose tissue-derived stem cells (ADSCs) were cultured in endothelial basal medium (EBM: white circles) or endothelial growth medium (EGM: black circles) that contains growth factors for VECs. Medium was replaced with serum-free medium and incubated for the indicated periods. Adrenomedullin (AM) accumulated in the medium was measured. ^a $P < 0.05$ vs 0 h, ^b $P < 0.01$ vs 0 h and ^d $P < 0.01$ vs EBM culture at each time point ($n = 6$ per group); **B:** Effect of knockdown and overexpression of AM on erectile function. ADSCs were infected with lentivirus expressing negative control siRNA (LV_NCSiRNA) that is predicted not to target any known vertebrate gene or lentivirus expressing AM siRNA (LV_AMsiRNA). ADSCs were cultured in EGM for 1 wk, and those LV_NCSiRNA-infected ADSCs (EGM_NCSiRNA) and LV_AMsiRNA-infected ADSCs (EGM_AMsiRNA) were injected in the cavernous body of STZ-induced diabetic rats. ICP was measured 4 wk after the ADSCs injection. An adenovirus expressing AM (AdAM) or adenovirus expressing green fluorescent protein (AdGFP) was also injected into the cavernous body of STZ-induced diabetic rats, and ICP was measured 4 wk after the infection. Nontreated STZ-injected diabetic rats were used as the negative control (NC). Bar graphs show ICP/MAP ($n = 5$ per group). ^a $P < 0.001$ vs NC, ^d $P < 0.001$ vs EGM_NCSiRNA injection and ^f $P < 0.001$ vs AdGFP infection; **C:** Effect of knockdown and overexpression of AM on the morphology of the cavernous body. Experiments were performed in the same way as described in the legend for Figure 1B. The cavernous body was stained by the Elastica van Gieson method. The histology of the root portion of the penis (longitudinal section) is shown. Bars are 200 μm . Note that the size of trabeculae of the cavernous body is smaller when EGM_AMsiRNA or AdGFP was injected into the penis of diabetic rats, compared with when EGM_NCSiRNA or AdAM was injected into the penis. ICP: Intracavernous pressure; STZ: Streptozotocin; MAP: Mean arterial pressure.

studies have reported that ADSCs injected into the penis or placed around the prostate gland migrated to the MPG^[35,39,45]. Fandel *et al.*^[35] demonstrated that ADSCs in-

jected into the penis migrated to the MPG, although ADSCs were not engrafted in the nerve tissue. Interestingly, the expression of stromal cell derived factor-1 (SDF-1)

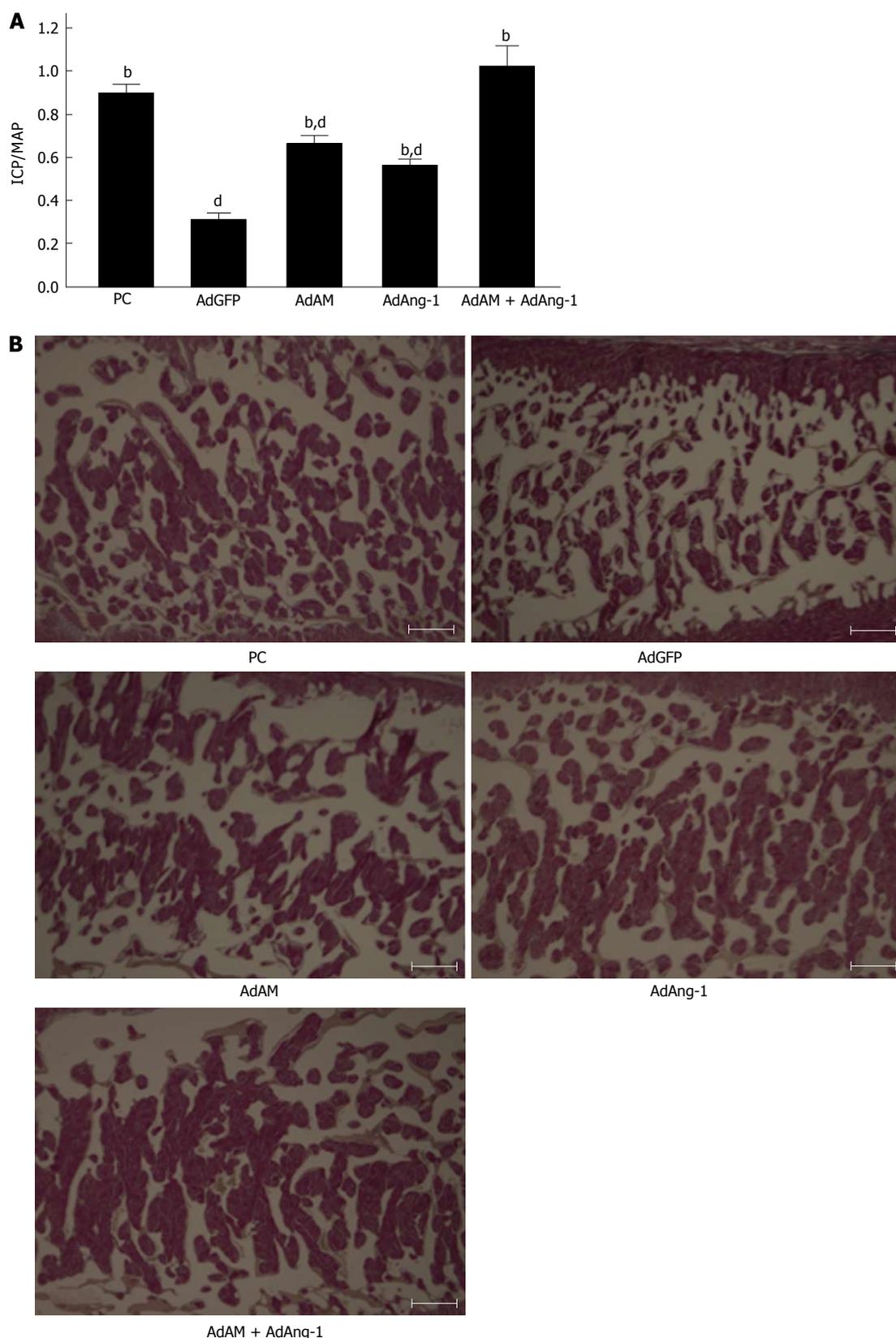


Figure 2 Effects of adenomedullin and angiotensin-1 on the function and morphology of the penis. A: Effects of adenovirus expressing adrenomedullin (AdAM), adenovirus expressing angiotensin-1 (AdAng-1) and AdAM plus AdAng-1 infection on erectile function. These adenoviruses were infected into the cavernous body of STZ-induced diabetic rats. Adenovirus expressing green fluorescent protein (AdGFP) was also infected as the negative control. Age-matched Wistar rats were used as the positive control (PC). Bar graphs demonstrate ICP/MAP ($n = 6$ per group). ^b $P < 0.001$ vs AdGFP infection and ^d $P < 0.001$ vs PC; B: Histological analysis of the cavernous body after adenoviral infection. Elastica van Gieson staining of the cavernous body isolated from age-matched Wistar rats (PC), and STZ-induced diabetic rats infected with AdGFP, AdAM, AdAng-1, and AdAM plus AdAng-1. The histology of the root portion of the penis (longitudinal section) is shown. Bars are 300 μm . Note that the size of trabeculae of the cavernous body is small when AdGFP is injected into the penis of diabetic rats, and that the size is restored to a similar level as observed in the age-matched control group when AdAM and/or AdAng-1 are injected. ICP: Intracavernous pressure; STZ: Streptozotocin; MAP: Mean arterial pressure.

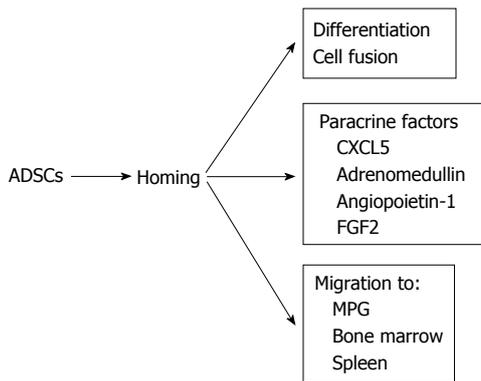


Figure 3 Possible mechanisms by which adipose tissue-derived stem cells stimulate the recovery of erectile function. FGF2: Fibroblast growth factor 2; MPG: Major pelvic ganglia; ADSCs: Adipose tissue-derived stem cells; CXCL5: Chemokine (C-X-C motif) ligand 5.

increased in MPG, suggesting that ADSCs preferentially migrated to the site of SDF-1 production. Qiu *et al.*^[39] also reported that intracavernously injected ADSCs migrated to the MPG, where they remained 17 wk after injection, although the number of ADSCs remaining in the MPG was quite low. You *et al.*^[45] showed that periprostatic implantation of ADSCs, but not their intracavernous injection, resulted in the migration of ADSCs to MPG. In summary, some ADSCs injected into the penis migrated to the tissues such as the bone marrow and MPG, and these cells may be implicated in the restoration of erectile function. The possible mechanisms by which ADSCs restore erectile function are summarized in Figure 3.

Endothelial progenitor cells

Endothelial progenitor cells (EPCs) were originally isolated from human peripheral blood by Asahara *et al.*^[57]. They isolated CD34-positive mononuclear blood cells and demonstrated that these cells obtained the characteristics of VECs when cultured on fibronectin-coated dishes. They also demonstrated that these cells were incorporated in ischemic tissues *in vivo* and expressed markers for VECs such as CD31 when introduced into the circulation using a hindlimb ischemia model. Furthermore, the authors showed that Flk-1-positive mononuclear blood cells were also integrated in the capillaries and small arteries when the hindlimb ischemia model was used. These cells were designated as EPCs. EPCs are progenitor cells whose differentiation potential is restricted to one lineage (VECs); therefore, they are not multipotent stem cells. Results of subsequent studies revealed that EPCs express three cell surface markers; CD133 (termed originally AC133), CD34, and Flk-1^[58-60]. Premature EPCs either in the bone marrow or immediately after entering into the systemic circulation are positive for CD133/CD34/Flk-1. However, when EPCs become more mature, they lose the expression of CD133 and begin to express CD31 and VE-cadherin. An attempt to use EPCs for the treatment of ED has been reported by Gou *et al.*^[61]. They transfected EPCs with the vascular endothelial growth factor gene and injected the transfected cells into the

penis of diabetic rats. The authors found that this treatment restored erectile function and the injected cells were integrated into the sites of neovascularization in the cavernous body^[61]. However, most researchers in this field regard EPCs as a marker for ED rather than a therapeutic tool for ED. Factors affecting the number of circulating EPCs or their functions have been reported. The number of circulating EPCs and their migratory activity are reportedly reduced in patients with coronary risk factors^[62,63]. EPCs isolated from type 2 diabetes patients have a decreased capacity for proliferation and the formation of capillary tubes *in vitro*^[64]. In contrast, the number of circulating EPCs rapidly increases after limb ischemia and acute myocardial infarction^[65,66]. Because atherosclerosis and ED share a common feature, *i.e.*, endothelial dysfunction, it has been speculated that the dynamics of EPCs might change in ED. Indeed, several studies have reported that the number of circulating EPCs decreased in ED patients^[67-69], suggesting that the decrease in the number of circulating EPCs can predict the presence of ED as well as cardiovascular diseases. Interestingly, the number of circulating EPCs increased when patients were administered statins or PDE5Is^[70-73] probably *via* the mobilization of EPCs from the bone marrow. Therefore, these drugs may improve erectile function *via* the mobilization of EPCs to the cavernous body.

Muscle-derived stem cells

Muscle-derived stem cells (MDSCs) are ASCs that exist in skeletal muscle. MDSCs can be obtained from autologous muscle biopsies and have been used for the treatment of ED in several studies. Nolzco *et al.*^[74] injected MDSCs into the penis of aged rats and found that MDSCs differentiated into VSMCs in the cavernous body, resulting in the recovery of erectile function. Woo *et al.*^[75] used a bilateral cavernous nerve injury model in rats and examined the effects of the injection of MDSCs into the penis on erectile function. They demonstrated that MDSCs remained in the cavernous body 4 wk after injection, and erectile function was significantly restored. Kovanecz *et al.*^[76] used a bilateral cavernous nerve resection model of rats and, following the injection of MDSCs into the cavernous body, found that erectile function was restored and α -smooth muscle actin expression was increased. The injection of MDSCs also increased the expression of neural nitric oxide synthase and brain-derived neurotrophic factor^[76]. In summary, MDSCs seem to be useful for the treatment of ED. However, because harvesting MDSCs from the skeletal muscle is relatively more invasive than the collection of ADSCs, the beneficial characteristics of MDSCs compared with ADSCs should be clarified before introducing them to clinical application.

Umbilical cord blood stem cells

Umbilical cord blood stem cells (UCBSCs) are an attractive type of stem cells in that they are youngest stem cells among a variety of ASCs. Because they are young, they have less possibility to have DNA damage than other ASCs^[77,78]. Bahk *et al.*^[79] used human UCBSCs to treat

diabetic patients with ED, and demonstrated that erectile function was restored and that blood glucose levels decreased in these patients, although the mechanisms remain unknown.

Brain-derived stem cells

Brain-derived stem cells (BDSCs) reportedly have capacity to differentiate into VSMCs^[80]. Song *et al*^[81] isolated fetal BDSCs from embryonal 12-d rats and injected them into the penis. They demonstrated that injected BDSCs obtained characteristics of VSMCs *in vivo* 6 wk after injection^[81], although they did not examine their effect on erectile function. Considering the source of BDSCs, it will be difficult to use them in clinical settings.

Neural crest stem cells

Neural crest stem cells (NCSCs) are the progenitor cells of several cell types that constitute the peripheral nervous system, including neurons, Schwann cells, adrenal chromaffin cells and smooth muscle cells. Transplantation of NCSCs could reportedly induce the regeneration of connective tissues, VSMCs, skeletal muscle and VECs^[82,83]. Song *et al*^[84] injected NCSCs into the penis of rats and demonstrated that they obtained markers for VECs and VSMCs 2 wk after injection, although their effects on erectile function was not analyzed^[84]. Clinical application of NCSCs may also be difficult considering the source of these cells.

Treatment of ED using ESCs and iPS cells

Because iPS cells have been created, clinical application of pluripotent stem cells will be intensively explored in the future. To our knowledge, no studies have been published in which iPS cells or iPS cells-derived cells were used to treat ED. Bochinski *et al*^[85] used ESCs that had differentiated into the neural cell line, and injected them into the MPG or cavernous body using a bilateral cavernous nerve injury model. They found that the injection into the both MPG and cavernous body restored erectile function. They also found that neurofilament staining was recovered in the ESCs-injected group^[85]. Therefore, ESCs and iPS cells may be useful for treatment of ED. However, these cells may not efficiently home and survive for a long period under persistent inflammation. For example, in diabetic conditions hyperglycemia and adipocytokines induce persistent inflammation in the tissues. Implanted cells may not home and survive under these conditions unless such an inflammation is sufficiently controlled.

FUTURE DIRECTIONS

As mentioned above, stem cell therapy for ED appears to be a promising strategy. However, several problems should be solved before moving to clinical application.

Tumorigenesis

It is well known that ESCs and iPS cells easily form tumors, because these cells are pluripotent. Although ASCs

seem to be less prone to forming tumors, ASCs can form malignant tumors when transplanted *in vivo*^[86]. Jeong *et al*^[86] injected BMMSCs into the peri-infarct area of myocardial infarction (MI) model of mice and hindlimb muscle of mice with diabetic neuropathy. They found sarcoma formation in 30% of hearts in the MI model and in 46% of hindlimbs in the diabetic neuropathy model^[86]. Therefore, it will be necessary to sufficiently investigate the malignant potential of stem cells prior to their clinical use and establish methodology to select “healthy” stem cells that will not form tumors.

Fate of injected cells

As described above, some (most) of stem cells injected into the penis do not remain in the penis and migrate to other tissues such as the bone marrow and spleen. Little is known about the fate of these cells that have migrated to non-diseased organs. Detailed examinations will be necessary to detect the fate of these cells before moving to clinical applications.

How to improve homing and survival of stem cells

Most studies suggested that injected stem cells disappeared from the penis in one month. It is crucially important to explore methods to improve homing and survival of stem cells. It was reported that expression of SDF-1 was increased in the MPG and SDF-1 might stimulate migration of ADSCs to the MPG^[35]. Therefore, SDF-1 is a candidate that stimulates migration and homing of stem cells into injured sites. Intensive studies will be necessary to identify molecules that are implicated in migration, homing and survival of stem cells.

Activation and mobilization of endogenous stem cells

It is suggested from EPC study that statins or PDE5Is can stimulate mobilization of EPCs from the bone marrow^[70-73]. Therefore, it may be possible to activate and/or mobilize tissue-residual endogenous stem cells by some drugs. If endogenous stem cells residing in the penis can be efficiently activated, it will help to regenerate the cavernous body. This possibility should be examined in the future.

Identification of paracrine factors

ASCs produce a variety of paracrine factors that potentially regenerate the cavernous body. However, paracrine factors that are implicated in the regeneration of the cavernous body have not been sufficiently identified. Furthermore, it remains unknown what combinations of these paracrine factors are most suitable to stimulate the regeneration of the cavernous body. If these problems are solved, administration of cytokines cocktail may be more effective to treat ED than ASCs injection.

CONCLUSION

Stem cells especially ASCs have been used in the treatment of ED, and stem cell therapy seems to be effective

at least in animal modes. Major, if not all, effects of ASCs on erectile function appear to be achieved by secretion of paracrine factors rather than their direct differentiation into cells residing in the cavernous body. Before moving to clinical application, malignant potential of stem cells should be carefully considered. It is also necessary to explore methods to improve homing and survival of stem cells.

REFERENCES

- Morrison SJ, Shah NM, Anderson DJ. Regulatory mechanisms in stem cell biology. *Cell* 1997; **88**: 287-298 [PMID: 9039255 DOI: 10.1016/S0092-8674(00)81867-X]
- Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. *Nature* 1981; **292**: 154-156 [PMID: 7242681 DOI: 10.1038/292154a0]
- Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006; **126**: 663-676 [PMID: 16904174 DOI: 10.1016/j.cell.2006.07.024]
- Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007; **131**: 861-872 [PMID: 18035408 DOI: 10.1016/j.cell.2007.11.019]
- Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K. Induced pluripotent stem cells generated without viral integration. *Science* 2008; **322**: 945-949 [PMID: 18818365 DOI: 10.1126/science.1162494]
- Yu J, Hu K, Smuga-Otto K, Tian S, Stewart R, Slukvin IL, Thomson JA. Human induced pluripotent stem cells free of vector and transgene sequences. *Science* 2009; **324**: 797-801 [PMID: 19325077 DOI: 10.1126/science.1172482]
- Zhou H, Wu S, Joo JY, Zhu S, Han DW, Lin T, Trauger S, Bien G, Yao S, Zhu Y, Siuzdak G, Schöler HR, Duan L, Ding S. Generation of induced pluripotent stem cells using recombinant proteins. *Cell Stem Cell* 2009; **4**: 381-384 [PMID: 19398399 DOI: 10.1016/j.stem.2009.04.005]
- Jiang Y, Vaessen B, Lenvik T, Blackstad M, Reyes M, Verfaillie CM. Multipotent progenitor cells can be isolated from postnatal murine bone marrow, muscle, and brain. *Exp Hematol* 2002; **30**: 896-904 [PMID: 12160841 DOI: 10.1016/S0301-472X(02)00869-X]
- Presnell SC, Petersen B, Heidar M. Stem cells in adult tissues. *Semin Cell Dev Biol* 2002; **13**: 369-376 [PMID: 12324219 DOI: 10.1016/S1084952102000939]
- Schäffler A, Büchler C. Concise review: adipose tissue-derived stromal cells--basic and clinical implications for novel cell-based therapies. *Stem Cells* 2007; **25**: 818-827 [PMID: 17420225 DOI: 10.1634/stemcells.2006-0589]
- Hipp J, Atala A. Sources of stem cells for regenerative medicine. *Stem Cell Rev* 2008; **4**: 3-11 [PMID: 18286392 DOI: 10.1007/s12015-008-9010-8]
- Herzog EL, Chai L, Krause DS. Plasticity of marrow-derived stem cells. *Blood* 2003; **102**: 3483-3493 [PMID: 12893756 DOI: 10.1182/blood-2003-05-1664]
- Grove JE, Bruscia E, Krause DS. Plasticity of bone marrow-derived stem cells. *Stem Cells* 2004; **22**: 487-500 [PMID: 15277695 DOI: 10.1634/stemcells.22-4-487]
- Gimble JM, Katz AJ, Bunnell BA. Adipose-derived stem cells for regenerative medicine. *Circ Res* 2007; **100**: 1249-1260 [PMID: 17495232 DOI: 10.1161/01.RES.0000265074.83288.09]
- Peroni D, Scambi I, Pasini A, Lisi V, Bifari F, Krampera M, Rigotti G, Sbarbati A, Galie M. Stem molecular signature of adipose-derived stromal cells. *Exp Cell Res* 2008; **314**: 603-615 [PMID: 18022619 DOI: 10.1016/j.yexcr.2007.10.007]
- Torensma R, Prins HJ, Schrama E, Verwiel ET, Martens AC, Roelofs H, Jansen BJ. The impact of cell source, culture methodology, culture location, and individual donors on gene expression profiles of bone marrow-derived and adipose-derived stromal cells. *Stem Cells Dev* 2013; **22**: 1086-1096 [PMID: 23145933 DOI: 10.1089/scd.2012.0384]
- Bivalacqua TJ, Deng W, Kendirci M, Usta MF, Robinson C, Taylor BK, Murthy SN, Champion HC, Hellstrom WJ, Kadowitz PJ. Mesenchymal stem cells alone or ex vivo gene modified with endothelial nitric oxide synthase reverse age-associated erectile dysfunction. *Am J Physiol Heart Circ Physiol* 2007; **292**: H1278-H1290 [PMID: 17071732 DOI: 10.1152/ajpheart.00685.2006]
- Song YS, Lee HJ, Park IH, Kim WK, Ku JH, Kim SU. Potential differentiation of human mesenchymal stem cell transplanted in rat corpus cavernosum toward endothelial or smooth muscle cells. *Int J Impot Res* 2007; **19**: 378-385 [PMID: 17460699 DOI: 10.1038/sj.ijir.3901539]
- Abdel Aziz MT, El-Haggar S, Mostafa T, Atta H, Fouad H, Mahfouz S, Rashed L, Sabry D, Senbel A, Ali GA. Effect of mesenchymal stem cell penile transplantation on erectile signaling of aged rats. *Andrologia* 2010; **42**: 187-192 [PMID: 20500748 DOI: 10.1111/j.1439-0272.2009.00977.x]
- Kendirci M, Trost L, Bakondi B, Whitney MJ, Hellstrom WJ, Spees JL. Transplantation of nonhematopoietic adult bone marrow stem/progenitor cells isolated by p75 nerve growth factor receptor into the penis rescues erectile function in a rat model of cavernous nerve injury. *J Urol* 2010; **184**: 1560-1566 [DOI: 10.1016/j.juro.2010.05.088]
- Qiu 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 streptozocin-induced diabetic rats. *J Sex Med* 2011; **8**: 427-436 [PMID: 21091881 DOI: 10.1111/j.1743-6109.2010.02118.x]
- Kim SJ, Choi SW, Hur KJ, Park SH, Sung YC, Ha YS, Cho HJ, Hong SH, Lee JY, Hwang TK, Kim SW. Synergistic effect of mesenchymal stem cells infected with recombinant adenovirus expressing human BDNF on erectile function in a rat model of cavernous nerve injury. *Korean J Urol* 2012; **53**: 726-732 [PMID: 23136635 DOI: 10.4111/kju.2012.53.10.726]
- Kim SJ, Park SH, Sung YC, Kim SW. Effect of mesenchymal stem cells associated to matrix on the erectile function in the rat model with bilateral cavernous nerve crushing injury. *Int Braz J Urol* 2012; **38**: 833-841 [PMID: 23302404 DOI: 10.1590/1677-553820133806833]
- Sun C, Lin H, Yu W, Li X, Chen Y, Qiu X, Wang R, Dai Y. Neurotrophic effect of bone marrow mesenchymal stem cells for erectile dysfunction in diabetic rats. *Int J Androl* 2012; **35**: 601-607 [PMID: 22428616 DOI: 10.1111/j.1365-2605.2012.01250.x]
- He Y, He W, Qin G, Luo J, Xiao M. Transplantation KCNMA1 modified bone marrow-mesenchymal stem cell therapy for diabetes mellitus-induced erectile dysfunction. *Andrologia* 2014; **46**: 479-486 [PMID: 23646921 DOI: 10.1111/and.12104]
- You D, Jang MJ, Lee J, Jeong IG, Kim HS, Moon KH, Suh N, Kim CS. Periprostatic implantation of human bone marrow-derived mesenchymal stem cells potentiates recovery of erectile function by intracavernosal injection in a rat model of cavernous nerve injury. *Urology* 2013; **81**: 104-110 [PMID: 23122545 DOI: 10.1016/j.urology.2012.08.046]
- Ryu JK, Kim DH, Song KM, Yi T, Suh JK, Song SU. Intracavernous delivery of clonal mesenchymal stem cells restores erectile function in a mouse model of cavernous nerve injury. *J Sex Med* 2014; **11**: 411-423 [PMID: 24251583 DOI: 10.1111/jsm.12380]
- Terada N, Hamazaki T, Oka M, Hoki M, Mastalerz DM, Nakano Y, Meyer EM, Morel L, Petersen BE, Scott EW. Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. *Nature* 2002; **416**: 542-545 [PMID: 11932747 DOI: 10.1038/nature730]
- Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM, Fike JR, Lee HO, Pfeffer K, Lois C, Morrison SJ, Alvarez-Buylla A.

- Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. *Nature* 2003; **425**: 968-973 [PMID: 14555960 DOI: 10.1038/nature02069]
- 30 **Yeghiazarians Y**, Zhang Y, Prasad M, Shih H, Saini SA, Takagawa J, Sievers RE, Wong ML, Kapasi NK, Mirsky R, Koskenvuo J, Minasi P, Ye J, Viswanathan MN, Angeli FS, Boyle AJ, Springer ML, Grossman W. Injection of bone marrow cell extract into infarcted hearts results in functional improvement comparable to intact cell therapy. *Mol Ther* 2009; **17**: 1250-1256 [PMID: 19384293 DOI: 10.1038/mt.2009.85]
- 31 **Albersen M**, Fandel TM, Lin G, Wang G, Banie L, Lin CS, Lue TF. Injections of adipose tissue-derived stem cells and stem cell lysate improve recovery of erectile function in a rat model of cavernous nerve injury. *J Sex Med* 2010; **7**: 3331-3340 [PMID: 20561166 DOI: 10.1111/j.1743-6109.2010.01875.x]
- 32 **Garcia MM**, Fandel TM, Lin G, Shindel AW, Banie L, Lin CS, Lue TF. Treatment of erectile dysfunction in the obese type 2 diabetic ZDF rat with adipose tissue-derived stem cells. *J Sex Med* 2010; **7**: 89-98 [PMID: 20104670 DOI: 10.1111/j.1743-6109.2009.01541.x]
- 33 **Huang YC**, Ning H, Shindel AW, Fandel TM, Lin G, Harraz AM, Lue TF, Lin CS. The effect of intracavernous injection of adipose tissue-derived stem cells on hyperlipidemia-associated erectile dysfunction in a rat model. *J Sex Med* 2010; **7**: 1391-1400 [PMID: 20141586 DOI: 10.1111/j.1743-6109.2009.01697.x]
- 34 **Lin G**, Albersen M, Harraz AM, Fandel TM, Garcia M, McGrath MH, Konety BR, Lue TF, Lin CS. Cavernous nerve repair with allogenic adipose matrix and autologous adipose-derived stem cells. *Urology* 2011; **77**: 1509.e1-1509.e8 [PMID: 21492917 DOI: 10.1016/j.urol.2010.12.076]
- 35 **Fandel TM**, Albersen M, Lin G, Qiu X, Ning H, Banie L, Lue TF, Lin CS. Recruitment of intracavernously injected adipose-derived stem cells to the major pelvic ganglion improves erectile function in a rat model of cavernous nerve injury. *Eur Urol* 2012; **61**: 201-210 [PMID: 21824718 DOI: 10.1016/j.eururo.2011.07.061]
- 36 **Nishimatsu H**, Suzuki E, Kumano S, Nomiya A, Liu M, Kume H, Homma Y. Adrenomedullin mediates adipose tissue-derived stem cell-induced restoration of erectile function in diabetic rats. *J Sex Med* 2012; **9**: 482-493 [PMID: 21951711 DOI: 10.1111/j.1743-6109.2011.02469.x]
- 37 **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**: 1968-1979 [PMID: 22642440 DOI: 10.1111/j.1743-6109.2012.02760.x]
- 38 **Qiu X**, Fandel TM, Ferretti L, Albersen M, Orabi H, Zhang H, Lin G, Lin CS, Schroeder T, Lue TF. Both immediate and delayed intracavernous injection of autologous adipose-derived stromal vascular fraction enhances recovery of erectile function in a rat model of cavernous nerve injury. *Eur Urol* 2012; **62**: 720-727 [PMID: 22397847 DOI: 10.1016/j.eururo.2012.02.003]
- 39 **Qiu X**, Villalta J, Ferretti L, Fandel TM, Albersen M, Lin G, Dai Y, Lue TF, Lin CS. Effects of intravenous injection of adipose-derived stem cells in a rat model of radiation therapy-induced erectile dysfunction. *J Sex Med* 2012; **9**: 1834-1841 [PMID: 22548750 DOI: 10.1111/j.1743-6109.2012.02753.x]
- 40 **Ryu JK**, Tumorbaatar M, Jin HR, Kim WJ, Kwon MH, Piao S, Choi MJ, Yin GN, Song KM, Kang YJ, Koh YJ, Koh GY, Suh JK. Intracavernous delivery of freshly isolated stromal vascular fraction rescues erectile function by enhancing endothelial regeneration in the streptozotocin-induced diabetic mouse. *J Sex Med* 2012; **9**: 3051-3065 [PMID: 23088258 DOI: 10.1111/j.1743-6109.2012.02962.x]
- 41 **Castiglione F**, Hedlund P, Van der Aa F, Bivalacqua TJ, Rigatti P, Van Poppel H, Montorsi F, De Ridder D, Albersen M. Intratunical injection of human adipose tissue-derived stem cells prevents fibrosis and is associated with improved erectile function in a rat model of Peyronie's disease. *Eur Urol* 2013; **63**: 551-560 [PMID: 23040209 DOI: 10.1016/j.eururo.2012.09.034]
- 42 **Jeong HH**, Piao S, Ha JN, Kim IG, Oh SH, Lee JH, Cho HJ, Hong SH, Kim SW, Lee JY. Combined therapeutic effect of udenafil and adipose-derived stem cell (ADSC)/brain-derived neurotrophic factor (BDNF)-membrane system in a rat model of cavernous nerve injury. *Urology* 2013; **81**: 1108.e7-1108.14 [PMID: 23522997 DOI: 10.1016/j.urol.2013.01.022]
- 43 **Kim IG**, Piao S, Lee JY, Hong SH, Hwang TK, Kim SW, Kim CS, Ra JC, Noh I, 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 Part A* 2013; **19**: 14-23 [PMID: 22834730 DOI: 10.1089/ten.tea.2011.0654]
- 44 **Nishimatsu H**, Suzuki E, Nomiya A, Niimi A, Suzuki M, Fujimura T, Fukuhara H, Homma Y. Adrenomedullin and angiopoietin-1 additively restore erectile function in diabetic rats: comparison with the combination therapy of vascular endothelial growth factor and angiopoietin-1. *J Sex Med* 2013; **10**: 1707-1719 [PMID: 23651347 DOI: 10.1111/jsm.12177]
- 45 **You D**, Jang MJ, Lee J, Suh N, Jeong IG, Sohn DW, Kim SW, Ahn TY, Kim CS. Comparative analysis of periprostatic implantation and intracavernosal injection of human adipose tissue-derived stem cells for erectile function recovery in a rat model of cavernous nerve injury. *Prostate* 2013; **73**: 278-286 [PMID: 22821215 DOI: 10.1002/pros.22567]
- 46 **Ning H**, Liu G, Lin G, Yang R, Lue TF, Lin CS. Fibroblast growth factor 2 promotes endothelial differentiation of adipose tissue-derived stem cells. *J Sex Med* 2009; **6**: 967-979 [PMID: 19207272 DOI: 10.1111/j.1743-6109.2008.01172.x]
- 47 **Zhang H**, Yang R, Wang Z, Lin G, Lue TF, Lin CS. Adipose tissue-derived stem cells secrete CXCL5 cytokine with neurotrophic effects on cavernous nerve regeneration. *J Sex Med* 2011; **8**: 437-446 [PMID: 21114767 DOI: 10.1111/j.1743-6109.2010.02128.x]
- 48 **Kitamura K**, Kangawa K, Kawamoto M, Ichiki Y, Nakamura S, Matsuo H, Eto T. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. *Biochem Biophys Res Commun* 1993; **192**: 553-560 [PMID: 8387282 DOI: 10.1006/bbrc.1993.1451]
- 49 **Sugo S**, Minamino N, Kangawa K, Miyamoto K, Kitamura K, Sakata J, Eto T, Matsuo H. Endothelial cells actively synthesize and secrete adrenomedullin. *Biochem Biophys Res Commun* 1994; **201**: 1160-1166 [PMID: 8024557 DOI: 10.1006/bbrc.1994.1827]
- 50 **Sugo S**, Minamino N, Shoji H, Kangawa K, Kitamura K, Eto T, Matsuo H. Production and secretion of adrenomedullin from vascular smooth muscle cells: augmented production by tumor necrosis factor-alpha. *Biochem Biophys Res Commun* 1994; **203**: 719-726 [PMID: 8074727 DOI: 10.1006/bbrc.1994.2241]
- 51 **Kubo A**, Minamino N, Isumi Y, Katafuchi T, Kangawa K, Dohi K, Matsuo H. Production of adrenomedullin in macrophage cell line and peritoneal macrophage. *J Biol Chem* 1998; **273**: 16730-16738 [PMID: 9642228 DOI: 10.1074/jbc.273.27.16730]
- 52 **Kim W**, Moon SO, Sung MJ, Kim SH, Lee S, So JN, Park SK. Angiogenic role of adrenomedullin through activation of Akt, mitogen-activated protein kinase, and focal adhesion kinase in endothelial cells. *FASEB J* 2003; **17**: 1937-1939 [PMID: 12897063]
- 53 **Iimuro S**, Shindo T, Moriyama N, Amaki T, Niu P, Takeda N, Iwata H, Zhang Y, Ebihara A, Nagai R. Angiogenic effects of adrenomedullin in ischemia and tumor growth. *Circ Res* 2004; **95**: 415-423 [PMID: 15242974 DOI: 10.1161/01.RES.0000138018.61065.d1]
- 54 **Tokunaga N**, Nagaya N, Shirai M, Tanaka E, Ishibashi-Ueda

- H, Harada-Shiba M, Kanda M, Ito T, Shimizu W, Tabata Y, Uematsu M, Nishigami K, Sano S, Kangawa K, Mori H. Adrenomedullin gene transfer induces therapeutic angiogenesis in a rabbit model of chronic hind limb ischemia: benefits of a novel nonviral vector, gelatin. *Circulation* 2004; **109**: 526-531 [PMID: 14732745 DOI: 10.1161/01.CIR.0000109700.81266.32]
- 55 **Takahashi M**, Suzuki E, Kumano S, Oba S, Sato T, Nishimatsu H, Kimura K, Nagano T, Hirata Y. Angiopoietin-1 mediates adipose tissue-derived stem cell-induced inhibition of neointimal formation in rat femoral artery. *Circ J* 2013; **77**: 1574-1584 [PMID: 23486192 DOI: 10.1253/circj.CJ-12-0930]
- 56 **Lin G**, Qiu X, Fandel T, Banie L, Wang G, Lue TF, Lin CS. Tracking intracavernously injected adipose-derived stem cells to bone marrow. *Int J Impot Res* 2011; **23**: 268-275 [PMID: 21796145 DOI: 10.1038/ijir.2011.38]
- 57 **Asahara T**, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997; **275**: 964-967 [PMID: 9020076 DOI: 10.1126/science.275.5302.964]
- 58 **Yin AH**, Miraglia S, Zanjani ED, Almeida-Porada G, Ogawa M, Leary AG, Olweus J, Kearney J, Buck DW. AC133, a novel marker for human hematopoietic stem and progenitor cells. *Blood* 1997; **90**: 5002-5012 [PMID: 9389720]
- 59 **Gehling UM**, Ergün S, Schumacher U, Wagener C, Pantel K, Otte M, Schuch G, Schafhausen P, Mende T, Kilic N, Kluge K, Schäfer B, Hossfeld DK, Fiedler W. In vitro differentiation of endothelial cells from AC133-positive progenitor cells. *Blood* 2000; **95**: 3106-3112 [PMID: 10807776]
- 60 **Peichev M**, Naiyer AJ, Pereira D, Zhu Z, Lane WJ, Williams M, Oz MC, Hicklin DJ, Witte L, Moore MA, Rafii S. Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. *Blood* 2000; **95**: 952-958 [PMID: 10648408]
- 61 **Gou X**, He WY, Xiao MZ, Qiu M, Wang M, Deng YZ, Liu CD, Tang ZB, Li J, Chen Y. Transplantation of endothelial progenitor cells transfected with VEGF165 to restore erectile function in diabetic rats. *Asian J Androl* 2011; **13**: 332-338 [PMID: 21113173 DOI: 10.1038/aja.2010.116]
- 62 **Vasa M**, Fichtlscherer S, Aicher A, Adler K, Urbich C, Martin H, Zeiher AM, Dimmeler S. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res* 2001; **89**: E1-E7 [PMID: 11440984 DOI: 10.1161/hh1301.093953]
- 63 **Hill JM**, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA, Finkel T. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med* 2003; **348**: 593-600 [PMID: 12584367 DOI: 10.1056/NEJ-Moa022287]
- 64 **Tepper OM**, Galiano RD, Capla JM, Kalka C, Gagne PJ, Jacobowitz GR, Levine JP, Gurtner GC. Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. *Circulation* 2002; **106**: 2781-2786 [PMID: 12451003 DOI: 10.1161/01.CIR.0000039526.42991.93]
- 65 **Takahashi T**, Kalka C, Masuda H, Chen D, Silver M, Kearney M, Magner M, Isner JM, Asahara T. Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nat Med* 1999; **5**: 434-438 [PMID: 10202935 DOI: 10.1038/7434]
- 66 **Shintani S**, Murohara T, Ikeda H, Ueno T, Honma T, Katoh A, Sasaki K, Shimada T, Oike Y, Imaizumi T. Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. *Circulation* 2001; **103**: 2776-2779 [PMID: 11401930 DOI: 10.1161/hc2301.092122]
- 67 **Foresta C**, Caretta N, Lana A, Cabrelle A, Palù G, Ferlin A. Circulating endothelial progenitor cells in subjects with erectile dysfunction. *Int J Impot Res* 2005; **17**: 288-290 [PMID: 15729373 DOI: 10.1038/sj.ijir.3901311]
- 68 **Baumhäkel M**, Werner N, Böhm M, Nickenig G. Circulating endothelial progenitor cells correlate with erectile function in patients with coronary heart disease. *Eur Heart J* 2006; **27**: 2184-2188 [PMID: 16926179 DOI: 10.1093/eurheartj/ehl202]
- 69 **Esposito K**, Ciotola M, Maiorino MI, Giugliano F, Autorino R, De Sio M, Jannini E, Lenzi A, Giugliano D. Circulating CD34+ KDR+ endothelial progenitor cells correlate with erectile function and endothelial function in overweight men. *J Sex Med* 2009; **6**: 107-114 [PMID: 19170841 DOI: 10.1111/j.1743-6109.2008.01042.x]
- 70 **Dimmeler S**, Aicher A, Vasa M, Mildner-Rihm C, Adler K, Tiemann M, Rütten H, Fichtlscherer S, Martin H, Zeiher AM. HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI 3-kinase/Akt pathway. *J Clin Invest* 2001; **108**: 391-397 [PMID: 11489932 DOI: 10.1172/JCI13152]
- 71 **Vasa M**, Fichtlscherer S, Adler K, Aicher A, Martin H, Zeiher AM, Dimmeler S. Increase in circulating endothelial progenitor cells by statin therapy in patients with stable coronary artery disease. *Circulation* 2001; **103**: 2885-2890 [PMID: 11413075 DOI: 10.1161/hc2401.092816]
- 72 **Foresta C**, Ferlin A, De Toni L, Lana A, Vinanzi C, Galan A, Caretta N. Circulating endothelial progenitor cells and endothelial function after chronic Tadalafil treatment in subjects with erectile dysfunction. *Int J Impot Res* 2006; **18**: 484-488 [PMID: 16541115 DOI: 10.1038/sj.ijir.3901465]
- 73 **Foresta C**, Caretta N, Lana A, De Toni L, Biagioli A, Vinanzi C, Ferlin A. Relationship between vascular damage degrees and endothelial progenitor cells in patients with erectile dysfunction: effect of vardenafil administration and PDE5 expression in the bone marrow. *Eur Urol* 2007; **51**: 1411-1417; discussion 1417-1419 [PMID: 17034932 DOI: 10.1016/j.euro.2006.08.052]
- 74 **Nolazco G**, Kovanecz I, Vernet D, Gelfand RA, Tsao J, Ferrini MG, Magee T, Rajfer J, Gonzalez-Cadavid NF. Effect of muscle-derived stem cells on the restoration of corpora cavernosa smooth muscle and erectile function in the aged rat. *BJU Int* 2008; **101**: 1156-1164 [PMID: 18294308 DOI: 10.1111/j.1464-410X.2008.07507.x]
- 75 **Woo JC**, Bae WJ, Kim SJ, Kim SD, Sohn DW, Hong SH, Lee JY, Hwang TK, Sung YC, Kim SW. Transplantation of muscle-derived stem cells into the corpus cavernosum restores erectile function in a rat model of cavernous nerve injury. *Korean J Urol* 2011; **52**: 359-363 [PMID: 21687398 DOI: 10.4111/kju.2011.52.5.359]
- 76 **Kovanecz I**, Rivera S, Nolazco G, Vernet D, Segura D, Gharib S, Rajfer J, Gonzalez-Cadavid NF. Separate or combined treatments with daily sildenafil, molsidomine, or muscle-derived stem cells prevent erectile dysfunction in a rat model of cavernous nerve damage. *J Sex Med* 2012; **9**: 2814-2826 [PMID: 22974131 DOI: 10.1111/j.1743-6109.2012.02913.x]
- 77 **Rübe CE**, Fricke A, Widmann TA, Fürst T, Madry H, Pfreundschuh M, Rübe C. Accumulation of DNA damage in hematopoietic stem and progenitor cells during human aging. *PLoS One* 2011; **6**: e17487 [PMID: 21408175 DOI: 10.1371/journal.pone.0017487]
- 78 **Schuler N**, Rübe CE. Accumulation of DNA damage-induced chromatin alterations in tissue-specific stem cells: the driving force of aging? *PLoS One* 2013; **8**: e63932 [PMID: 23691119 DOI: 10.1371/journal.pone.0063932]
- 79 **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**: 150-160 [PMID: 20565373]
- 80 **Oishi K**, Ogawa Y, Gamoh S, Uchida MK. Contractile responses of smooth muscle cells differentiated from rat neural stem cells. *J Physiol* 2002; **540**: 139-152 [PMID: 11927676 DOI: 10.1113/jphysiol.2001.013278]
- 81 **Song Y**, Mehta N, Sheh B, Saljoogue F, U HS, Rajasekaran M. Transdifferentiation of rat fetal brain stem cells into penile smooth muscle cells. *BJU Int* 2009; **104**: 257-262 [PMID:

- 19220248 DOI: 10.1111/j.1464-410X.2009.08352.x]
- 82 **Trentin A**, Glavieux-Pardanaud C, Le Douarin NM, Dupin E. Self-renewal capacity is a widespread property of various types of neural crest precursor cells. *Proc Natl Acad Sci USA* 2004; **101**: 4495-4500 [PMID: 15070746 DOI: 10.1073/pnas.0400629101]
- 83 **Maurer J**, Fuchs S, Jäger R, Kurz B, Sommer L, Schorle H. Establishment and controlled differentiation of neural crest stem cell lines using conditional transgenesis. *Differentiation* 2007; **75**: 580-591 [PMID: 17381545 DOI: 10.1111/j.1432-0436.2007.00164.x]
- 84 **Song YS**, Lee HJ, Park IH, Lim IS, Ku JH, Kim SU. Human neural crest stem cells transplanted in rat penile corpus cavernosum to repair erectile dysfunction. *BJU Int* 2008; **102**: 220-224; discussion 224 [PMID: 18284412 DOI: 10.1111/j.1464-410X.2008.07469.x]
- 85 **Bochinski D**, Lin GT, Nunes L, Carrion R, Rahman N, Lin CS, Lue TF. The effect of neural embryonic stem cell therapy in a rat model of cavernosal nerve injury. *BJU Int* 2004; **94**: 904-909 [PMID: 15476533 DOI: 10.1111/j.1464-410X.2003.05057.x]
- 86 **Jeong JO**, Han JW, Kim JM, Cho HJ, Park C, Lee N, Kim DW, Yoon YS. Malignant tumor formation after transplantation of short-term cultured bone marrow mesenchymal stem cells in experimental myocardial infarction and diabetic neuropathy. *Circ Res* 2011; **108**: 1340-1347 [PMID: 21493893 DOI: 10.1161/CIRCRESAHA.110.239848]

P- Reviewer: Abdel-Salam OME, Carcas-Sansuan AJ, El-Assmy A, Saeki K

S- Editor: Ji FF **L- Editor:** A **E- Editor:** Liu SQ





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: bpgoffice@wjgnet.com

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>

