Construction of a recombinant eukaryotic expression vector containing a leptin gene and its expression in HPMSCs

Julou Jin · Bowei Wang · Zhenwei Zhu ·
Yifu Chen · Zhi Mao · Jinglong Wang ·
Bing Du · Shuping Wang · Zhihui Liu

Received: 20 February 2013 / Accepted: 3 June 2013 / Published online: 27 June 2013
© Springer Science+Business Media Dordrecht 2013

Abstract Leptin gene fragments were amplified from human adipose tissue using reverse transcription polymerase chain reaction technology. The leptin gene was reconstructed in pIRES2-EGFP and transfected into human placenta-derived mesenchymal stem cells (HPMSCs) using a liposome-mediated method. Leptin mRNA and protein was detected in the transfected cells using RT-PCR and Western blot analysis, and the results showed that HPMSCs transfected with pIRES2-EGFP-leptin expressed significantly more leptin mRNA and protein than HPMSCs transfected with pIRES2-EGFP. EGFP expression was observed under a fluorescence microscope, and results showed the report gene to have been successfully transferred into the target cells. The biological activity of leptin and the cell proliferation activity of HPMSCs transfected with pIRES2-EGFP-leptin was detected using an MTT assay, which showed that leptin can promote

the proliferation of HPMSCs. However, leptin in HPMSCs transfected with pIRES2-EGFP-leptin showed significantly more activity than HPMSCs transfected with pIRES2-EGFP. Identification of multipotency showed that HPMSCs transfected with pIRES2-EGFP-leptin maintained their multipotency.

Keywords Transfection · Human placenta-derived mesenchymal stem cells · Leptin

Introduction

Leptin is an adipose cell factor secreted by adipose tissue. Studies have shown that leptin plays a very important role in wound healing by acting on wound healing cells and wound healing factors. Leptin plays an especially important role in wound healing during the inflammatory reaction stage (Fantuzzi and Faggioni 2000), angiogenesis (Liapakis et al. 2008; Ring et al. 2003), hyperplasia of granulation tissue, the contraction of wounds, epithelial regeneration, and the proliferation of T-lymphocytes. Leptin may be a suitable target for new drugs meant to improve and regulate wound healing and trauma metabolism, but its half-life is very short, only 9.4 ± 3.0 min. This makes it difficult for leptin to play a long-term role in improving wound healing when applied locally. For this reason, determining whether leptin can be transfected into stem cells and then expressed continuously may be valuable. In recent years, researchers have paid

The authors Julou Jin and Bowei Wang equally contributed to this paper.

J. Jin · Z. Zhu · Y. Chen · Z. Mao · J. Wang ·
B. Du · S. Wang · Z. Liu (✉)
Department of Prosthodontics, Stomatological Hospital,
Jilin University, 1500 Qinghua Road, Changchun 130021,
China
e-mail: liuzhihui1975@sina.com

B. Wang
Department of Obstetrics and Gynecology, The Second
Hospital, Jilin University, 218 ZiQiang Street,
Changchun 130021, China

attention to the value of stem cells when applied in the field of wound healing. Studies have confirmed that bone marrow stromal stem cells (BMSCs) and adipose-derived stem cells (ADSCs) play some role in the process of wound healing and that they do so by changing the microenvironment of the wound, homing to the wound, and directionally differentiating into wound repair cells. HPMSCs have become a new star in the field of stem cell research because of the advantages of their plentiful supply, hematopoietic support, immune suppression, considerable multidirectional differentiation, reliability, safety, and specifically because of the fact that they are not teratogenic carcinoma. For these reasons, HPMSCs have applications in the medical industry. The purpose of this study was to transfect leptin into HPMSCs, because if the gene is transfected correctly and expressed at a high level and if HPMSCs transfected with leptin retain their multipotency, then it may be possible to promote wound healing using HPMSCs transfected with leptin.

Materials and methods

Materials

Adipose tissue and the written informed consent for its use were obtained from patients in the stomatology operating room of the Jilin University Hospital. An RNAiso Plus, High Fidelity Prime ScriptTM RT-PCR Kit, TaKaRa Agarose Gel DNA Purification Kit Ver. 2.0, DL10,000 DNA Marker, DNA A-Tailing Kit, pMD19-T Simple Vector, and DNA Ligation Kit Ver. 2.0 were purchased from TAKARA (Otsu, Shiga, Japan). *Escherichia coli* DH5 α strains were purchased from Beijing Cowin Bioscience Co., Ltd (Beijing, China); pMD18-T-simple was obtained from TAKARA (Japan); liposome LipofectamineTM 2000 and carrier pIRES2-EGFP were purchased from Invitrogen Co. (Shanghai, China); the restriction enzymes EcoR I, NheI, BamH I, and T4 DNA ligase were obtained from New England BioLabs, Inc. (Ipswich, MA, USA). PCR mix was acquired from Beijing Cowin Bioscience Co., Ltd. (China); human adipose tissue was obtained from SUN Bin of the Jilin University School of Stomatology. Human placental mesenchymal stem cells (HPMSCs) were cultured and identified in the cell culture room of the Jilin

University School of Stomatology. G418 selective medium were obtained from Baotaike Biotechnology Co. (Changchun, China). Agarose was purchased from Changchun Cell Clone Biological Science and Technology Co., Ltd. (Changchun, China); 1 Kb DNA Marker, DNA Marker DL2000, and DNA Marker III were obtained from Beijing Cowin Bioscience Co., Ltd. (China). Ethidium bromide (EB), bromophenol blue, and all antibiotics used in the present work were purchased from Sigma (St. Louis, MO, USA). Peptone and yeast extract powder were obtained from OXOID (Cambridge, U.K.). An Agarose Gel DNA Extraction Kit and EndoFree Plasmid Midi Kit were obtained from Beijing Cowin Bioscience Co., Ltd. (China). Western blot reagents and ECL were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). L-DMEM, H-DMEM, Collagenase II, trypsin, and FBS were obtained from Gibco (Life Technologies, Carlsbad, CA, USA).

Methods

Total RNA extraction and leptin cDNA synthesis

Total RNA from adipose tissue was extracted with RNAiso Plus according to the manufacturer's instructions. First, 1 μ g of total RNA was used to synthesize full-length leptin using a High Fidelity Prime ScriptTM RT-PCR Kit. Two primers were designed based on the sequence of the leptin gene, the multiple cloning sites on vector pIRES2-EGFP, and pMD18-T-simple, which contains NheI and BamHI restriction enzyme cleavage sites: forward: 5'-GCGGCTAGCATGCATTGGGGAACCCTGTGCG3' and reverse: 5'-GCGGGATCCTCAGCACCCAGGGCTGAGGTCC3'.

Purification of leptin cDNA and ligation with pMD18-T simple vector

The RT-PCR products were separated with 1.5 % agarose gel electrophoresis, and the target fragments were retrieved and purified using a TaKaRa Agarose Gel DNA Purification Kit v.2.0. The target fragments were polyadenylated using a DNA A-Tailing Kit. These fragments were then ligated into pMD18-T Simple Vector with a DNA Ligation Kit v.2.0 (TA Cloning Kit) (Invitrogen, Carlsbad, CA, USA). The recombinant pMD18-T-leptin was transformed into *E. coli* DH5 α competent cells for amplification.

Recombinant vectors were isolated from transformants using TaKaRa MiniBEST Plasmid Purification Kit v. 2.0, and the pMD18-T-leptin was sequenced by Tiangen Biotech Co., Ltd. (Beijing, China).

Construction of recombinant pIRES2-EGFP-leptin eukaryotic expression vector

The pMD18-T-leptin plasmids were digested by *NheI* and *BamHI* restriction enzymes, and the target fragments, full-length leptin cDNAs, were isolated and purified. The pIRES2-EGFP eukaryotic expression vectors were also digested by *NheI* and *BamHI* and then ligated into leptin cDNA with a DNA Ligation Kit v. 2.0. The recombinant pIRES2-EGFP-leptin was amplified in *E. coli* DH5 α -competent cells and isolated with a TaKaRa MiniBEST Plasmid Purification Kit v. 2.0.

Expression of the recombinant pIRES2-EGFP-leptin eukaryotic expression vector in HPMSCs

Cell transfection

Human placenta-derived mesenchymal stem cells were cultured in DMEM containing 10 % neonatal bovine serum at 37 °C in a humidified atmosphere of 5 % CO₂. Cells were passaged and plated (12-well plates for mRNA assay, 6-well plates for Western blot, and 96-well plates for growth curve assay) for 24 h before transfection at 80–90 % confluence. Cells were divided into three groups: HPMSCs (control), LipofectamineTM 2000 + pIRES2-EGFP (pIRES2), and LipofectamineTM 2000 + pIRES2-EGFP-leptin (leptin). Transfection was carried out according to the instructions included with the LipofectamineTM 2000. 72 h after transfection, transfection of the target gene was observed under a confocal fluorescence microscope using the reporter gene EGFP. Then the cells were cultured in G418 selection medium to screen for resistant cells. After 14 days, the positive cell clones were screened. Then the positive cells were cultivated for an additional 14 days and were collected and used for subsequent assays.

Detection of leptin mRNA by RT-PCR

Total RNA was isolated from the HPMSCs of the pIRES2 group and the leptin group, digested with

DNA enzyme, reverse-transcribed to cDNA using standard methods, and amplified by polymerase chain reaction (PCR) with the appropriate primers. Then the RT-PCR products were separated using agarose (1.5 %) gel electrophoresis. The gel was stained with 0.5 μ g/ml ethidium bromide for 30 min, then observed under 254 nm UV lamp. The DNA bands were evidenced by orange red fluorescence.

Western blot assay

After transfection, cells were collected and lysed, and the protein concentration was detected using a BCA protein assay kit. Supernatants were loaded on a 12 % SDS-PAGE gel, and they were then electrically transferred onto PVDF membranes, blocked for 2 h, and washed three times in PBS for 10 min each. The membrane was incubated with primary antibodies (rabbit anti-human leptin antibody) for 10 min at room temperature and washed three times in PBS for 10 min each. The sample was then incubated with HRP-conjugated secondary antibody for 10 min at room temperature and, washed three times in PBS for 10 min each. The bands were visualized with ECL Plus and exposed to X-ray film.

Assessment of the proliferative activity of HPMSCs transfected with leptin

Cells that grew well were selected and seeded on 96-well plates. The appropriate cell suspension (200 μ l) for each group plus 20 μ l MTT solution (5 mg/ml) was added to each well. After culturing for 3–6 h, the cell mixture was added to the 150 μ l DMSO. Optical density was measured at 490 nm. Statistical data are presented as mean $\pm$ standard deviation ($\times \pm S$). The t test was used for inter-group comparison.

Assessment of the biological activity of HPMSCs transfected with leptin

Human endothelial cells from ECV304 (obtained from ATCC, Manassas, VA, USA) were cultured in DMEM containing 10 % fetal bovine serum, at 37 °C, in a humidified atmosphere of 5 % CO₂, and seeded on 96-well plates for 24 h. Serum-free medium was changed and the cells were cultured for an additional 12 h, then replaced with 10 % FBS containing

medium, and different dilutions of transfected MSC culture medium (20 μ l) were added to each well. Then the cells were continuously cultured for 48 h, centrifuged, and the supernatant was discarded. When cells grew to 80–90 % confluence, 20 μ l MTT solution was added to each well. The cells were continuously cultured for 4 h, then killed. The supernatant was carefully removed by aspiration, and 100 μ l DMSO solution was added. The mixture was shaken until the blue sediment had fully dissolved. D_{480} values were measured, and statistical data are presented as mean $\pm$ standard deviation ($\times \pm S$). The *t* test was used for inter-group comparison.

Assessment of multipotency of HPMSCs transfected with pIRES2-EGFP-leptin

Lipoblast induction A DMEM-HG induction system containing 10 % FBS, dexamethasone (1 μ M), anti-inflammatory pain agent (200 μ M), IBMX (0.5 mM), and insulin (10 μ g/ml) was used. Oil red staining was also performed. The cytoplasmic inclusions of neutral lipids were then stained with oil red O (0.5 %, ProChem, Inc., Rockford, IL, USA). Cells were fixed with 4 % paraformaldehyde (PFA) solution and incubated in 60 % isopropanol for 5 min at room temperature and then aspirated. Cells were stained with oil red O solution for 30–60 min.

Osteogenic induction The system used here has a DMEM-HG induction feature containing 10 % FBS, dexamethasone (1 μ M), β -glycerophosphate (10 μ M),

vitamin C (50 mg/l). Alizarin red staining was also performed. Cell cultures were washed twice in PBS and were fixed in 70 % ethanol for 30 minutes. The cultures were then stained with alizarin red (0.1 %, ProChem, Inc.) for 30 minutes and were evaluated by light microscopy. Calcium deposits were confirmed by the formation of red nodules.

Results

Evaluation of RT-PCR products and recombinant pIRES2-EGFP-leptin eukaryotic expression vector

The RT-PCR products were loaded on 1.5 % agarose gels, and the band of full-length leptin cDNA was located at 500 bp (Fig. 1a). After the leptin cDNA fragment was inserted into the pIRES2-EGFP plasmid (5,428 bp), the fragment was confirmed by *Nhe*I and *Bam*HI digestion and electrophoresis (Fig. 1b). The cDNA sequence was confirmed by DNA sequencing, as shown in Fig. 2.

mRNA and protein expression of leptin in HPMSCs

After transfection, the expression of leptin was analyzed by RT-PCR and Western blot. Results showed that the HPMSCs in the leptin group expressed leptin, whereas the HPMSCs of the pIRES2 group did not (Fig. 3a, b).

Fig. 1 Identification of Leptin. **a** Electrophoresis of full-length target gene RT-PCR product. M: DNA Marker DL10,000. 1: Leptin. **b** *Nhe*I and *Bam*HI digestion and electrophoresis of pIRES2-EGFP-leptin eukaryotic expression vector. M: DNA Marker DL10,000. 1: pIRES2-EGFP-leptin plasmid digested by *Nhe*I and *Bam*HI

MTT array results showed the biological activity of leptin expressed by HPMSCs in the leptin group to be more prominent than in the pIRES2 and control groups. The biological activity of the pIRES2 and control groups were similar (Table 2 and Fig. 5).

Table 1 Assessment of the proliferation activity of HPMSCs by MTT array

Group	Optical density value (OD value)
Control	0.54 ± 0.0124
LEPTIN	0.87 ± 0.0285
PIRES2	0.53 ± 0.0876

Separately, *t* test was performed on the leptin and pIRES2 groups ($p < 0.01$); *t* test of the leptin and control groups was performed ($p < 0.01$); *t* test of the pIRES2 and control group was also performed ($p > 0.05$)

Fluorescence microscopy and flow cytometry analysis of transfection and transfection efficiency

A large number of human placenta-derived mesenchymal stem cells expressing green fluorescence were observed in the leptin and pIRES2 groups after 48 h of transfection. Expression peaked between 48 and 72 h. Expression decreased after 1 week but remained visible until the third week (Fig. 6). Transfection efficiency was 26.8 % 72 h after transfection, as detected by flow cytometry analysis.

Assessment of the multipotency of HPMSCs transfected with pIRES2-EGFP-leptin

Under adipose cell induction conditions, fat generated in cells was stained red (Fig. 7b). Bone nodules were observed under osteogenic induction conditions using an inverted microscope, and violet complexes appeared in the cells after alizarin red staining (Fig. 7a).

Fig. 4 Assessment of the proliferation activity of HPMSCs by MTT array **Table 2** Assessment of the biology activity of HPMSCs transfected with leptin

Group	Optical density value (OD value)
Control	0.46 ± 0.0135
LEPTIN	0.97 ± 0.0142
PIRES2	0.44 ± 0.0126

Separately, *t* test was performed on the leptin and pIRES2 groups ($p < 0.01$); *t* test of the leptin and control groups was performed ($p < 0.01$); *t* test of the pIRES2 and control group was also performed ($p > 0.05$)

Discussion

Leptin is a cellular factor involved in wound healing. It plays an important role in the wound healing process. There is a significant correlation between the concentration of leptin and those of IL-1 β (Kino 2003), IL-6 (Hobson et al. 2004; Brown et al. 2008; Maruna et al. 2001; Wallace et al. 2000), and TNF- α (Schoof et al. 2003; Cho et al. 2003; Kaska and Zivny 2002). Leptin may participate in the systemic inflammatory response to severe burns and surgical injury, like these proinflammatory cytokines. Leptin can induce the expression of angiotensin-2 in adipose tissue (Cohen et al. 2001). New studies have found that leptin can increase the activity of the mitochondria and increase the rate of biotransformation, which may promote wound healing. In this way, leptin may be suitable for use in treatments meant to improve and promote skin regeneration, and it may stop or even reverse some parts of aging of the skin (Poeggeler et al. 2010). In recent years, there have been many studies on the role of leptin in wound healing. These have involved injecting

Fig. 5 HPMSCs culture supernatant and hECV304 proliferation

Fig. 6 Green fluorescence in HPMSCs transfected with pIRES2-EGFP-leptin. **a** Phase contrast image. **b** Fluorescence image

Fig. 7 HPMSCs transfected with pIRES2-EGFP-leptin were induced into **a** osteoblasts (alizarin red staining $\times 100$) and **b** grease (oil red staining, $\times 100$) in vitro. The arrow in **a** shows calcified nodule and in **b** shows lipid droplet

leptin into the site of a spinal cord injury and local injection near burns (Wen et al. 2012). However, leptin degrades rapidly in the body and has a short effective period, making single injections less than ideal. In order to establish a sustained leptin expression system, some researchers transferred the human

leptin gene into African green monkey kidney fibroblasts (COS-7), which were found to express the leptin gene successfully. However, GM COS-7 allografts have a high immune rejection rate. HPMSCs are more practical for clinical use. They are multipotent mesenchymal stem cells derived from placental

parenchyma (Koo et al. 2012). They express the specific surface markers of MSCs (Pasquinelli et al. 2007). They can also differentiate into various types of tissues and cells, such as adipocytes, endothelial cells, osteocytes, chondrocytes, and muscle and nerve cells under appropriate conditions (Soncini et al. 2007; Portmann-Lanz et al. 2006; Alviano et al. 2007; Ventura et al. 2007; In't Anker et al. 2004). HPMSCs inhibit the proliferation of T cells. PDL-1 (Programmed Cell Death Ligand -1) is a B7-related protein that inhibits cell-mediated immune responses by reducing the secretion of IL-2 and IL-10 from memory T cells. This suggests that PDL-1 may be useful in reducing allogenic CD4⁺ memory T-cell responses to endothelial cells, thereby reducing the likelihood of host immune responses to allografts. It can inhibit T cell activation during the immune response, and it plays an important negative role in the regulation of T-cells during the immune response (Eppihimer et al. 2002; Sica et al. 2003). Studies have shown HPMSCs to have low immunogenicity, considerable multipotency, and a pronounced ability to proliferate. They are suitable for convenient, non-invasive procedures. This has made them an important source of cells for regenerative medicine and clinical applications. For these reasons, the present experiment were designed to transfer exogenous leptin gene into HPMSCs, and establish a sustained and stable gene expression system. This system may be suitable for the healing of wounds and for use under the skin flap during radiotherapy, but further studies are required.

The transfection of adenoviruses and LipofectamineTM 2000 are the most widely used gene transfer techniques. LipofectamineTM 2000 transfection has some advantages over adenoviral transduction. These include a wide range of possible host cells, few side effects, repeatability, and the relative ease of the transfection process. In addition, Lipofectamine does not activate any known cancer genes. However, its transfection efficiency is lower than that of adenoviral transfection. For security reasons, we adopted the method of LipofectamineTM 2000 transfection method. pIRES2-EGFP eukaryotic expression vector has enhanced green fluorescent protein (EGFP) gene and neomycin and kanamycin resistance genes (Liang et al. 2011). These two characteristics facilitate the observation and screening of positive cells under a microscope. The amplified target gene leptin was acquired from human adipose tissue by RT-PCR.

pIRES2-EGFP-leptin was constructed and identified. RT-PCR and Western blot analysis confirmed that HPMSCs transfected with pIRES2-EGFP-leptin expressed more leptin than HPMSCs transfected with pIRES2-EGFP, which indicated that leptin had been successfully transferred to human placenta-derived mesenchymal stem cells. It also indicated that the HPMSCs were not experiencing any of the endocrine effects of leptin. Cells were observed using a phase contrast fluorescence microscope, and green fluorescence was observed in the leptin and pIRES2 groups, which suggested that the reported genes had been successfully transfected into target cells. This proved that it was feasible to transfect HPMSCs with pIRES2-EGFP-leptin using LipofectamineTM 2000 transfection.

The effects of human placenta-derived mesenchymal stem cell culture supernatant on hECV304 proliferation activity were detected using an MTT assay, and the results suggested that leptin was expressed continuously and effectively after transfection into human placenta-derived mesenchymal stem cells. These findings are similar to those of a previous experiment (Liu et al. 2011), suggesting that leptin may have a similar type of promoting effect on HPMSCs that VEGF has on endothelial cells. HPMSCs alone and HPMSCs transfected with pIRES2-EGFP showed no leptin expression, suggesting that human placenta-derived mesenchymal stem cells did not experience any of the endocrine effects of leptin. Changes in the cell proliferation activity of human placenta-derived mesenchymal stem cells were detected using an MTT assay, and the results suggested that pIRES2-EGFP-leptin had been successfully transferred into HPMSCs and had a prominent role in the proliferation process. We further carried out a multiple differentiation induction identification on HPMSCs transfected with pIRES2-EGFP-leptin in order to prevent it from generating genetic mutation and losing multipotency. The results showed that the transgenic HPMSCs retained their multipotency.

To sum up, pIRES2-EGFP-leptin was constructed successfully and the liposome transfection method was used to transfer it into HPMSCs, which then expressed leptin. This indicated that HPMSCs can be used as carrier cells for leptin gene therapy. HPMSCs transfected with leptin showed long-term release of leptin. HPMSCs also showed no endocrine leptin function. This experiment lays a foundation for further

experimentation into practical applications of HPMSCs carrying the leptin gene (but inducible) for the treatment of refractory wound healing.

Acknowledgments This study was supported by the basic scientific and research operational funds of Jilin University (4500-60323446) and Youth Scientific Foundation of Science and Technology Department of Jilin Province (20080165).

Conflict of interest The authors report no conflicts of interest. The authors alone are responsible for the content and composition of the paper with the exception that they have engaged a professional English editing service.

References

- Alviano F, Fossati V, Marchionni C, Arpinati M, Bonsi L, Franchina M, Lanzoni G, Cantoni S, Cavallini C, Bianchi F, Tazzari PL, Pasquinelli G, Foroni L, Ventura C, Grossi A, Bagnara GP (2007) Term amniotic membrane is a high throughput source for multipotent mesenchymal stem cells with the ability to differentiate into endothelial cells in vitro. *BMC Dev Biol* 7:11–15
- Brown R, Thompson HJ, Imran SA, Ur E, Wilkinson M (2008) Traumatic brain injury induces adipokine gene expression in rat brain. *Neurosci Lett* 432:73–78
- Cho YM, Kim MS, Shin CS, Park DJ, Park KS, Yang HK, Lee KU, Cho BY, Lee HK, Kim SY (2003) Dynamic change in plasma leptin level during the perioperative period. *Horm Res* 59:100–104
- Cohen B, Barkan D, Levy Y, Goldberg I, Fridman E, Kopolovic J, Rubinstein M (2001) Leptin induction angiopoietin-2 expression in adipose tissues. *Biol Chem* 276:7697–7700
- Eppihimer MJ, Gunn J, Freeman GJ, Greenfield EA, Chernova T, Erickson J, Leonard JP (2002) Expression and regulation of the PD-L1 immunoinhibitory molecule on microvascular endothelial cells. *Microcirculation* 9:133–145
- Fantuzzi G, Faggioni R (2000) Leptin in the regulation of immunity, inflammation, and hematopoiesis. *J Leukoc Biol* 68:437–446
- Hobson KG, Havel PJ, McMurtry AL, Lawless MB, Palmieri TL, Greenhalgh DD (2004) Circulating leptin and cortisol after burn injury: loss of diurnal pattern. *Burn Care Rehabil* 25:491–499
- In't Anker PS, Scherjon SA, Kleijburg-van der Keur C, de Groot-Swings GM, Claas FH, Fibbe WE, Kanhai HH (2004) Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. *Stem Cells* 22:1338–1345
- Kaska M, Zivny P (2002) Serum leptin levels in the perioperative period in surgery patients. *Rozhl Chir* 81:22–24
- Kino Y, Kato M, Ikehara Y, Asanuma Y, Akashi K, Kawai S (2003) Plasma leptin levels in patients with burn injury: a preliminary report. *Burns* 29:449–453
- Koo BK, Park IY, Kim J, Kim JH, Kwon A, Kim M, Kim Y, Shin JC, Kim JH (2012) Isolation and characterization of chorionic mesenchymal stromal cells from human full term placenta. *Korean Med Sci* 27:857–863
- Liang QL, Wang BR, Li ZY, Chen GQ, Zhou Y (2011) Construction of eukaryotic expression vector of TSLC1 gene. *Arch Med Sci* 7:579–585
- Liapakis IE, Anagnostoulis S, Karayiannakis AJ, Korkolis DP, Lambropoulou M, Arnaud E, Simopoulos CE (2008) Recombinant leptin administration improves early angiogenesis in full-thickness skin flaps: an experimental study. *In vivo* 22:247–252
- Liu ZH, Meng GW, Wang BW, Liu CL, Zhou YM, Wang Y (2011) Research on human placenta-derived mesenchymal stem cells transfected with pIRES2-EGFP-VEGF165 using liposome. *Afr J Biotechnol* 10:7727–7735
- Maruna P, Gurlich R, Frasko R (2001) Dynamics of leptin plasma levels after abdominal surgical procedures. *Rozhl Chir* 80:299–303
- Pasquinelli G, Tazzari P, Ricci F, Vaselli C, Buzzi M, Conte R, Orrico C, Foroni L, Stella A, Alviano F, Bagnara GP, Lucarelli E (2007) Ultrastructural characteristics of human mesenchymal stromal(stem) cells derived from bone marrow and term placenta. *Ultrastruct Pathol* 31:23–31
- Poeggeler B, Schulz C, Pappolla MA, Bodó E, Tiede S, Lehnert H, Paus R (2010) Leptin and the skin: a new frontier. *Exp Dermatol* 19:12–18
- Portmann-Lanz CB, Schoeberlein A, Huber A, Sager R, Malek A, Holzgreve W, Surbek DV (2006) Placental mesenchymal stem cells as potential autologous graft for pre- and perinatal neuroregeneration. *Am J Obstet Gynecol* 194:664–673
- Ring BD, Scully S, Davis CR, Baker MB, Cullen MJ, Pelley-mounter MA, Danilenko DM (2003) Systemically and topically administered leptin both accelerate wound healing in diabetic ob/ob mice. *Endocrinology* 141:446–449
- Schoof E, Stuppy A, Harig F, Singer H, Carbon R, Horbach T, Kratzsch J, Rascher W, Dötsch J (2003) No influence of surgical stress on postoperative leptin gene expression in different adipose tissues and soluble leptin receptor plasma levels. *Horm Res* 59:184–190
- Sica GL, Choi IH, Zhu G, Tamada K, Wang SD, Tamura H, Chapoval AI, Flies DB, Bajorath J, Chen L (2003) B7-H4, a molecule of the B7 family, negatively regulates T cell immunity. *Immunity* 18:849–861
- Soncini M, Vertua E, Gibelli L, Zorzi F, Denegri M, Albertini A, Wengler GS, Parolini O (2007) Isolation and characterization of mesenchymal cells from human fetal membranes. *Tissue Eng Regen Med* 1:296–305
- Ventura C, Cantoni S, Bianchi F, Lionetti V, Cavallini C, Scarlata I, Foroni L, Maioli M, Bonsi L, Alviano F, Fossati V, Bagnara GP, Pasquinelli G, Recchia FA, Perbellini A (2007) Hyaluronan mixed esters of butyric and retinoic acid drive cardiac and endothelial fate in term placenta human mesenchymal stem cells and enhance cardiac repair in infarcted rat hearts. *Biol Chem* 282:14243–14252
- Wallace AM, Sattar N, Mcmillan DC (2000) The co-ordinated cytokine/hormone response to acute injury incorporates leptin. *Cytokine* 12:1042–1045
- Wen H, Wu G, Chen W, Yang H, Fu J (2012) Topical application of leptin promotes burn wound healing in rats. *Nan Fang Yi Ke Da Xue Xue Bao* 32:703–706