Asian Journal of Andrology (2017) 19, 543–547
www.asiaandro.com; www.ajandrology.com

Open Access

ORIGINAL ARTICLE

Reproduction

Mesenchymal stem cells from human umbilical cord ameliorate testicular dysfunction in a male rat hypogonadism model

Zhi-Yuan Zhang, Xiao-Yu Xing, Guan-Qun Ju, Liang Zhong, Jie Sun

Androgen deficiency is a physical disorder that not only affects adults but can also jeopardize children's health. Because there are many disadvantages to using traditional androgen replacement therapy, we have herein attempted to explore the use of human umbilical cord mesenchymal stem cells for the treatment of androgen deficiency. We transplanted CM-DiI-labeled human umbilical cord mesenchymal stem cells into the testes of an ethane dimethanesulfonate (EDS)-induced male rat hypogonadism model. Twenty-one days after transplantation, we found that blood testosterone levels in the therapy group were higher than that of the control group ($P = 0.037$), and using immunohistochemistry and flow cytometry, we observed that some of the CM-DiI-labeled cells expressed Leydig cell markers for cytochrome P450, family 11, subfamily A, polypeptide 1, and 3-β-hydroxysteroid dehydrogenase. We then recovered these cells and observed that they were still able to proliferate *in vitro*. The present study shows that mesenchymal stem cells from human umbilical cord may constitute a promising therapeutic modality for the treatment of male hypogonadism patients.

Asian Journal of Andrology (2017) **19**, 543–547; doi: 10.4103/1008-682X.186186; published online: 30 August 2016

Keywords: Leydig cells; stem cells; testicular dysfunction

INTRODUCTION

Androgen deficiency is a very common physical disorder that not only affects adults but can also jeopardize children's health. It is estimated that about 0.5% of children worldwide suffer from androgen deficiency.[1] For children undergoing puberty who suffer from androgen deficiency, traditional androgen replacement therapy cannot mimic their natural testosterone fluctuations,[2] and this may then lead to primary hypogonadism and altered sexual development.[3] There may, however, be other treatments available, such as stem cell therapy.

As stem cells exhibit the potential to differentiate into multiple cell types, mesenchymal stem cells (MSCs) have been used widely in the treatment of organ dysfunction. Researchers have transplanted rat adipose-derived MCSs into D-galactose-treated aging rats and found that some of the MSCs differentiated into 3-β-hydroxysteroid dehydrogenase (HSD3B1)-positive Leydig-like cells, and that testicular dysfunction was ameliorated.[4] However, Ren *et al.*[5] transplanted human bone marrow mesenchymal stem cells (BMSCs) into mice and found that none of the cells differentiated into Leydig-like cells. We, therefore, used human umbilical mesenchymal stem cells (HUMSCs) to treat ethane dimethanesulfonate (EDS)-induced male rat hypogonadism to study the value of HUMSCs in treating androgen deficiency.

MATERIALS AND METHODS

Isolation and culture of HUMSCs

Umbilical cords were collected aseptically with patient consent from women at full term during Cesarean section, at the Shanghai Ninth People's Hospital, Shanghai, China. HUMSC isolation was performed as previously described.[6] Umbilical cord Wharton's jelly was cut into 2–3 mm^3 pieces, which were then cultured in a 37°C incubator in 10 cm cell culture dishes (Corning Inc., Acton, MA, USA) containing growth medium prepared from Dulbecco's modified Eagle's medium-low glucose medium (DMEM-LG; HyClone, Logan, UT, USA), supplemented with 10% (*v/v*) fetal bovine serum (FBS; HyClone, Logan, UT, USA), 2 mmol l^{-1} L-glutamine, 100 U ml^{-1} penicillin, and 100 mg ml^{-1} streptomycin. Cells were harvested using 0.25% trypsin and medium was changed every 2 days.

Identification of MSC marker expression by flow cytometry

Cell surface antigen phenotyping was performed on human umbilical cord MSCs. Cells were harvested using 0.25% trypsin, washed in phosphate-buffered saline (PBS), and incubated for 30 min at room temperature in the dark using a Human MSC Analysis Kit (BD Pharmingen, San Diego, CA, USA) that contained mouse anti-human CD90 FITC, CD105 PerCP-Cy5.5, CD73 APC, CD44 PE, CD34 PE, CD11b PE, CD19 PE, CD45 PE, and HLA-DR PE; mIgG1, κ FITC, mIgG1, κ PerCP-Cy5.5, mIgG1 κ APC mIgG1, κ PE, mIgG2a, and κ PE were used as isotype controls. After washing in PBS, the fluorescence of 1×10^4 cells was analyzed using a FACS flow cytometer (BECTON DICKINSON, Newark, NJ, USA).

Donor cell preparation

When HUMSCs after the third passage reached 70%–80% confluency, they were incubated with 5 μg ml^{-1} CM-DiI at 37°C for 30 min and

Department of Urology, Shanghai Children's Medical Center Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 200127, China.
Correspondence: Dr. J Sun (sunjiefan@hotmail.com)
Received: 31 December 2015; Revised: 30 March 2016; Accepted: 01 July 2016

then washed with PBS 3 times. The efficiency of the CM-Dil staining was detected with a fluorescence microscope (Leica, Heidelberg, Deutschland).

Animal preparation and cell transplantation

Forty male Sprague-Dawley rats were purchased at 8 weeks of age from the Shanghai Slack Experimental Animal Center. All rats were kept under conditions of controlled temperature (24 ± 1°C), relative humidity (50%–60%), and a light/dark cycle of 12 h light/12 h dark. Standard rodent diet and drinking water were available *ad libitum*. All surgical procedures and postoperative care were approved by the Research Ethics Committee of Shanghai Jiao Tong University Affiliated Shanghai Children's Medical Center.

The forty rats were randomly divided into control group (twenty rats) and therapy group (twenty rats), and EDS was injected into the abdominal cavities of all forty rats at a dose of 75 mg kg^{-1} to destroy Leydig cell function. After 4 days of EDS injections, HUMSCs stained with CM-Dil were washed twice with PBS and incubated with 0.25% trypsin-EDTA for 1.5 min at 37°C. Cells were gently dissociated, resuspended manually, and collected in a 15 ml Corning tube. Cells were rinsed twice with PBS following centrifugation at 1000 rpm for 5 min, and finally, cells were resuspended in PBS and loaded into a 1 ml syringe for injection into the testes of adult rats. Approximately 1×10^6 cells in a 250 μl volume of PBS were injected into the mesenchyme of recipient testes of rats from the therapy group. The rats from the control group received 250 μl of PBS buffer. Twenty-one days after transplantation, testes from the six rats of the therapy group were tested in a random fashion using immunohistochemistry, and the testes from the six rats of the therapy group were prepared for flow cytometric sorting, which was also done in a random fashion.

Immunohistochemistry

Immunohistochemistry was performed on modified Davidson's fluid-fixed and paraffin-embedded testicular sections from grafted rats. Testicular sections were briefly deparaffinized, hydrated in a successive series of decreasing ethanol concentrations, and rinsed in distilled water. Sections were then placed in an EDTA buffer solution using a pressure cooker for 3 min for heat antigen retrieval. After rinsing in PBS, sections were blocked with 5% normal horse serum for 2 h at 37°C to prevent nonspecific binding of IgG and were subsequently incubated with a 1:100 dilution of a monoclonal anti-CYP11A1 antibody (Abcam, Cambridge, UK) at 4°C overnight. The secondary antibody was donkey anti-rabbit IgG H&L (Alexa Fluor® 488) in a 1:2000 dilution (Abcam, Cambridge, UK), and this was used to incubate the sections for 1 h at 37°C. Nuclei were stained with DAPI. Finally, slides were dehydrated and coverslipped with neutral balsam. All slides were photographed with a fluorescence microscope.

Transplanted HUMSCs sorted by flow cytometry

Cells in the interstitial tissues of testes were isolated from rats 21 days after transplantation as described previously.[7] Briefly, rats were humanely killed by cervical dislocation, and the testes were extracted. The testes were then incubated in a centrifuge tube containing 0.03% collagenase NB4 and digested for 15 min at 37°C with shaking at 1500 rpm. The supernatant was discarded, and a fresh collagenase NB4 solution was added for a second 15-min digestion, during which time the rotational speed was lowered to 130 rpm. Cells in the supernatant were then centrifuged and resuspended in PBS. The cell suspension was then used to isolate CM-Dil-positive cells using a flow cytometry sorting instrument (MoFlo XDP, Beckman Coulter, Brea, CA, USA), and these CM-Dil-positive cells were cultured in DMEM for morphologic analysis.

Detection of HUMSC-derived Leydig-like cells after sorting

After 3 days of culture, the CM-Dil-positive cells were harvested using 0.25% trypsin, resuspended in 0.01% formaldehyde, and permeabilized with 0.5% Tween 20. Finally, cells were incubated with anti-HSD3B1 antibody (1:1000; Abcam, Cambridge, UK) for 30 min and then incubated with donkey anti-rabbit IgG H&L (1:2000; Alexa Fluor® 488; Abcam) for 30 min. Stained cells were examined with a flow cytometer.

Evaluation of testosterone concentrations

We collected blood from all forty rats at 7 days, 18 days, and 25 days after EDS injection (3 days, 14 days, and 21 days after cell transplantation), and measured the concentrations of testosterone in serum the same day that blood was collected, using a chemiluminescence assay (Shanghai Children's Medical Center).

Statistical analyses

Data were expressed as mean ± one standard deviation (s.d.) of the mean. Statistical analyses were performed using independent sample Student's *t*-tests to compare control and therapy groups.

RESULTS

Identification of MSC marker expression by flow cytometry

Isolated UCMSCs adhered to plastic surfaces and showed a typical spindle-shaped appearance (data not shown).

Flow cytometric analysis showed that HUMSCs were positive for CD105, CD90, and CD73, which are considered typical MSC-positive markers. Cells were negative for CD45, CD34, CD11b, CD19, and HLA-DR, which are typical markers of non-MSCs (**Figure 1**). HUMSCs were examined for expression of MSC markers; the area surrounded by the red line represents the blank control for background fluorescence, and the area surrounded by the blue line indicates signals from antibodies to human MSC markers. Cells were stained with phycoerythrin (PE), allophycocyanin (APC), peridinin-chlorophyll-protein complex-Cy5.5 (PerCP-Cy5.5), or isothiocyanate (FITC)-conjugated antibodies to indicate the following MSC markers: CD90-FITC, CD105-PerCP-Cy5.5, CD73-APC, CD34-PE, CD11b-PE, CD19-PE, CD45-PE, or HLA-DR-PE, respectively.

Morphologic assessment and immunohistochemistry of UCMSCs after transplantation

To evaluate the morphologic and histochemical changes in HUMSCs after transplantation, 21 days after transplantation, the testes of the therapy group were examined by immunohistochemistry using CYP11A1 antibody. HUMSCs, which were CM-Dil-positive cells under the fluorescence microscope, acquired a small and round Leydig cell-like appearance, and CM-Dil-positive cells were also CYP11A1-positive, indicating that they had differentiated into Leydig-like cells (**Figure 2**).

Detection and sorting of transplanted HUMSCs by flow cytometry

To examine the differentiation status of CM-Dil-stained HUMSCs after transplantation, we digested testes from rats 21 days after transplantation to acquire the cells located in the interstitium and found that these CM-Dil-positive cells were still able to proliferate (**Figure 3**) and showed HSD3B1-antibody staining (**Figure 4**). This showed that these cells expressed HSD3B1, which indicated that UCMSCs acquired

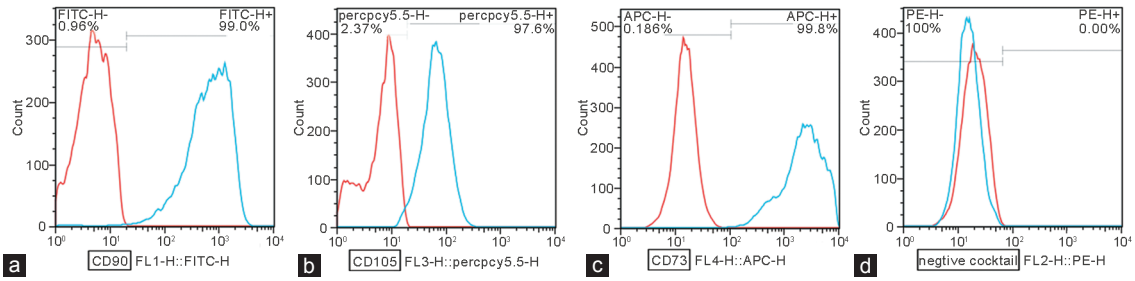

Figure 1: Surface marker analysis of cultured HUMSCs. CD90 (**a**)/CD105 (**b**)/CD73 (**c**) expression indicated the presence of HUMSCs; the negative control contained few non-MSCs (**d**). MSCs: mesenchymal stem cells; HUMSCs: human umbilical mesenchymal stem cells.

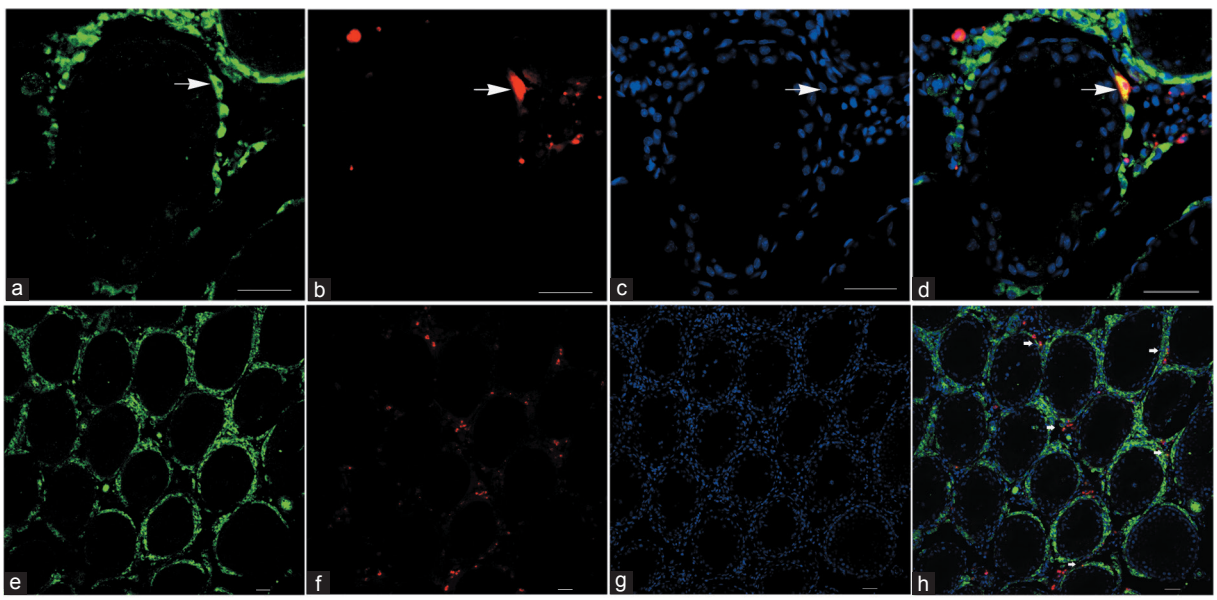

Figure 2: Immunohistochemical fluorescence staining showed the distribution of HSD3B-positive cells (**a** and **e**), CM-Dil-stained cells (**b** and **f**), and nuclei (**c** and **g**). CM-Dil-positive cells co-expressing CYP11A1 are shown by white arrows (**d** and **h**) (scale bar = 100 μm).

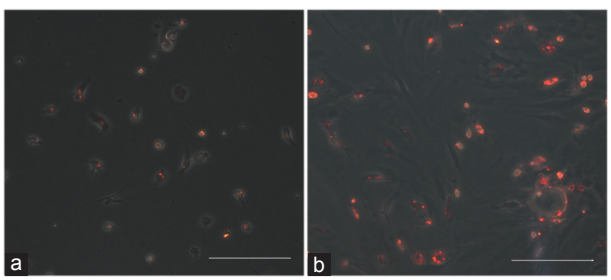

Figure 3: CM-Dil-positive HUMSCs sorted by flow cytometry. After 6 h of culture, cells adhered to the substratum and showed small rounded shapes (**a**). After 3 days of culture, CM-Dil-positive cells were observed to be spindle-shaped, and they proliferated and formed colonies (**b**) (scale bar = 100 μm). HUMSCs: human umbilical mesenchymal stem cells.

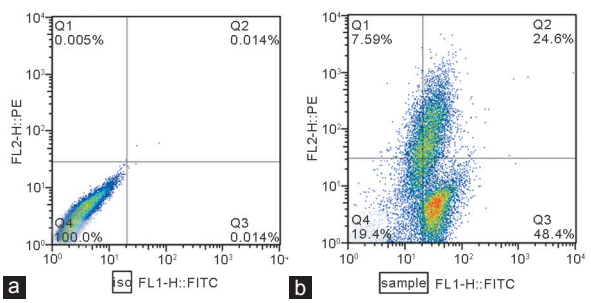

Figure 4: HSD3B staining for cultured CM-Dil-positive cells. Contrasted with the isotype control (**a**), after sorting, some of the cells may not have expressed red fluorescence because they lost CM-Dil during mitotic divisions. Alternatively, cells incubated with the Q3 dose did not come from HUMSCs, but rather from rat Leydig cells (**b**). HUMSCs: human umbilical mesenchymal stem cells.

properties of Leydig cells after they were transplanted to testes from EDS-treated rats for 21 days.

Evaluation of testosterone in rat serum

Eighteen days after EDS injections, serum testosterone concentrations in the therapy group were only slightly higher (and not statistically different) compared to the control group ($P = 0.643$). However, after 25 days of EDS injections, serum testosterone in the therapy group was greatly augmented relative to the control group ($P = 0.037$) (**Figure 5**).

DISCUSSION

It is estimated that about 1 child in 200 suffers from androgen deficiency.[1] Trauma, infections, tumor growth, and radiation therapy can be the reasons for androgen deficiency in children.[8] The current clinical treatment for androgen deficiency – that of androgen replacement therapy – can cause a number of adverse reactions.[9] To avoid these side effects, pediatricians need to have at their disposal

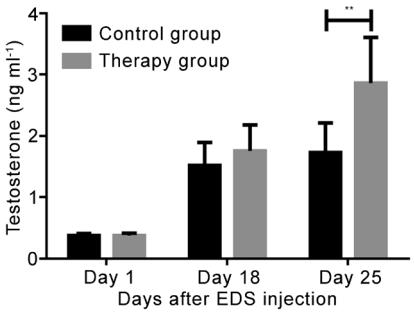

Figure 5: Twenty-five days after EDS injection, serum testosterone concentrations in the therapy group were higher than that in the control group ($P = 0.037$). EDS: ethane dimethanesulfonate.

alternative therapeutic options that simulate natural developmental fluctuations.[2] Therefore, we transplanted HUMSCs into testes of EDS-treated rats to investigate their therapeutic potential, as HUMSCs have been widely used in tissue repair, and they can be induced into Leydig-like cells *in vitro*.[10]

Our study showed that after transplantation, HUMSCs survived in an EDS-treated male rat hypogonadism model without obvious immunologic rejection. Moreover, some of the CM-Dil-marked HUMSCs expressed the Leydig cell marker CYP11A1. We sorted and cultured CM-Dil-positive HUMSCs from rat testes 3 weeks after transplantation and found that these cells were still able to proliferate and that they expressed HSD3B1. Finally, we analyzed blood samples from the therapy and control groups, and the results showed that the therapy group's serum testosterone levels were higher than in the control group ($P = 0.037$). Our results suggest that HUMSCs are able to differentiate into Leydig-like cells with normal function.

EDS, as an alkylating agent, is able to specifically eliminate adult Leydig cells from rat testes. The elimination of adult Leydig cells is then normally followed by a Leydig cell regeneration process, which is similar to the Leydig cell differentiation process observed for human puberty.[11] During the process of Leydig cell regeneration, and as a result of low testosterone, the levels of other hormones and cytokines may be upregulated.[12] For example, luteinizing hormone has been used to induce HUMSCs into Leydig-like cells *in vitro*,[13] nerve growth factor can promote the regeneration and differentiation of Leydig cells,[14] and protein kinase inhibitor β may interact with the catalytic subunit of cAMP-dependent protein kinase and act as a competitive inhibitor, thus stimulating cell growth and androgen secretion.[15] In addition, as a cell that is important in the construction of the testicular microenvironment, Sertoli cells can promote Leydig cell differentiation via the secretion of cellular factors such as epidermal growth factor,[16] insulin-like growth factors-1,[17] and platelet-derived growth factor.[18] We hypothesize that the combined effects of the upregulation of cellular factors and the testicular microenvironment can induce HUMSCs into Leydig-like cells.

Our study showed that transplanting HUMSCs into EDS-treated rats promoted the recovery of blood testosterone levels back to normal. The flow cytometric analyses indicated that some of the HUMSCs also expressed HSD3B1, and these HSD3B1-positive cells may be considered functional Leydig-like cells involved in the accelerated recovery of serum testosterone. However, it should be noted that undifferentiated MSCs might also promote the regeneration of Leydig cells. It has been reported that MSCs are able to secrete various cellular factors and adhesive molecules, including vascular endothelial growth factor, basic fibroblast growth factor, hepatocyte growth factor, and insulin-like growth factor-I that exert beneficial paracrine effects on surrounding cells.[19–21] These cellular factors can then promote the regeneration of Leydig cells and enhance their functions.[17,18,22] In the present experiment, transplanted HUMSCs may have released or promoted the release of certain factors that guided the regeneration of Leydig cells. HUMSCs, compared with BMSCs, possess a higher proliferative potential, and HUMSCs are more capable of differentiating into Leydig cells.[6] In our study, we transplanted MSCs from humans instead of rats because some researchers have claimed that human BMSCs do not differentiate into Leydig cells in mouse testes.[5] This raised the question of whether MSCs are able to change into Leydig cells or differentiated MSCs. The matter has not yet been resolved because of technical problems that may cause a failure of differentiation. To answer this question, we used HUMSCs instead of rat MSCs, and we marked HUMSCs with CM-Dil, which is a good cell tracer that is not only unaffected by cell proliferation and differentiation[23] but is also able to keep cells stained for longer than 4 weeks *in vivo*.[24] We also did not find any obvious immunologic rejection after the transplantation. As an immunologically privileged organ, the testis contains few immunocytes, and HUMSCs do not express the major histocompatibility complex (MHC) class II (HLA-DR) antigens.[25–27] Previous studies have shown that umbilical mesenchymal stem cells are still viable and not rejected 4 months after transplantation as xenografts, without the need for immune suppression;[28] this also proved the feasibility of heteroplastic transplantation using HUMSCs.

In our study, although we did not find cells with double nuclei, we were still not able to rule out the possibility of cellular fusion between HUMSCs and recipient testicular Leydig cells or their progenitor cells. In addition, as we did not determine the origin of the cells staining positive for HSD3B1 by double-staining for human nuclear antigen, it is possible that not all of the CM-Dil-labeled cells were HSD3B1 positive originated from human tissues. However, we believe that the elevation in serum testosterone still highlights the value of using MSCs in the treatment of androgen deficiency.

CONCLUSION

HUMSCs can promote recovery of serum testosterone levels in EDS-treated rats via differentiation into normally functioning Leydig-like cells. Based on our results, HUMSCs may constitute a promising therapy for the treatment of both young and old male hypogonadism patients.

AUTHOR CONTRIBUTIONS

ZYZ carried out the immunohistochemistry and flow cytometric analyses, participated in cell culture, and drafted the manuscript. XYX carried out the cell culture. LZ carried out the animal experimentation. GQJ participated in the design of the study and performed the statistical analysis. JS conceived the study, participated in its design and coordination, and helped draft the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing interests.

ACKNOWLEDGMENTS

This research was supported by the grants from the National Natural Science Foundation of China (81270689) and Research Project of Shanghai Science and Technology Committee (12ZR1419200). We are grateful for the assistance of Professor Zhang Zhen in the area of experimental methods. We would like to thank Accdon for its linguistic assistance during the preparation of this manuscript.

REFERENCES

1 Bojesen A, Juul S, Gravholt CH. Prenatal and postnatal prevalence of Klinefelter syndrome: a national registry study. *J Clin Endocrinol Metab* 2003; 88: 622–6.
2 Rogol AD. Pubertal androgen therapy in boys. *Pediatr Endocrinol Rev* 2005; 2: 383–90.
3 Sun J, Xi YB, Zhang ZD, Shen P, Li HY, *et al*. Leydig cell transplantation restores androgen production in surgically castrated prepubertal rats. *Asian J Androl* 2009; 11: 405–9.
4 Yang C, Du YK, Wang J, Luan P, Yang QL, *et al*. Transplanted adipose-derived stem cells ameliorate testicular dysfunction in a D-galactose-induced aging rat model. *J Cell Physiol* 2015; 230: 2403–14.
5 Ren SQ, Deng S, Wu YJ, Hou L, Dong T, *et al*. [Differentiation of human bone marrow mesenchymal stem cells into Leydig or steroidogenic cells *in vivo*]. *Sichuan Da Xue Xue Bao Yi Xue Ban* 2014; 45: 192–5, 201.
6 Wei X, Peng G, Zheng S, Wu X. Differentiation of umbilical cord mesenchymal stem cells into steroidogenic cells in comparison to bone marrow mesenchymal stem cells. *Cell Prolif* 2012; 45: 101–10.
7 Zhong L, Sun J, Liu GH, Zhu YJ, Zhu J. Research on the steroidogenesis of proliferated Leydig cells *in vitro*. *J Artif Organs* 2013; 16: 229–33.
8 Hagenas L. [Normal and deviating puberty in boys]. *Tidsskr Nor Laegeforen* 2008; 128: 1284–8.
9 Bardin CW, Swerdloff RS, Santen RJ. Androgens: risks and benefits. *J Clin Endocrinol Metab* 1991; 73: 4–7.
10 Yazawa T, Mizutani T, Yamada K, Kawata H, Sekiguchi T, *et al*. Differentiation of adult stem cells derived from bone marrow stroma into Leydig or adrenocortical cells. *Endocrinology* 2006; 147: 4104–11.
11 Payne AH, Hardy MP, Russell LD. The Leydig Cell. Vienna: Cache River Press; 1996.
12 Kerr JB, Bartlett JM, Donachie K, Sharpe RM. Origin of regenerating Leydig cells in the testis of the adult rat. An ultrastructural, morphometric and hormonal assay study. *Cell Tissue Res* 1987; 249: 367–77.
13 Wu YJ, Dong Q, Li SF, Wei X, Long D, *et al*. [Differentiation of human bone marrow mesenchymal stem cells into Leydig or steroidogenic cells *in vitro*]. *Sichuan Da Xue Xue Bao Yi Xue Ban* 2012; 43: 493–7, 506.
14 Zhang L, Wang H, Yang Y, Liu H, Zhang Q, *et al*. NGF induces adult stem Leydig cells to proliferate and differentiate during Leydig cell regeneration. *Biochem Biophys Res Commun* 2013; 436: 300–5.
15 Zhang YF, Yuan KM, Liang Y, Chu YH, Lian QQ, *et al*. Alterations of gene profiles in Leydig-cell-regenerating adult rat testis after ethane dimethane sulfonate-treatment. *Asian J Androl* 2015; 17: 253–60.
16 Naz RK, Kaplan P. Effects of epidermal growth factor on human sperm cell function. *J Androl* 1993; 14: 240–7.
17 Lin T, Wang D, Hu J, Stocco DM. Upregulation of human chorionic gonadotrophin-induced steroidogenic acute regulatory protein by insulin-like growth factor-I in rat Leydig cells. *Endocrine* 1998; 8: 73–8.
18 Odeh HM, Kleinguetl C, Ge R, Zirkin BR, Chen H. Regulation of the proliferation and differentiation of Leydig stem cells in the adult testis. *Biol Reprod* 2014; 90: 123.
19 Son BR, Marquez-Curtis LA, Kucia M, Wysoczynski M, Turner AR, *et al*. Migration of bone marrow and cord blood mesenchymal stem cells *in vitro* is regulated by stromal-derived factor-1-CXCR4 and hepatocyte growth factor-c-met axes and involves matrix metalloproteinases. *Stem Cells* 2006; 24: 1254–64.
20 Peled A, Kollet O, Ponomaryov T, Petit I, Franitza S, *et al*. The chemokine SDF-1 activates the integrins LFA-1, VLA-4, and VLA-5 on immature human CD34(+) cells: role in transendothelial/stromal migration and engraftment of NOD/SCID mice. *Blood* 2000; 95: 3289–96.
21 Mbalaviele G, Orcel P, Bouizar Z, Jullienne A, De Vernejoul MC. Transforming growth factor-beta enhances calcitonin-induced cyclic AMP production and the number of calcitonin receptors in long-term cultures of human umbilical cord blood monocytes in the presence of 1,25-dihydroxycholecalciferol. *J Cell Physiol* 1992; 152: 486–93.
22 Ricci G, Guglielmo MC, Caruso M, Ferranti F, Canipari R, *et al*. Hepatocyte growth factor is a mouse fetal Leydig cell terminal differentiation factor. *Biol Reprod* 2012; 87: 146.
23 Weir C, Morel-Kopp MC, Gill A, Tinworth K, Ladd L, *et al*. Mesenchymal stem cells: isolation, characterisation and *in vivo* fluorescent dye tracking. *Heart Lung Circ* 2008; 17: 395–403.
24 Wu SH, Huang SH, Lo YC, Chai CY, Lee SS, *et al*. Autologous adipose-derived stem cells attenuate muscular atrophy and protect spinal cord ventral horn motor neurons in an animal modelof burn injury. *Cytotherapy* 2015; 17: 1066–75.
25 Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringden O. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. *Exp Hematol* 2003; 31: 890–6.
26 Nasef A, Mathieu N, Chapel A, Frick J, Francois S, *et al*. Immunosuppressive effects of mesenchymal stem cells: involvement of HLA-G. *Transplantation* 2007; 84: 231–7.
27 Le Blanc K, Tammik L, Sundberg B, Haynesworth SE, Ringden O. Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex. *Scand J Immunol* 2003; 57: 11–20.
28 Zhang YN, Lie PC, Wei X. Differentiation of mesenchymal stromal cells derived from umbilical cord Wharton's jelly into hepatocyte-like cells. *Cytotherapy* 2009; 11: 548–58.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

©The Author(s)(2017)