European Review for Medical and Pharmacological Sciences 2019; 23: 4954-4960

Effects of human umbilical cord mesenchymal stem cells-derived exosomes on fracture healing in rats through the Wnt signaling pathway

J. ZHOU[1], H.-X. LIU[1], S.-H. LI[2], Y.-S. GONG[3], M.-W. ZHOU[4], J.-H. ZHANG[5], G.-Y. ZHU[5]

[1]Department of Osteomyelitis, Gansu Hospital of Traditional Chinese Medicine, Gansu, China
[2]Gansu Hospital of Traditional Chinese Medicine, Gansu, China
[3]Department of Soft Tissue Injury, Gansu Hospital of Traditional Chinese Medicine, Gansu, China
[4]Department of Orthopedic, Gansu Hospital of Traditional Chinese Medicine, Gansu, China
[5]Department of Oncology, Gansu Hospital of Traditional Chinese Medicine, Gansu, China

Abstract. – OBJECTIVE: To investigate the role of human umbilical cord mesenchymal stem cell (hucMSC)-derived exosomes in the Wnt signaling pathway and their effects on fracture healing in rats.

MATERIALS AND METHODS: A total of 24 healthy male Sprague-Dawley (SD) rats were randomly divided into 3 groups, of which the experimental groups were injected with Phosphate-Buffered Saline (PBS) and hUCMSC-derived exosomes, respectively, at the fracture site, and a blank control group was set. At 2 and 3 w after treatment, respectively, the healing condition at the fracture site in the rats was detected by micro-computed tomography (CT). The protein expressions of β-catenin and Wnt3a of the Wnt signaling pathway in the bone tissue were measured via Western blotting (WB) assay. Quantitative Real Time-fluorescence Polymerase Chain Reaction (qRT-PCR) was performed to determine the expressions of osteogenic marker genes [collagen type I (COL-1), osteopontin (OPN) and runt-related transcription factor 2 (RUNX2)].

RESULTS: The results of the micro-CT scan showed that the rats treated with exosomes had better apposition of the fracture site, and the appearance of cortical bone was continuous. The fracture sites in the blank control group and PBS injection group were not healed, and the appearance of cortical bone was discontinuous, with significant fracture lines. According to the WB results, the protein expression levels of β-catenin and Wnt3a in exosome treatment group were significantly higher than those in the blank control group and PBS injection group ($p<0.01$). The qRT-PCR results indicated that the expression levels of COL-1, OPN and RUNX2 in exosome treatment group were increased evidently compared with those in the other two groups ($p<0.01$).

CONCLUSIONS: HucMSC-derived exosomes are probably involved in the repair of fracture in rats through the Wnt signaling pathway.

Key Words:
Human umbilical cord mesenchymal stem cells, Exosomes, Wnt signaling pathway, Fracture.

Introduction

Fracture is a fairly common clinical manifestation of completely or partially broken continuity of bone structure caused by violence or cumulative strain. Healing in about 10% of patients with fracture is delayed, which will bring physiological and psychological discomfort and great distress to the patients[1]. Therefore, stimulating the quick repair and healing of fracture are of critical importance. Fracture healing is a complex process involving a series of physiological activities, among which the proliferation and differentiation of osteoprogenitor cells and various stem cells at the fracture site play crucial roles in healing[2]. With the emergence of stem cell technology, an increasing number of studies are applying the stem cells to treat numerous diseases, and growing attention and research have been attached to exosomes, as derivatives of the stem cells, by researchers in recent years[3]. Exosomes refer to vesicle-like bodies containing complicated ribonucleic acids (RNAs) and proteins, with a diameter of 40-100 nm, which were first discovered in sheep reticulocytes in 1983. Exosomes participate in the transmission of signals and transport of substances between the internal environment and cells, and exert very important effects in tissue homeostasis, interactions between organs and intercellular communication[4,5]. In the field of tissue regeneration, large quantities of studies on the treatment of diseases with exosomes have been conducted by more

Corresponding Author: Guoyan Zhu, MD; e-mail: 375146748@qq.com

and more scientific researchers due to their stable components and fewer potential safety hazards[6]. Numerous researchers have verified that mesenchymal stem cell-derived exosomes have positive protective effects and manifest efficacy in animal models of myocardial infarction[7], liver failure[8] and ischemia/reperfusion injury[9]. In addition, some studies have indicated that the Wnt signaling pathway performs vital functions in cell growth and differentiation and play crucial regulatory roles in bone development and formation[10-12]. Activated Wnt/β-catenin signaling pathway can promote osteocyte proliferation and differentiation as well as further fracture repair[13]. Although there are studies on fracture repair involving exosomes and Wnt/β-catenin signaling pathway, the function of exosomes on the Wnt/β-catenin signaling pathway and their impacts on fracture repair are rarely investigated. This research aims to explore the impacts of human umbilical cord mesenchymal stem cell (hucMSC)-derived exosomes on fracture healing by acting on the Wnt signaling pathway, providing theoretical bases and potential therapeutic methods for studies and clinical treatments related to fracture healing.

Materials and Methods

Main Materials

Sprague-Dawley rats, pentobarbital sodium (Shanghai Longsheng Chemical Co., Ltd., Shanghai, China), SYBR Green Real Time-Polymerase Chain Reaction (RT-PCR) Master Mix kit (TaKaRa, Tokyo, Japan), hucMSCs (Shanghai Cell Bank of Chinese Academy of Sciences, Shanghai, China), Dulbecco's Modified Eagle Medium/nutrient mixture F-12 (DMEM/F12, Hyclone, South Logan, UT, USA) medium, fetal bovine serum (FBS, Hyclone, South Logan, UT, USA) and Phosphate-Buffered Saline (PBS, Gibco, Grand Island, NY, USA), HyStem®-HP Cell Culture Scaffold Kit (Sigma-Aldrich, St. Louis, MO, USA), TRIzol (Invitrogen, Carlsbad, CA, USA), cluster of differentiation 9 (CD9) antibody, CD63 antibody, CD81 antibody, β-catenin antibody, Wnt3a antibody and β-actin antibody (Abcam, Cambridge, MA, USA), and 0.22 μm pinhole filter (Millipore, Billerica, MA, USA).

Establishment of Rat Model of Fracture

The rat model of fracture was established according to the experimental methods of femoral fracture. This study was approved by the Animal Ethics Committee of Gansu Hospital of Traditional Chinese Medicine Animal Center. All the rats were deprived of food and water for 8 h before the experiment and then fixed after anesthesia by intraperitoneal injection of 0.75% pentobarbital sodium. The operation was performed at the anterior patella of the knee joint of the right hind leg, during which an approximately 1 cm-long straight incision was made, and the muscle and fascia were separated carefully. The shaft of the middle femur was cut off using a dental saw, the fracture sites were aligned *via* a Kirschner wire, and the incision was sutured carefully layer by layer. After the modeling operation was completed, the rats were fed under a clean environment with regular disinfection and provided with routine food and water.

Culture of HucMSCs and Extraction of Exosomes

HucMSCs were cultured in an incubator with a DMEM/F12 medium containing 10% FBS at 37°C with 5% CO_2. When 90% of the bottom of the dish was covered with the cells, they were washed with PBS twice, digested with 0.25% trypsin and subcultured at a density of 1:3. The extraction methods for exosomes are as follows[14]: the medium of hucMSCs was collected once every other day, transferred into a centrifuge tube and centrifuged at 300 g and 4°C for 10 min. The supernatant was taken and centrifuged at 16,500 g and 4°C for 20 min. Next, the supernatant was collected and filtered using the 0.22 μm filter, the filtrate was centrifuged at 120,000 g and 4°C for 70 min, and the supernatant was absorbed immediately, followed by washing with PBS, ultracentrifugation again and collection of precipitation.

Treatment with Exosome Injection

A HyStem-HP hydrogel carrier was used to deliver the exosomes. According to the methods in the manufacturer's instructions of hydrogel, 100 μg exosomes were sufficiently mixed with 100 μL of hydrogel, and the mixture was injected into the fracture site of the rats in the experimental group before the incision at the femur was closed through the operation. PBS was used as a control of exosomes in the experimental group, and the group injected with nothing was set as a blank control group.

X-Ray Examination and Analysis

After fracture modeling and at 2 and 3 w after the operation, an X-ray perspective apparatus was

applied to examine the fracture site; the fracture modeling and fracture conditions after operation and treatment were observed. Photography conditions: tube projection distance of 100 cm, 100 mA and 50 kV. X-ray images were analyzed using medical image analysis software.

Extraction of Total RNA and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

The sequences of collagen type I (COL-1), osteopontin (OPN), runt-related transcription factor 2 (RUNX2) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were obtained from the website of NCBI database (http://www.ncbi.nlm.nih.gov/). The primers were designed using Premier 6.0 software and synthesized by Sangon Biotech Co. (Shanghai, China). The primer sequences for COL-1, OPN, RUNX2 and GAPDH are shown in Table I. TRIzol reagent was utilized to extract the total RNA, and its concentration was measured *via* a NanoDrop spectrophotometer. Then, the total RNA was synthesized into complementary deoxyribonucleic acid (cDNA) using the random primers from Reverse Transcription (RT) Master Mix kit. Next, quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was performed using the SYBR-Green RT-PCR Master Mix kit and ABI 7500 sequence detection system (Applied Biosystems, Foster City, CA, USA) in accordance with the manufacturer's protocol. The transcription level was evaluated based on cycle threshold (Ct) value. The target amount of standardized internal reference was calculated using the $2^{-\Delta\Delta Ct}$ method.

Western Blotting (WB)

Total proteins were extracted from the exosomes and fracture site, whose concentration was determined by bicinchoninic acid (BCA) protein assay (Pierce, Waltham, MA, USA). Then, the proteins were separated *via* 8% dodecyl sulfate, sodium salt-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA), followed by sealing with 5% skim milk powder and 0.1% Tris-Buffered Saline and Tween 20 (TBST; Sigma-Aldrich, St. Louis, MO, USA). Subsequently, primary antibodies for CD9, CD63, CD81, β-catenin, Wnt3a and β-actin were added and gently shaken at 4°C overnight. After that, Horseradish-peroxidase (HRP)-labeled secondary antibodies were added for incubation, and the proteins to be detected were subjected to exposure using enhanced chemiluminescence (ECL; Thermo Fisher Scientific, Waltham, MA, USA) reagent. β-actin detected using the same WB was taken as the control.

Table I. Primer sequences for COL-1, OPN, RUNX2 and GAPDH detection.

Name	Sequence
COL-1 F	5'-GTACATCAGCCCAAACCCCA-3'
COL-1 R	5'-CAGGATCGGAACCTTCGCTT-3'
OPN F	5'-GCCAGCCAAGGACCAACTA-3'
OPN R	5'-AGTGTTTGCTGTAATGCGCC-3'
RUNX2 F	5'-GCCAATCCCTAAGTGTGGCT-3'
RUNX2 R	5'-AACAGAGAGCGAGGGGGTAT-3'
GAPDH F	5'-AGTGCCAGCCTCGTCTCATA-3'
GAPDH R	5'-GGTAACCAGGCGTCCGATAC-3'

Statistics Analysis

Statistical Product and Service Solutions (SPSS) 20.0 software (SPSS, Chicago, IL, USA) was used for data record and processing. The data in each group was presented as mean ± standard deviation ($\bar{\chi}\pm s$), and independent-sample *t*-test was adopted for comparison between groups. $p<0.05$ suggested that the difference was statistically significant.

Results

Detection of HucMSC-Derived Exosomes

After amplification and culture, hucMSCs were in a good growth status, with plump morphology (Figure 1A). Next, the supernatant medium for cell culture was collected to successfully extract the exosomes by means of ultracentrifugation. The purified exosomes manifested a regular spheroidal shape under the transmission electron microscope, with a diameter of 30-100 (Figure 1B). The WB results showed that CD9, CD63 and CD81 were expressed by the exosomes (Figure 1C).

X-Ray Analysis for Femoral Fracture Healing in Rats

After the establishment of the rat model of fracture, the hucMSC-derived exosomes, PBS and blank control were injected separately. The photography results of X-ray after 3 weeks of feeding displayed that the fracture was not healed in the blank control group (Figure 2A) and PBS injection group (Figure 2B), while it was healed well in the exosome injection group (Figure 2C).

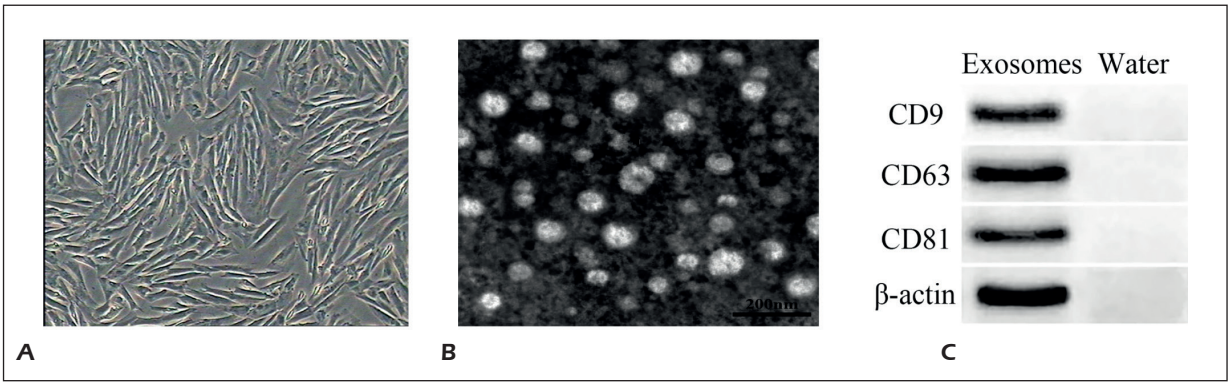

Figure 1. HucMSCs and their exosomes. **A**, Morphology of hucMSCs observed under an inverted microscope. **B**, Morphology of hucMSC-derived exosomes observed under a transmission electron microscope. **C**, Protein expressions of CD9, CD63 and CD81 in hUCMSC-derived exosomes detected *via* WB.

Wnt Signaling Pathway Analysis

The expression levels of the Wnt signaling pathway-related proteins β-catenin and Wnt3a in tissues at the fracture site in different treatment groups were analyzed (Figure 3A). The results showed that injecting hucMSC-derived exosomes could markedly increase the expression levels of β-catenin and Wnt3a ($p<0.01$; Figure 3B), which were maintained at relatively high levels after injection for 2 and 3 w. However, the protein expression levels of β-catenin and Wnt3a in the blank control group and PBS injection group were lower than those in the exosome injection group, and they were higher at 3 w than those at 2 w, displaying an increasing trend with the prolonged recovery time.

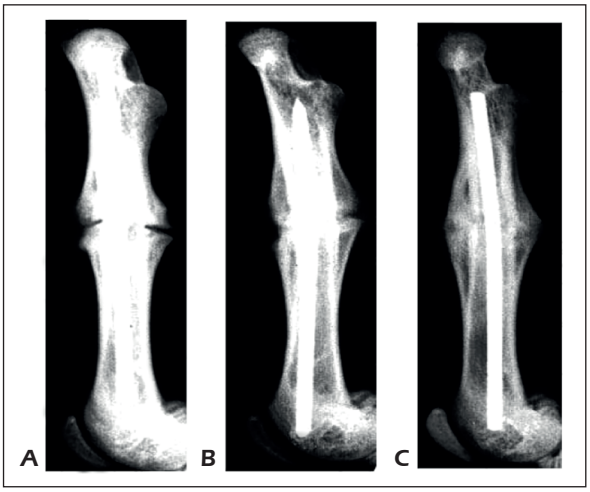

Figure 2. Rat femurs observed *via* X-ray 3 w later. **A**, Blank control group, fracture non-union. **B**, PBS + HyStem-HP hydrogel carrier injection group, fracture non-union. **C**, HucMSC-derived exosomes + HyStem-HP hydrogel carrier injection group, significant fracture healing.

Expressions of Osteogenic Marker Genes at the Fracture Site in Different Treatment Groups Detected Via qRT-PCR

The expressions of osteogenic marker genes (COL-1, OPN and RUNX2) at the fracture site in different treatment groups were detected *via* qRT-PCR at 3 w. It was indicated that the exosome injection group had markedly higher expression levels of COL-1, OPN and RUNX2 at the fracture site than the blank control group and PBS injection group ($p<0.01$; Figure 4). There were no significant differences between the blank control group and PBS injection group ($p>0.05$; Figure 4).

Discussion

The patients with fracture, a relatively common disease, suffer from great physiological distress and inconvenience to life. The successful healing of fracture is of important significance for the patients, but it is a complex process involving inflammation, angiogenesis and bone anabolism[15]. Exosomes are extracellular vesicles secreted by cells, including proteins and nucleic acids, which are bioactive substances in essence and ultimately released into the extracellular matrix (ECM)[16,17]. Yin et al[18] have revealed that the genetic information contained in the stem cell-derived exosomes can be shared by maturate osteocytes and stem cells and promote bone regeneration. COL-1, the most abundant protein in bone ECM, is synthesized by the osteocytes and serves as a crucial biomarker of bone formation[19]. RUNX2, expressed in pre-osteoblastic cells, is a key transcription factor in the process of osteoblast differentiation, whose inactivation can inhibit the osteoblast differen-

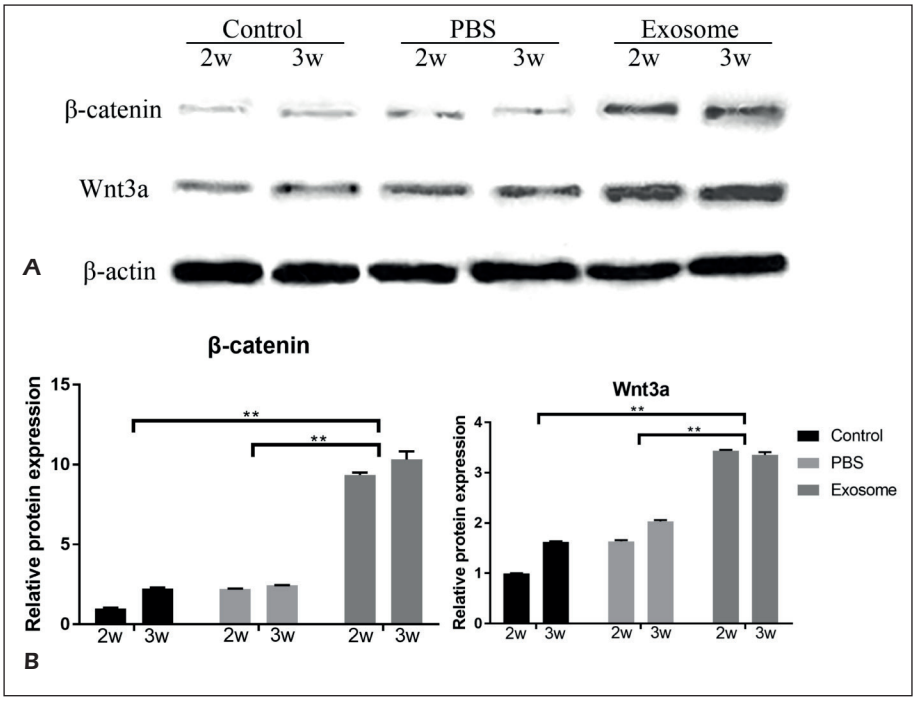

Figure 3. Expressions of the Wnt signaling pathway-related proteins at the fracture site in rats detected *via* WB. **A**, WB assay results for β-catenin and Wnt3a at the fracture site in rats at 2 and 3 w in blank control group, PBS injection group and exosome injection group, with β-actin as the internal reference. **B**, Histogram for relative expressions of β-catenin and Wnt3a. The expression levels of β-catenin and Wnt3a in exosome injection group are notably higher than those in the blank control group and PBS injection group ($p<0.01$), **stands for extremely significant.

tiation[20]. As a vital component of bone matrix, OPN is persistently expressed during the growth and remodeling of endochondral bone and highly expressed at the site of osteoclast resorption[21]. In this research, it was shown that hucMSC-derived exosomes could improve the expressions of osteogenic marker genes such as COL-1, OPN and RUNX2, indicating that they can effectively stimulate the osteoblast differentiation and accelerate fracture healing in the rats.

Whether hucMSC-derived exosomes may accelerate skeletal healing through the Wnt signaling pathway was further explored in this research. The Wnt/β-catenin signaling pathway is a highly conserved pathway mainly composed of multiple protein factors, including extracellular ligands (Wnt), β-catenin in the cytoplasm, seven-transmembrane receptor proteins [frizzled (Frz)] and glycogen synthase kinase-3 (GSK-3)[22]. It regulates a variety of physiological processes of cells, such as development, differentiation, growth and apoptosis, which is highly similar among different species. A study[10] has demonstrated that the Wnt/β-catenin signaling pathway plays a dominant regulatory role in bone development and formation during the embryonic development. Wnts are secretory glycoproteins that promote downstream signal transduction by virtue of their co-receptors (LDL-receptor-related protein 5/6 and seven-transmembrane receptor complex Frz) primarily under the regulation of GSK-3. β-catenin is a subunit of the cadherin protein complex capable of transducing intracellular signals in the Wnt signaling pathway. It can be transported into the nucleus by the cytoplasm and then activate the transcription of osteogenesis-re-

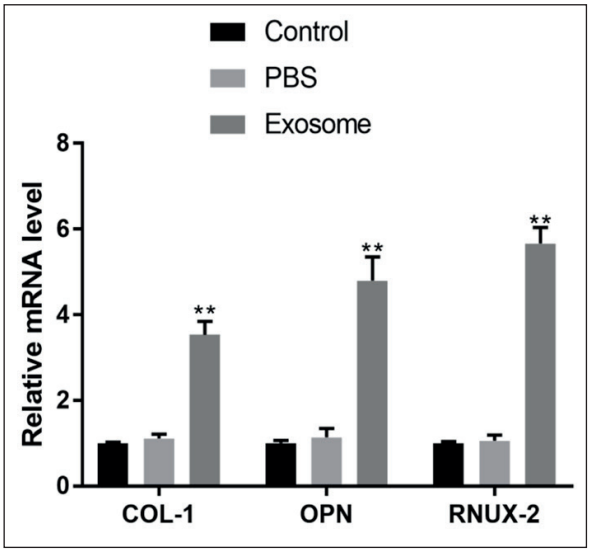

Figure 4. Expressions of osteogenic marker genes COL-1, OPN and RUNX2 at the fracture site in different treatment groups detected via qRT-PCR at 3 w. **Stands for significant differences between the exosome injection group and blank control group or PBS injection group.

lated genes by binding to T cell factor/lymphoid enhancer factor, a transcription factor[23]. It has been testified that the Wnt signal transduction is a key molecular mechanism in the process of fracture healing. Leucht et al[24] have illustrated that the reduced Wnt signal transduction will trigger the bone marrow stromal cell to differentiate into adipocytes, while increased Wnt signal transduction will promote osteogenic differentiation. The bone marrow mesenchymal stem cell-derived exosomes can alleviate radiation-induced bone loss and restore the osteogenic differentiation by improving the expression of β-catenin[25]. In addition, β-catenin is able to positively regulate the differentiation of mesenchymal stem cells into osteoblastic lineage[26]. Relevant studies have manifested that Wnt3a can enhance the ALP activity induced by BMP9, increase the expressions of OC and OPN and accelerate mineral (calcium) deposition and bone formation *in vivo*[27], illustrating that inducing the expressions of β-catenin and Wnt3a in the Wnt signaling pathway can promote the bone formation.

Conclusions

We found that hucMSC-derived exosomes could increase the protein expressions of β-catenin and Wnt3a in the Wnt signaling pathway, indicating that hucMSC-derived exosomes probably participate in repairing fracture in rats through the Wnt signaling pathway. This research can provide effective therapeutic approaches for fracture in clinic and theoretical bases for related studies.

Disclosure of interest

The authors declare that they have no conflict of interest.

References

1) Zhang N, Zhang RF, Zhang AN, Dong GX, Suo N, Wu ZP, Liu YM, Wang LT. MiR-204 promotes fracture healing via enhancing cell viability of osteoblasts. Eur Rev Med Pharmacol Sci 2018; 22: 29-35.
2) Wehrle E, Liedert A, Heilmann A, Wehner T, Bindl R, Fischer L, Haffner-Luntzer M, Jakob F, Schinke T, Amling M, Ignatius A. The impact of low-magnitude high-frequency vibration on fracture healing is profoundly influenced by the oestrogen status in mice. Dis Model Mech 2015; 8: 93-104.
3) Hessvik NP, Llorente A. Current knowledge on exosome biogenesis and release. Cell Mol Life Sci 2018; 75: 193-208.
4) El Andaloussi S, Mager I, Breakefield XO, Wood MJ. Extracellular vesicles: biology and emerging therapeutic opportunities. Nat Rev Drug Discov 2013; 12: 347-357.
5) Beit-Yannai E, Tabak S, Stamer WD. Physical exosome: exosome interactions. J Cell Mol Med 2018; 22: 2001-2006.
6) Zhou Y, Xu H, Xu W, Wang B, Wu H, Tao Y, Zhang B, Wang M, Mao F, Yan Y, Gao S, Gu H, Zhu W, Qian H. Exosomes released by human umbilical cord mesenchymal stem cells protect against cisplatin-induced renal oxidative stress and apoptosis in vivo and in vitro. Stem Cell Res Ther 2013; 4: 34.
7) Khan M, Nickoloff E, Abramova T, Johnson J, Verma SK, Krishnamurthy P, Mackie AR, Vaughan E, Garikipati VN, Benedict C, Ramirez V, Lambers E, Ito A, Gao E, Misener S, Luongo T, Elrod J, Qin G, Houser SR, Koch WJ, Kishore R. Embryonic stem cell-derived exosomes promote endogenous repair mechanisms and enhance cardiac function following myocardial infarction. Circ Res 2015; 117: 52-64.
8) Borrelli DA, Yankson K, Shukla N, Vilanilam G, Ticer T, Wolfram J. Extracellular vesicle therapeutics for liver disease. J Control Release 2018; 273: 86-98.
9) Lin KC, Yip HK, Shao PL, Wu SC, Chen KH, Chen YT, Yang CC, Sun CK, Kao GS, Chen SY, Chai HT, Chang CL, Chen CH, Lee MS. Combination of adipose-derived mesenchymal stem cells (ADMSC) and ADMSC-derived exosomes for protecting kidney from acute ischemia-reperfusion injury. Int J Cardiol 2016; 216: 173-185.
10) Church VL, Francis-West P. Wnt signalling during limb development. Int J Dev Biol 2002; 46: 927-936.
11) Fischer L, Boland G, Tuan RS. Wnt signaling during BMP-2 stimulation of mesenchymal chondrogenesis. J Cell Biochem 2002; 84: 816-831.
12) Zhou L, An N, Jiang W, Haydon R, Cheng H, Zhou Q, Breyer B, Feng T, He TC. Fluorescence-based functional assay for Wnt/beta-catenin signaling activity. Biotechniques 2002; 33: 1126-1128, 1130, 1132.
13) Gaur T, Lengner CJ, Hovhannisyan H, Bhat RA, Bodine PV, Komm BS, Javed A, van Wijnen AJ, Stein JL, Stein GS, Lian JB. Canonical WNT signaling promotes osteogenesis by directly stimulating Runx2 gene expression. J Biol Chem 2005; 280: 33132-33140.
14) Zhang Y, Hao Z, Wang P, Xia Y, Wu J, Xia D, Fang S, Xu S. Exosomes from human umbilical cord mesenchymal stem cells enhance fracture healing through HIF-1α-mediated promotion of angiogenesis in a rat model of stabilized fracture. Cell Proliferat 2018; 52: e12570.
15) Einhorn TA, Gerstenfeld LC. Fracture healing: mechanisms and interventions. Nat Rev Rheumatol 2015; 11: 45-54.
16) Bjorge IM, Kim SY, Mano JF, Kalionis B, Chrzanowski W. Extracellular vesicles, exosomes and shedding vesicles in regenerative medicine - a new paradigm for tissue repair. Biomater Sci 2017; 6: 60-78.

17) Simons M, Raposo G. Exosomes--vesicular carriers for intercellular communication. Curr Opin Cell Biol 2009; 21: 575-581.
18) Yin P, Lv H, Li Y, Deng Y, Zhang L, Tang P. Exosome-mediated genetic information transfer, a missing piece of osteoblast-osteoclast communication puzzle. Front Endocrinol (Lausanne) 2017; 8: 336.
19) Wei J, Shimazu J, Makinistoglu MP, Maurizi A, Kajimura D, Zong H, Takarada T, Lezaki T, Pessin JE, Hinoi E, Karsenty G. Glucose uptake and runx2 synergize to orchestrate osteoblast differentiation and bone formation. Cell 2015; 161: 1576-1591.
20) Long F. Building strong bones: molecular regulation of the osteoblast lineage. Nat Rev Mol Cell Biol 2011; 13: 27-38.
21) Chen J, Singh K, Mukherjee BB, Sodek J. Developmental expression of osteopontin (OPN) mRNA in rat tissues: evidence for a role for OPN in bone formation and resorption. Matrix 1993; 13: 113-123.
22) Day TF, Guo X, Garrett-Beal L, Yang Y. Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. Dev Cell 2005; 8: 739-750.
23) Chen Y, Alman BA. Wnt pathway, an essential role in bone regeneration. J Cell Biochem 2009; 106: 353-362.
24) Leucht P, Minear S, Ten BD, Nusse R, Helms JA. Translating insights from development into regenerative medicine: the function of Wnts in bone biology. Semin Cell Dev Biol 2008; 19: 434-443.
25) Zuo R, Liu M, Wang Y, Li J, Wang W, Wu J, Sun C, Li B, Wang Z, Lan W, Zhang C, Shi C, Zhou Y. BM-MSC-derived exosomes alleviate radiation-induced bone loss by restoring the function of recipient BM-MSCs and activating Wnt/beta-catenin signaling. Stem Cell Res Ther 2019; 10: 30.
26) Chen Y, Whetstone HC, Lin AC, Nadesan P, Wei Q, Poon R, Alman BA. Beta-catenin signaling plays a disparate role in different phases of fracture repair: implications for therapy to improve bone healing. PLoS Med 2007; 4: e249.
27) Zhang X, Lin LB, Xu DJ, Chen RF, Tan JX, Liang X, Hu N, Huang W. Wnt3a enhances bone morphogenetic protein 9-induced osteogenic differentiation of C3H10T1/2 cells. Chin Med J (Engl) 2013; 126: 4758-4763.