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RESEARCH ARTICLE

Transplantation of placenta-derived mesenchymal stem cells in type 2 diabetes: a pilot study

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Abstract Mesenchymal stem cells (MSC) have been used in clinical trials for severe diabetes, a chronic disease with high morbidity and mortality. Bone marrow is the traditional source of human MSC, but human term placenta appears to be an alternative and more readily available source. Here, the therapeutic effect of human placenta-derived MSC (PD-MSC) was studied in type 2 diabetes patients with longer duration, islet cell dysfunction, high insulin doses as well as poor glycemic control in order to evaluate the safety, efficacy and feasibility of PD-MSC treatment in type 2 diabetes (T2D). Ten patients with T2D received three intravenous infusions of PDSC, with one month interval of infusion. The total number of PDSC for each patient was $(1.22–1.51) \times 10^6$/kg, with an average of 1.35×10^6/kg. All of the patients were followed up after therapy for at least 3 months. A daily mean dose of insulin used in 10 patients was decreased from 63.7 ± 18.7 to 34.7 ± 13.4 IU ($P < 0.01$), and the C-peptide level was increased from 4.1 ± 3.7 ng/mL to 5.6 ± 3.8 ng/mL ($P < 0.05$) respectively after therapy. In 4 of 10 responders their insulin doses reduced more than 50% after infusion. The mean levels of insulin and C-peptide at each time point in a total of 10 patients was higher after treatment ($P < 0.05$). No fever, chills, liver damage and other side effects were reported. The renal function and cardiac function were improved after infusion. The results obtained from this pilot clinical trial indicate that transplantation of PD-MSC represents a simple, safe and effective therapeutic approach for T2D patients with islet cell dysfunction. Further large-scale, randomized and well-controlled clinical studies will be required to substantiate these observations.

Keywords placenta stem cells; treatment of type 2 diabetes

1 Introduction

Diabetes has been a major public health problem in the world, especially in China. More than 92 million Chinese men and women have diabetes and 148 million have pre-diabetes, in which type 2 diabetes (T2D) accounts for more than 90% [1]. Diabetes can result in multi-system chronic complications, particularly micro- and macrovascular complications, with high morbidity and mortality [2–4]. The islet transplantation is an efficient therapy for T2D, especially for those who had a long duration, serious islet cell dysfunction, or poor blood glucose control even with large dose of insulin application. However, the application of this treatment is greatly restricted by the limited availability of primary human islets, the high cost and the immune rejection response [5,6].

Human mesenchymal stem cells (MSC) represent a relatively rare stem cell population that resides primarily in the bone marrow but can be isolated also from other adult and fetal tissues, including adipose tissue, umbilical cord blood and tissue, placenta, and fetal lung [7–13]. These cells can self-renew and differentiate to multilineage cells and can secrete several cytokines, growth factors, and extracellular matrix molecules that play important roles in the regulation of hematopoiesis, angiogenesis and immune and inflammatory responses [11,14–18]. The function properties of MSC make them unique and being used increasingly in clinical trials for a range of regenerative diseases including diabetes [19–26]. MSC have been

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shown to possess potential to differentiate into pancreatic islet cells and animal studies have shown that they have clinical effects on the prevention and treatment of diabetes [27–31], which presents a new hope for the treatment of diabetes. Recently, autologous bone marrow-derived MSC have been used to treat T2D [26] and adipose derived MSC modified with Pdx1 gene have been applied in the treatment of type 1 diabetes [27], and both had achieved encouraging efficacy.

In making new cell therapy-based strategies a clinical reality, it is fundamental to identify which type or source of stem cells is preferable for a particular therapeutic application, either in terms of ethical consideration or risks-to-benefits ratio. Although bone marrow remains the main source of MSC for clinical investigation, human term placenta has been considered as one of ideal sources due to their accessibility without ethical conflicts, painless procedures to donors, and lower risk of viral contamination [32–39]. In the present study, we performed a pilot phase I clinical trial using PD-MSC to treat a total of 10 patients with T2D in the aim of evaluating the safety and clinical feasibility of PD-MSC.

2 Materials and Methods

2.1 Study design

The pilot clinical study was approved by the Ethical Committee of Liaoyang Diabetic Hospital, Liaoning Province, China. All patients signed informed consents before treatment.

2.2 Patients

A total of 10 patients (7 males) who fulfilled the criteria were included. The inclusion criteria were patients with T2D diagnosed from November 2008 to November 2009 in Liaoyang Diabetes Hospital, between 30 and 85 years of age, duration of diabetes $\geqslant 3$ years, requiring insulin for optimal glycemic control in a dose of $\geqslant 0.7$ U/kg/day at least for 1 year, having insulin dysfunction, poorly controlled blood glucose fluctuation with insulin-based treatment, and willingness to participate in the study.

In this study, the 10 patients were between 45 and 82 years of age, with an average of 66 years of age; duration of diabetes from 3 years to 20 years, with an average of 11 years; daily insulin requirement from 38 units to 90 units, with an average of 63.7 units. And all of them were complicated with coronary artery insufficiency, multiple lacunar infarction, kidney disease, atherosclerosis and large limbs neuropathy.

2.3 Examination

The following examinations were carried out before and 3 months after cell transplantation. They include simultaneous glucose tolerance test, insulin release test, C-peptide stimulation test, the determination of glycosylated hemoglobin, islet cell antibodies, insulin antibodies, glutamic acid decarboxylase antibody. The cardiac, liver and kidney function tests were performed. The adverse events and side effects were observed during treatment. We considered it effective if daily insulin requirement reduced by $\geqslant 50\%$ after treatment and lasted more than 3 months according to the evaluation criteria formulated in the Fourth National Conference on the development of islet transplantation, 1995.

2.4 Human placenta derived stem cells (PD-MSC)

Placentas were obtained from healthy mothers, that is to say, the contributors had no genetic family history, no cancer history, no hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), Epstein-Barr virus (EBV), cytomegalovirus (CMV) and syphilis in serum. Placenta collection was approved by the Institutional Medical Research Ethics Committee of the local maternity hospitals. Fully informed consent was obtained several weeks prior to delivery from each mother. A blood sample was tested for specific human pathogens including HIV1/2, HBV, HCV and syphilis. Placentas were immersed in D-MEM/F-12 (1∶1) (Gibco) supplemented with gentamicin (100 μg/mL) and amphotericin-B (5μg/mL) (Sigma) and immediately transferred to the laboratory. The shipment temperature was 4–10°C.

The treatment of placenta tissue and isolation of PD-MSC were performed in the GMP laboratory of Beijing Health-biotech Co. Ltd using a method previously described [11,32], with some modifications. Briefly, the whole placenta was washed with phosphate buffered saline (PBS) twice and then dissected with scissors into pieces approximately 1–2 g in size. These tissue pieces were then treated with 0.075% collagenase (Sigma) at 37°C for 30 min, and further digested with 0.125% trypsin (Gibco) at 37°C for 30 min with gentle agitation. Fetal bovine serum (FBS) (Hyclone) was added to neutralize the excess trypsin. The digested mixture was passed through a 100 μm filter to obtain cell suspensions. The cells were plated at a density of 10 000 to 15 000 cells/cm^2 in a T75 cell culture flask (Corning) in D-MEM/F-12 medium supplemented with 10% FBS and 10ng/mL epidermal growth factor (Sigma). Cell cultures were maintained in a humidified atmosphere with 5% CO_2 at 37°C. After 3 days of culture, the medium was replaced to remove non-adherent cells, and changed twice weekly thereafter. Once confluence had been reached, the adherent cells (passage 0) were detached with 0.125% trypsin and passaged in the T-75 flask. The adherent MSC obtained from passage 2 were harvested for cell banking followed by a series of safety evaluation. The PD-MSC released from the cell banking were cultured and expanded in Good Manufacturing

Practice (GMP) laboratory for 5 passages to prepare final cell products which should be sterile and all qualified for the examinations including mycoplasma, HBV, HCV, HIV, EBV, CMV, syphilis, and endotoxin testing. For the sterility and mycoplasma assay, a 14-day sterility culture and 28-day mycoplasma culture were performed according to the Chinese Pharmacopoeia. To test the sterility, an additional Gram stain was performed to detect any gross contamination before release of the final product. Endotoxin levels were detected by the gel-clot limulus amebocyte lysate test method according to the Chinese Pharmacopoeia.

An aliquot of PD-MSC was stained with phycoerythrin (PE)-conjugated antibodies against Nestin, CD73, CD90, CD105, CD151, OCT4, Sox2 and HLA-DR, or fluorescein isothiocyanate (FITC)-conjugated antibodies against CD 31, CD34 and CD45. Mouse isotypic antibodies served as the control. Antibodies used were purchased from Becton Dickinson, except for CD105 (Serotech). Cells were stained with a single label and then analyzed by flow cytometry with a FACScan (Becton Dickinson).

The passage 5 products of PD-MSC prepared from two placentas were used in this pilot clinical study. These cells expressed highly Nestin, CD151, CD105, CD73, CD166, Oct4 and Sox2 but not CD34, CD31, CD45, CD184 and HLA-DR (Fig. 1). Chromosomal karyotype of PD-MSC

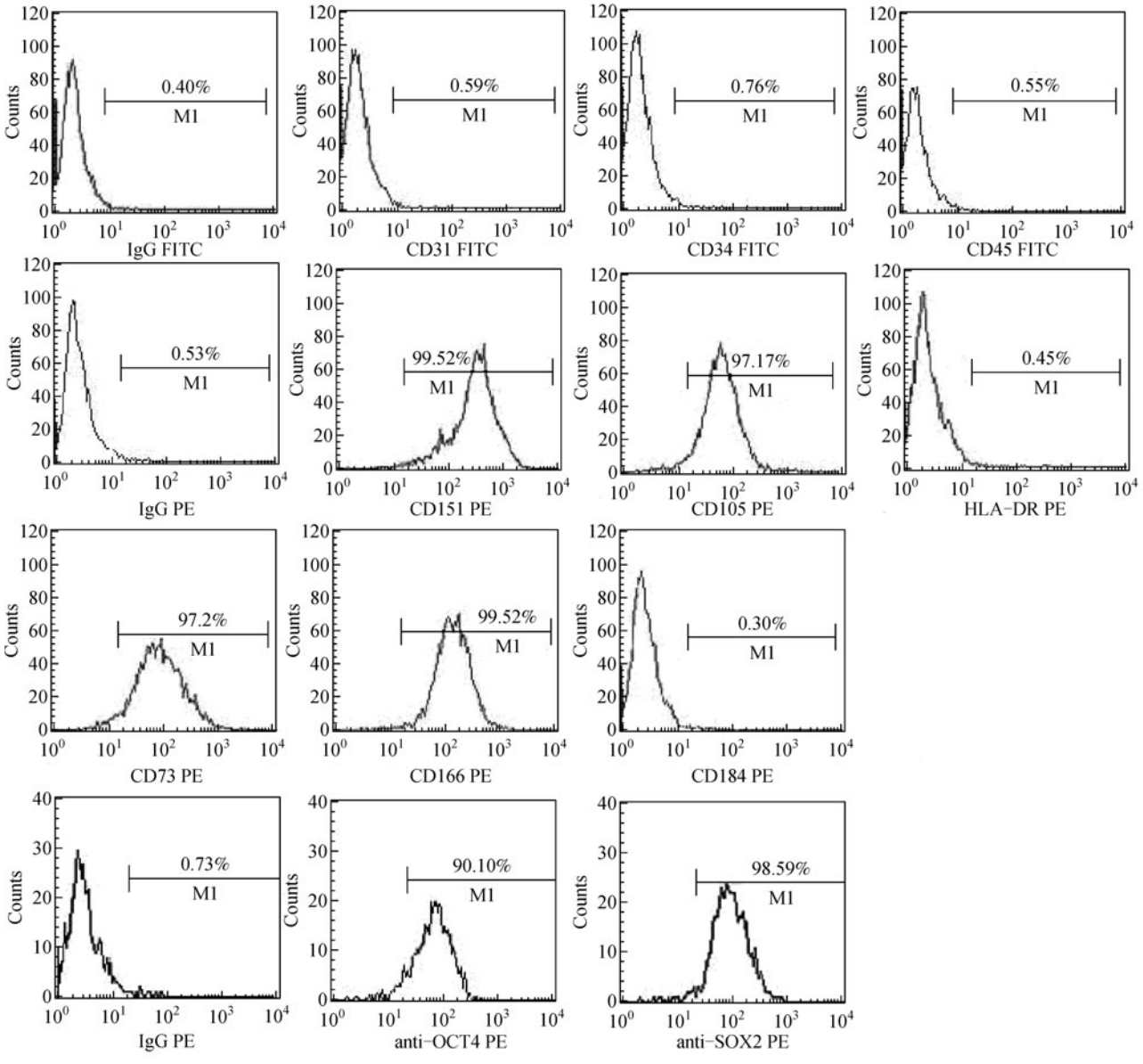

Fig. 1 Immunophenotype of placenta derived mesenchymal stem cells (PD-MSC). The PD-MSC were isolated from tissues of human placenta, cultured and expanded in GMP Laboratory for 5 passages, and analyzed by FACS.

was normal. The preparation of PD-MSC was sterile and the examinations of mycoplasma, HBV, HCV, HIV, EBV, CMV, syphilis, and endotoxin testing were all qualified.

2.5 Treatment

The patients received three intravenous infusions of PD-MSC, with one month interval of infusion. The total number of PD-MSC for each patient was at (1.22–1.51) × 10^6 / kg, with an average of 1.35 × 10^6/kg. At same time, patients continued to apply insulin, and adjusted the insulin dose according to blood glucose levels. For complications, the original general treatments were maintained. All of the patients were followed up after therapy for at least 3 months.

2.6 Statistical analysis

The statistical program for the Social Sciences (Release 13.0, PC Windows; SPSS Inc., Chicago, IL) was used for the data analysis. Data were expressed as mean ± SD. Paired *t*-test was used for tests of significance and Pearson correlation analysis was used to find correlation between independent variables. A P value < 0.05 was regarded statistically significant.

3 Results

A total of 10 patients with T2D (7 men) were included in this study. Table 1 shows a reduction in mean insulin requirement from 63.7 to 34.7 IU after 3 times of treatment with PD-MSC in this group of 10 patients, which was statistically significant ($P < 0.01$). Four patients had a reduction of insulin requirement by > 50%, including 3 cases of anti-islet cell antibodies and anti-glutamic acid decarboxylase antibody-positive patients.

Treatment of PD-MSC also significantly improved the level of glycosylated hemoglobin of patients with T2D. As can be seen in Table 2, all the 10 patients' mean values of glycosylated hemoglobin significantly decreased from 9.8% to 6.7% after treatment with PD-MSC ($P < 0.05$).

Correlation analysis between C peptide and insulin before and after treatment with PD-MSC was performed and a significant correlation was observed for C peptide and insulin, with $\gamma = 0.992$ ($P < 0.01$), and $\gamma = 0.931$ ($P < 0.01$) respectively.

The patients did not stop the application of insulin during the period of treatment with human PD-MSC. To exclude the impact of exogenous insulin, which could positively assess islet cell function, the values of insulin and C peptide were simultaneously measured. Tables 3 and 4 show the results of assessments. At each time point after

Table 1 Insulin dosage (IU) before and after treatment

Patient	1	2	3	4	5	6	7	8	9	10	mean±SD
Pre-treatment	48	60	38	76	76	90	66	58	87	38	63.7±18.7
6 months after treatment	20	36	18	54	46	50	36	32	39	16	34.7±13.4*

* $P < 0.01$

Table 2 Changes in glycosylated hemoglobin (%) before and after treatment

Patient	1	2	3	4	5	6	7	8	9	10	mean±SD
Pre-treatment	8.1	9.5	8.9	9.1	8.9	13.6	13.6	8.2	11.0	7.5	9.8±2.2
6 months after treatment	6.5	6.1	4.6	6.0	6.0	7.5	7.8	6.3	9.1	6.9	6.7±1.2*

* $P < 0.01$

Table 3 Levels of insulin (μIU/mL) before and after treatment (n = 10)

Time (min)	0	30	60	120	mean±SD
Pre-treatment	7.6±4.8	12.3±6.2	18.2±11.4	24.7±20.1	16.6±13.5
6 months after treatment	11.1±5.1*	17.7±7.4*	24.6±12.2*	31.0±19.8*	21.6±13.7*

The levels of insulin were measured before and after treatment. At each time point after treatment, insulin levels were higher those that before treatment, which was statistically significant. * $P < 0.01$

Table 4 Levels of C-peptide (ng/mL) before and after treatment (n = 10)

Time (min)	0	30	60	120	mean±SD
Pre-treatment	2.6±2.1	3.3±2.4	4.5±3.0	5.6±4.0	4.1±3.7
6 months after treatment	3.5±2.3*	5.3±3.3*	6.4±3.3*	8.2±3.9*	5.6±3.8*

The levels of C peptide were measured at different time points after treatment. At each time point after treatment, C peptide levels were higher than those before treatment, which was statistically significant. * $P < 0.05$

treatment, insulin and C peptide levels were higher than those before treatment, which was statistically significant ($P < 0.05$).

No obvious side effects including fever, chills, liver damage and immune rejection response were observed after cell transplantation. Moreover, renal and cardiac functions showed varying degrees of improvement after infusion of PD-MSC. The ten patients were all anti-insulin-antibody-negative before and after treatment. Three patients had positive anti-islet cell antibodies and anti-glutamic acid decarboxylase antibody before treatment. Two cases turned into suspected positive, while the other one had no change after cell treatment.

4 Discussion

This pilot study evaluates the safety and efficacy of allogeneic PD-MSC in patients with T2D. The group of 10 patients received this treatment had a long history of T2D. They were applied an average of 63.7 units of insulin before treatment. All these patients had pancreatic islet cell dysfunction at different levels, large fluctuations in blood glucose and complications with heart, brain and kidney damages. The transplantation of PD-MSC reduced the daily insulin requirement, controlled their blood glucose fluctuations and improved their quality of life. As can be observed during the study, the blood glucose began to decrease at the 7th day after transplantation of PD-MSC, consequently the insulin requirement reduced gradually. After three times of PD-MSC administration, the mean insulin dose had reduced from 63.7 to 34.7 IU which lasted three months. Thus, the transplantation PD-MSC could be considered as an effective therapeutic approach for type 2 diabetes. Four patients showed a reduction of daily insulin requirement by $\geqslant 50\%$, which reached the efficacy level as same as the islet cell transplantation, but far lower cost and without immune response.

Glycosylated hemoglobin (HbA1c) is a good indicator for the blood glucose levels for its characteristics of more objectiveness and less susceptibility to diet, exercises and other interference factors. Thus, it always acts as a complementary value to the point values of blood glucose. In this group of patients, HbA1c decreased from 9.8% to 6.7% on average, closing to the normal level. This further supports the concept that allogeneic transplantation of PD-MSC is effective in treating type 2 diabetes.

Insulin release test results showed that PD-MSC had a significant clinical effect on improving islet cell function and reducing blood glucose fluctuations for patients with T2D. To rule out the possibility of exogenous insulin interferences in the test and to reflect accurately the function of islet β cells, we evaluated the efficacy of PD-MSC mainly according to values of C-peptide. After transplantation of PD-MSC, C peptide level increased at every time point, and there were statistically significant differences between values of every two time points, indicating that β-cell function was improved and insulin secretion was therefore increased. To determinate the interferences of exogenous insulin in the insulin release test, we made simultaneous determinations of insulin and C-peptide before and after cell transplantation, and analyzed their correlation. There was a positive correlation between insulin and C-peptide. Therefore, it was confirmed that exogenous insulin had little effect on the test, yet for some uncertain reasons.

We were interesting to note that three patients with positive anti-islet cell antibodies and anti-glutamic acid decarboxylase antibodies also had a reduction in daily insulin dose by $\geqslant 50\%$ after transplantation of PD-MSC. This may suggest that PD-MSC play an immunoregulatory role. It has been reported that among T2D patients, some of them may have latent autoimmune diabetes in adult (LADA) [2,6,26,40], whose anti-islet cell antibodies and anti-glutamic acid decarboxylase antibodies are positive due to a possible immune response. However, so far, there is no uniform standard for diagnosing the LADA, so we do not exclude these three cases.The mechanism of β-cell function's improvement after transplantation of PD-MSC remains unclear at present. First, previously reported studies indicate that PD-MSC can differentiate into islet β cells and then secret insulin. Some experiments have confirmed that several different sources of MSC could differentiate into β cells [28–31]. In addition, transplanted PD-MSC in the pancreas can differentiate into vascular endothelial cells and improve blood supply of pancreas tissue to restore β cell function [16,41,42]. Finally, PD-MSC can secrete a number of cytokines, which may directly or indirectly improve islet function and related complications [43]. Phinney and Prockop believed that a variety of soluble cytokines secreted by MSC did much more to tissue repair than cell differentiation [42]. In our another study (not published yet), we observed that PD-MSC can secrete more insulin-like growth factor (IFG), vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) than bone marrow MSC, and these cytokines probably play a role in pancreatic tissue repair. To our knowledge, this is the first clinical trial in the world to treat T2D by transplanting allogeneic PD-MSC. The results obtained from the initial ten patients with T2D suggest that transplantation of PD-MSC represents a simple, safe, efficient therapeutic approach for T2D. This treatment seems even able to improve the damaged renal and cardiac functions in most of the patients. Actually, a randomized and placebo well controlled phase II clinical trial has been initiated in multiple centers in order to substantiate the role of transplantation of allogeneic PD-MSC in the management of T2D.

References

1. Yang W, Lu J, Weng J P, Jia W, Ji L, Xiao J, Shan Z, Liu J, Tian H, Ji Q, Zhu D, Ge J, Lin L, Chen L, Guo X, Zhao Z, Li Q, Zhou Z, Shan G, He J. Prevalence of diabetes among men and women in China. N Engl J Med, 2010, 362(12): 1090–1101
2. Hu D, Sun L, Fu P, Xie J, Lu J, Zhou J, Yu D, Whelton P K, He J, Gu D. Prevalence and risk factors for type 2 diabetes mellitus in the Chinese adult population: the InterASIA Study. Diabetes Res Clin Pract, 2009, 84(3): 288–295
3. Kobayashi N, Yuasa T, Okitsu T. Regenerative medicine for diabetes mellitus. Cell Transplant, 2009, 18(5): 491–496
4. Li G, Zhang P, Wang J, Gregg EW, Yang W, Gong Q, Li H, Li H, Jiang Y, An Y, Shuai Y, Zhang B, Zhang J, Thompson TJ, Gerzoff RB, Roglic G, Hu Y, Bennett PH. The long-term effect of lifestyle interventions to prevent diabetes in the China Da Qing Diabetes Prevention Study: a 20-year follow-up study. Lancet, 2008; 24:371 (9626):1783–1789
5. Ciceri F, Piemonti L.Bone marrow and pancreatic islet: an old story with new perspectives. Cell Transplant, 2010 Aug 17
6. Kodama S, Kühtreiber W, Fujimura S, Dale E A, Faustman D L. Islet regeneration during the reversal of autoimmune diabetes in NOD mice. Science, 2003, 302(5648): 1223–1227
7. Pittenger M F, Mackay A M, Beck S C, Jaiswal R K, Douglas R, Mosca J D, Moorman M A, Simonetti D W, Craig S, Marshak D R. Multilineage potential of adult human mesenchymal stem cells. Science, 1999, 284(5411): 143–147
8. Deans R J, Moseley A B. Mesenchymal stem cells: biology and potential clinical uses. Exp Hematol, 2000, 28(8): 875–884
9. Gronthos S, Franklin D M, Leddy H A, Robey P G, Storms R W, Gimble J M. Surface protein characterization of human adipose tissue-derived stromal cells. J Cell Physiol, 2001, 189(1): 54–63
10. Erices A, Conget P, Minguell J J. Mesenchymal progenitor cells in human umbilical cord blood. Br J Haematol, 2000, 109(1): 235–242
11. Lu L L, Liu Y J, Yang S G, Zhao Q J, Wang X, Gong W, Han Z B, Xu Z S, Lu Y X, Liu D, Chen Z Z, Han Z C. Isolation and characterization of human umbilical cord mesenchymal stem cells with hematopoiesis-supportive function and other potentials. Haematologica, 2006, 91(8): 1017–1026
12. In 't Anker P S, Scherjon S A, Kleijburg-van der Keur C, de Groot-Swings G M, Claas F H, Fibbe W E, Kanhai H H. Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. Stem Cells, 2004, 22(7): 1338–1345
13. Zheng C L, Yang S G, Guo Z X, Liao W, Zhang L, Yang R, Han Z C. Human multipotent mesenchymal stromal cells from fetal lung expressing pluripotent markers and differentiating into cell types of three germ layers. Cell Transplant, 2009, 18(10): 1093–1109
14. Chen K, Wang D, Du W T, Han Z B, Ren H, Chi Y, Yang S G, Zhu D, Bayard F, Han ZC. Human umbilical cord mesenchymal stem cells hUC-MSCs exert immunosuppressive activities through a PGE_2-dependent mechanism. Clin Immunol, 2010, 135(3): 448–458
15. Wang D, Chen K, Du W T, Han Z B, Ren H, Chi Y, Yang S G, Bayard F, Zhu D, Han Z C. CD14+ monocytes promote the immunosuppressive effect of human umbilical cord matrix stem cells. Exp Cell Res, 2010, 316(15): 2414–2423
16. Liao W B, Xie J, Zhong J, Liu Y J, Du L, Zhou B, Xu J, Liu P X, Yang S G, Wang J M, Han Z B, Han Z C. Therapeutic effect of human umbilical cord multipotent mesenchymal stromal cells in a rat model of stroke. Transplantation, 2009, 87(3): 350–359
17. Zhao Q J, Ren H Y, Li X Y, Chen Z, Zhang X Y, Gong W, Liu Y J, Pang T X, Han Z C. Differentiation of Human umbilical cord mesenchymal stem cells into low immunogenic hepatocyte-like cells. Cytotherapy, 2009, 11(4): 414–426
18. Wu K H, Mo X M, Zhou B, Lu S H, Yang S G, Liu Y L, Han Z C. Cardiac potential of stem cells from whole human umbilical cord tissue. J Cell Biochem, 2009, 107(5): 926–932
19. Garcia-Olmo D, Herreros D, Pascual I, Pascual J A, Del-Valle E, Zorrilla J, De-La-Quintana P, Garcia-Arranz M, Pascual M. Expanded adipose-derived stem cells for the treatment of complex perianal fistula: a phase II clinical trial. Dis Colon Rectum, 2009, 52 (1): 79–86
20. Hare J M, Traverse J H, Henry T D, Dib N, Strumpf R K, Schulman S P, Gerstenblith G, DeMaria A N, Denktas A E, Gammon R S, Hermiller J B Jr, Reisman M A, Schaer G L, Sherman W. A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. J Am Coll Cardiol, 2009, 54(24): 2277–2286
21. Horwitz E M, Prockop D J, Fitzpatrick L A, Koo W W, Gordon P L, Neel M, Sussman M, Orchard P, Marx J C, Pyeritz R E, Brenner M K. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. Nat Med, 1999, 5(3): 309–313
22. Kebriaei P, Isola L, Bahceci E, Holland K, Rowley S, McGuirk J, Devetten M, Jansen J, Herzig R, Schuster M, Monroy R, Uberti J. Adult human mesenchymal stem cells added to corticosteroid therapy for the treatment of acute graft-versus-host disease. Biol Blood Marrow Transplant, 2009, 15(7): 804–811
23. Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, Lanino E, Sundberg B, Bernardo M E, Remberger M, Dini G, Egeler R M, Bacigalupo A, Fibbe W, Ringdén O. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. Lancet, 2008, 371(9624): 1579–1586
24. Le Blanc K, Rasmusson I, Sundberg B, Götherström C, Hassan M, Uzunel M, Ringdén O. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet, 2004, 363(9419): 1439–1441
25. Lee P H, Kim J W, Bang O Y, Ahn Y H, Joo I S, Huh K. Autologous mesenchymal stem cell therapy delays the progression of neurological deficits in patients with multiple system atrophy. Clin Pharmacol Ther, 2008, 83(5): 723–730
26. Bhansali A, Upreti V, Khandelwal N, Marwaha N, Gupta V, Sachdeva N, Sharma R R, Saluja K, Dutta P, Walia R, Minz R, Bhadada S, Das S, Ramakrishnan S. Efficacy of autologous bone marrow-derived stem cell transplantation in patients with type 2 diabetes mellitus. Stem Cells Dev, 2009, 18(10): 1407–1416
27. Lin G, Wang G, Liu G, Yang L J, Chang L J, Lue T F, Lin C S. Treatment of type 1 diabetes with adipose tissue-derived stem cells expressing pancreatic duodenal homeobox 1. Stem Cells Dev, 2009, 18(10): 1399–1406
28. Liu M, Han Z C. Mesenchymal stem cells: biology and clinical

potential in type 1 diabetes therapy. J Cell Mol Med, 2008, 12(4): 1155–1168

29. Santana A, Enseñat-Waser R, Arribas M I, Reig J A, Roche E. Insulin-producing cells derived from stem cells: recent progress and future directions. J Cell Mol Med, 2006, 10(4): 866–883
30. Wang H S, Shyu J F, Shen W S, Hsu H C, Chi T C, Chen C P, Huang S W, Shyr Y M, Tang K T, Chen T H. Transplantation of insulin producing cells derived from umbilical cord stromal mesenchymal stem cells to treat NOD mice. Cell Transplant, 2010 Aug 18
31. Zhang Y, Dou Z. Transdifferentiation of bone marrow mesenchymal stem cell into islet cells to treat diabetes mellitus. J Cell Biol, 2007, 29: 1–5
32. Brooke G, Rossetti T, Pelekanos R, Ilic N, Murray P, Hancock S, Antonenas V, Huang G, Gottlieb D, Bradstock K, Atkinson K. Manufacturing of human placenta-derived mesenchymal stem cells for clinical trials. Br J Haematol, 2009, 144(4): 571–579
33. Tran T C, Kimura K, Nagano M, Yamashita T, Ohneda K, Sugimori H, Sato F, Sakakibara Y, Hamada H, Yoshikawa H, Hoang S N, Ohneda O. Identification of human placenta-derived mesenchymal stem cells involved in re-endothelialization. J Cell Physiol, 2011, 226(1): 224–235
34. Fukuchi Y, Nakajima H, Sugiyama D, Hirose I, Kitamura T, Tsuji K. Human placenta-derived cells have mesenchymal stem/progenitor cell potential. Stem Cells, 2004, 22(5): 649–658
35. Hwang J H, Shim S S, Seok O S, Lee H Y, Woo S K, Kim B H, Song H R, Lee J K, Park Y K. Comparison of cytokine expression in mesenchymal stem cells from human placenta, cord blood, and bone marrow. J Korean Med Sci, 2009, 24(4): 547–554
36. Parolini O, Alviano F, Bagnara G P, Bilic G, Bühring H J, Evangelista M, Hennerbichler S, Liu B, Magatti M, Mao N, Miki T, Marongiu F, Nakajima H, Nikaido T, Portmann-Lanz C B, Sankar V, Soncini M, Stadler G, Surbek D, Takahashi T A, Redl H, Sakuragawa N, Wolbank S, Zeisberger S, Zisch A, Strom S C. Concise review: isolation and characterization of cells from human term placenta: outcome of the first international Workshop on Placenta Derived Stem Cells. Stem Cells, 2008, 26(2): 300–311
37. Dzierzak E, Robin C. Placenta as a source of hematopoietic stem cells. Trends Mol Med, 2010, 16(8): 361–367
38. Lee L K, Ueno M, Van Handel B, Mikkola H K. Placenta as a newly identified source of hematopoietic stem cells. Curr Opin Hematol, 2010, 17(4): 313–318
39. Evangelista M, Soncini M, Parolini O. Placenta-derived stem cells: new hope for cell therapy? Cytotechnology, 2008, 58(1): 33–42
40. Zhou J, Ma X J, Bao Y Q, Pan X P, Lu W, Hu C, Xiang K S, Jia W P. Study on prevalence of latent autoimmune diabetes in adults and its relationship with metabolic syndrome. Zhonghua Yi Xue Za Zhi, 2009, 89(18): 1250–1254
41. Salem H K, Thiemermann C. Mesenchymal stromal cells: current understanding and clinical status. Stem Cells, 2010, 28(3):585–596
42. Phinny D G, Prockop D J. Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair–current views. Stem Cells, 2007, 25(11):2896–2902
43. Horwitz E M, Prather W R. Cytokines as the major mechanism of mesenchymal stem cell clinical activity: expanding the spectrum of cell therapy. Isr Med Assoc J, 2009, 11(4): 209–211