

Stromal cell-derived factor-1 induces matrix metalloproteinase expression in human endplate chondrocytes, cartilage endplate degradation in explant culture, and the amelioration of nucleus pulposus degeneration *in vivo*

HUA ZHANG¹, TING ZHU¹, LI ZHANG² and QIONGHUA WU¹

¹Department of Orthopaedic Surgery, The Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, Zhejiang 310009; ²Department of Clinical Laboratory, Hangzhou Red Cross Hospital, Zhejiang Chinese Medical University, Hangzhou, Zhejiang 310000, P.R. China

Received February 10, 2016; Accepted November 13, 2017

DOI: 10.3892/ijmm.2017.3278

Abstract. Intervertebral disc (IVD) degeneration is a strong etiological factor in chronic lower back pain. Stem cell migration toward the site of IVD degeneration for regeneration is restricted by avascularity and distance. Our previous study indicated that the expression of stromal cell-derived factor-1 (SDF-1) and its receptor, C-X-C chemokine receptor type 4 (CXCR4) was upregulated in degenerated cartilage endplate (CEP) and nucleus pulposus (NP). In the present study, SDF-1 increased CXCR4 mRNA and protein expression in human endplate chondrocytes in a dose-dependent manner. The results of reverse transcription-quantitative polymerase chain reaction, western blotting and zymography indicated that SDF-1 increased matrix metalloproteinase (MMP)-1, -3 and -13 mRNA and protein expression in human endplate chondrocytes in a dose-dependent manner. The results of zymography suggested that SDF-1 also increased MMP-2 and -9 protein expression in a dose-dependent manner. The CXCR4-specific chemical inhibitor AMD3100 significantly decreased the levels of MMP-1, -2, -3, -9 and -13 expression. In a human cartilage explant culture model, SDF-1 accelerated the degradation of extracellular matrix (ECM), and AMD3100 decreased cartilage cleavage. However, in a rat tail disc degeneration model, the injection of SDF-1 into the NP resulted in the retention of dense areas of proteoglycan matrix and enhanced NP regeneration. These results suggest that SDF-1, as an inflammatory cytokine, induces MMP expression in human endplate chondrocytes and that ECM remodeling in

the CEP may be a favorable factor of endogenous stem cell homing into the NP for regeneration *in vivo*.

Introduction

Chronic lower back pain is a worldwide problem that may lead to loss of physical function, decreased quality of life and psychological distress (1). Lower back pain is a multifactorial condition for which intervertebral disc (IVD) degeneration has been indicated as a strong etiological factor (1). Pathological changes associated with IVD degeneration include the local accumulation of matrix metalloproteinases (MMPs) and the decreased synthesis of extracellular matrix (ECM) (2-5). A previous study by the present research team indicated that the expression of stromal cell-derived factor-1 (SDF-1) and its receptor, C-X-C chemokine receptor type 4 (CXCR4), is upregulated in degenerated cartilage endplate (CEP) and nucleus pulposus (NP) tissue (6). Cells in the CEP and NP express CXCR4 protein, and there is a positive correlation between the level of SDF-1 expression and the percentage of CXCR4-positive cells (6). Previous studies indicate that the induction of MMP-3, -9 and -13 expression occurs in joint chondrocytes via the combination of SDF-1 and CXCR4 (7-9). However, whether the SDF-1/CXCR4 pathway induces MMP expression and ECM degradation in the CEP remains unclear.

In most organs, the main sources of stem cells for tissue regeneration that maintain a high self-repair capability are the vascular system and bone marrow (10,11). However, since the IVD is avascular, the migration of stem cells from blood vessels and bone marrow to tissue defect sites is restricted by inaccessibility and distance. Sakai *et al* (12) established a tail-looping disc degeneration model in mice. They reported that only limited numbers of bone marrow-derived mesenchymal stem cells (BMSCs) were recruited into the IVD during disc degeneration, presumably because of its avascular nature. Illien-Jünger *et al* (13) reported that the exposure of IVDs to degenerative conditions induces the release of factors that promote BMSC recruitment in *ex vivo* organ culture. The migration of mesenchymal stem cells (MSCs) toward the NP

Correspondence to: Dr Hua Zhang, Department of Orthopaedic Surgery, The Second Affiliated Hospital, School of Medicine, Zhejiang University, 88 Jiefang Road, Hangzhou, Zhejiang 310009, P.R. China
E-mail: zhanghua068@zju.edu.cn

Key words: stromal cell-derived factor-1, matrix metalloproteinase, cartilage endplate, nucleus pulposus, stem cell

mediated through SDF-1 has been investigated using a disc explant culture model (14). A hyaluronan-based hydrogel release system containing SDF-1 was implanted into a partially nucleotomized bovine disc, and the migration of exogenous BMSCs through the CEP was significantly enhanced in discs implanted with SDF-1 hydrogel (14).

As mentioned above, SDF-1 may be an inflammatory cytokine involved in ECM degradation and also a factor for the homing of endogenous stem cells into the IVD. Therefore, improved knowledge concerning the inflammatory and homing effects of SDF-1 is likely to facilitate the development of stem cell therapy for diseases caused by disc degeneration. The present study was performed to determine whether the SDF-1/CXCR4 pathway induces MMP expression in endplate chondrocytes and ECM degradation in CEP explant culture, and whether SDF-1 implantation into the NP improves the regeneration process *in vivo*.

Materials and methods

Materials. Unless otherwise specified, all materials were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany).

Endplate chondrocyte isolation and culture. Primary endplate chondrocytes were isolated from the CEPs of young patients (18-29 years old; female to male proportion, 20:80) with fresh burst spinal fractures, for which magnetic resonance imaging of the IVDs indicated Pfirrmann grade I to II disc degeneration (15). Sample collection was realized 1-2 days after fracture; the samples were collected during surgery in the operation room. All patients provided signed informed consent for their participation in the study. The study was approved by the Ethics Committee of the Second Affiliated Hospital of the School of Medicine of Zhejiang University (Hangzhou, China). Briefly, the CEPs were harvested and adjacent tissues stripped off. Following immersion in PBS containing penicillin (100 U/ml) and streptomycin (100 mg/ml) for 10 min, the cartilage was cut into small pieces followed by digestion with 0.25% (w/v) collagenase (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. After 3 h, the residual tissue was removed and the cell suspension was centrifuged at 300 x g at room temperature for 5 min. The harvested cells were maintained in Dulbecco's modified Eagle's medium (Gibco; Thermo Fisher Scientific, Inc.) containing 10% (v/v) fetal bovine serum (FBS) and the medium was changed every 3 days. When the cultured primary cells reached 80% confluence, they were detached by treatment with 0.25% (w/v) trypsin and 0.1% (w/v) ethylenediaminetetraacetic acid (Gibco; Thermo Fisher Scientific, Inc.) and subcultured at a density of 1x10⁴ cells/cm². Cultured cells before passage 2 were used for the experiments.

Cell treatment. Prior to use in the experiments, all cell samples were starved of serum for 12 h. The cells were seeded in 6-well plates at an initial density of 2x10⁵/well for gene expression analysis and in 60-mm dishes at an initial density of 5x10⁵/well for protein experiments. When the chondrocytes reached 70-80% confluency, they were incubated with SDF-1 (50, 100

Table I. Primer sequences used in this study.

Gene	Primer sequences
CXCR4	F: 5'-CTCCTGCTGACTATTCCCGAC-3' R: 5'-GATAAGGCCAACCATGATGTGC-3'
MMP-1	F: 5'-CCA AAT GGG CTT GAA GCT G-3' R: 5'-GGT ATC CGT GTA GCA CAT TCT GTC-3'
MMP-3	F: 5'-TTT CCA GGG ATTGAC TCA AAG A-3' R: 5'-AAG TGC CCATAT TGT GCC TTC-3'
MMP-13	F: 5'-ATGCAGTCTTTCTTCGGCTTAG-3' R: 5'-ATGCCATCGTGAAGTCTGGT-3'
GAPDH	F: 5'-GAAGGTGAAGGTCTGGAGTC-3' R: 5'-GAAGATGGTGATGGGATTTC-3'

F, forward; R, reverse; CXCR4, C-X-C chemokine receptor type 4; MMP, matrix metalloproteinase. The sequences were blasted (<http://www.ncbi.nlm.gov/BLAST/>).

or 200 ng/ml; PeproTech, Inc., Rocky Hill, NJ, USA) at 37°C in a humidified atmosphere of 5% CO₂ and 95% air for 24 h. In order to examine the downstream signaling pathways affected by the SDF-1 treatment, the endplate chondrocytes were pretreated with 500 ng/ml AMD3100 (Selleck Chemicals, Houston, TX, USA) at 37°C in a humidified atmosphere of 5% CO₂ and 95% air for 30 min prior to the application of SDF-1.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted using TRIzol reagent (Thermo Fisher Scientific, Inc.) and qualified by measuring the absorbance at 260 nm. cDNA was synthesized from 1 µg RNA using a Takara RNA PCR kit (Takara Bio., Inc., Otsu, Japan) in accordance with the manufacturer's protocol. qPCR was performed using SYBR-Green Master mix (Takara Bio, Inc.) on an ABI StepOnePlus system (v2.3; Applied Biosystems; Thermo Fisher Scientific, Inc.). The primer sequences used are listed in Table I. Samples were examined in triplicate and the results were averaged. The relative expression levels of genes were normalized to the value of GAPDH using the $\Delta\Delta$ cycle threshold ($\Delta\Delta C_q$) method (16), and differences in gene expression were calculated using ABI software (Applied Biosystems; Thermo Fisher Scientific, Inc.). The thermocycling conditions used for qPCR were as follows: stage 1, 95°C for 30 sec; stage 2, 95°C for 5 sec, 60°C for 30 sec (stage 2 was repeated for 40 times); stage 3, 95°C for 15 sec, 60°C for 1 min, 95°C for 15 sec.

Western blot analysis. Cells were seeded at a density of 5x10⁵ cells in 60-mm dishes and treated with serum-free medium for 12 h. The samples were lysed in radioimmuno-precipitation assay lysis buffer containing a protease inhibitor cocktail (Sigma-Aldrich; Merck KGaA) for 30 min on ice. Cell lysates were centrifuged at 12,000 x g for 15 min, and the supernatant was collected. Protein content was quantified using a BCA protein assay kit (Pierce; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Equal amounts of protein (40 µg) were separated by 10% SDS-PAGE

at 100 V for 1.5 h. The proteins were then transferred onto polyvinylidene difluoride membranes at 250 mA for 2 h. The membranes were blocked with 5% bovine serum albumin (Sigma-Aldrich; Merck KGaA) for 1 h at room temperature and then incubated with primary antibody at 4°C overnight. The primary antibodies targeted human CXCR4 (60042-1-Ig), MMP-1 (10371-2-AP), MMP-3 (66338-1-Ig) and MMP-13 (18165-1-AP) (dilution, 1:1,000; all from ProteinTech Group, Inc., Chicago, IL, USA) and GAPDH (dilution, 1:1,000; ab8245; Abcam, Cambridge, MA, USA). After washing five times with TBS and Tween-20, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody (dilution, 1:1,000; ab205719) for 1 h at room temperature. The target proteins were visualized using an enhanced chemiluminescence detection system (ChemiDoc™ XRS+ imaging system; EMD Millipore, Billerica, MA, USA) and hyper-enhanced chemiluminescence film. Band density analysis was performed using Quantity One software (version 4.6.2; Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Zymography. The chondrocyte culture medium was collected, centrifuged at 1,000 × g for 30 min at 4°C, and concentrated 100-fold with a Centrprep centrifugal filter concentrator (EMD Millipore). The sample was loaded into an SDS-PAGE gel containing 1-mg/ml gelatin (for the detection of MMP-2 and -9) or collagen (for the detection of MMP-1, -3 and -13) and subjected to electrophoresis at a constant voltage. The gels were washed with 2.5% Triton X-100 to remove SDS, rinsed with 50 mM Tris-HCl (pH 7.5), and then incubated overnight at room temperature with developing buffer (50 mM Tris-HCl, pH 7.5, 5 mM CaCl₂, 1 μM ZnCl₂, 0.02% thimerosal and 1% Triton X-100). The zymographic activities were visualized by staining with 1% Coomassie Blue. Zymography band density analysis was performed using Quantity One software.

CEP explant culture. According to the method described by Chen *et al* (17), the CEPs harvested from young patients were initially cultured at 37°C with 5% CO₂ in 10% FBS (v/v), antibiotics [penicillin (100 U/ml) and streptomycin (100 mg/ml)] and 2 mM L-glutamine (Gibco; Thermo Fisher Scientific, Inc.). After 2 days, the explants were washed in serum-free medium and placed in 24-well culture dishes with fresh serum-free medium containing different concentrations of SDF-1 (50, 100 or 200 ng/ml) or 500 ng/ml AMD3100 (with or without 100 ng/ml SDF-1) and cultured for a further 2 days. In the combined treatment group, in order to examine the downstream signaling pathways underlying the effects of SDF-1 treatment, the CEPs were pretreated with AMD3100 for 30 min prior to SDF-1 administration.

Animal model. A total of 20 female Sprague-Dawley rats (200-250 g; 8 weeks old; Shanghai Laboratory Animal Center of Chinese Academy of Sciences) were used. The principles of laboratory animal care (18) were followed. The animals were housed in animal-holding units at 24°C in a 12/12-h light/dark cycle. The experiments were approved by the Ethics Committee of the Second Affiliated Hospital of the School of Medicine of Zhejiang University. The animals were anesthetized by the intraperitoneal injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). Coccygeal intervertebral level 2-3 was selected for

the study. A rat tail disc degeneration model was induced by percutaneous needle puncture as described by Mao *et al* (19). A 30-gauge needle was inserted at coccygeal intervertebral level 2-3 at the level of the annulus fibrosus (AF), crossing the NP up to the contralateral AF. When full penetration was achieved, the needle was rotated 360° twice and held in place for 30 sec. The rats were randomly divided into four groups (n=5) and injected with 3 μl PBS (control group), or 3 μl PBS containing 200 ng/ml SDF-1, 500 ng/ml AMD3100, or 200 ng/ml SDF-1 plus 500 ng/ml AMD3100 using a Hamilton micro-syringe. The animals were sacrificed by CO₂ inhalation 30 days after disc puncture.

Histological examination. The samples (the coccygeal intervertebral discs cut from the sacrificed animals) were fixed at room temperature in 4% paraformaldehyde for 4 days and decalcified with 10% buffered formic acid for 2 months. Decalcified specimens (at room temperature) were dehydrated in alcohol, embedded in paraffin blocks, and cut into 5-μm sections. The sections were stained with Safranin-O (for 8 min)/Fast Green (for 5 min) at room temperature. Photographic images of the stained sections were captured using a DP70 CCD camera coupled to an AX-70 microscope (both from Olympus Corporation, Tokyo, Japan).

Statistical analysis. Data are expressed as the mean ± standard deviation from experiments performed at least in triplicate. Data were analyzed using one way anova followed by Tukey's Multiple Comparison test. All statistical analyses were performed using SPSS software (version 6.0; SPSS, Inc., Chicago, IL, USA). P-values were two-tailed, and P<0.05 was considered to indicate a statistically significant result.

Results

SDF-1 upregulates CXCR4 expression in human endplate chondrocytes. RT-qPCR demonstrated that SDF-1 increased CXCR4 mRNA expression in human endplate chondrocytes, and this effect appeared to be dose-dependent. In subconfluent monolayer culture, the stimulatory effect of SDF-1 on CXCR4 mRNA expression was detected at levels as low as 50 ng/ml. When used at a dose of 200 ng/ml, SDF-1 induced a 6-fold increase in CXCR4 mRNA expression (Fig. 1). To confirm the effects of SDF-1 on CXCR4 protein expression, western blot analysis was performed following the treatment of endplate chondrocytes with SDF-1 for 24 h. The results demonstrated that SDF-1 increased the level of CXCR4 protein, and the increase appeared to be dose-dependent (Figs. 2 and 3). Furthermore, the results of RT-qPCR and western blotting indicated that AMD3100 (500 ng/ml), a CXCR4-specific chemical inhibitor, significantly increased the mRNA and cell-surface expression of CXCR4.

SDF-1/CXCR4 interaction is responsible for MMP-1, -2, -3, -9 and -13 expression in human endplate chondrocytes. The results of RT-qPCR, western blotting and zymography indicate that SDF-1 increased the mRNA and protein expression levels of MMP-1, -3 and -13 in human endplate chondrocytes, and the increases appeared to be dose-dependent (Figs. 1-5). The zymography results also suggest that SDF-1 increased

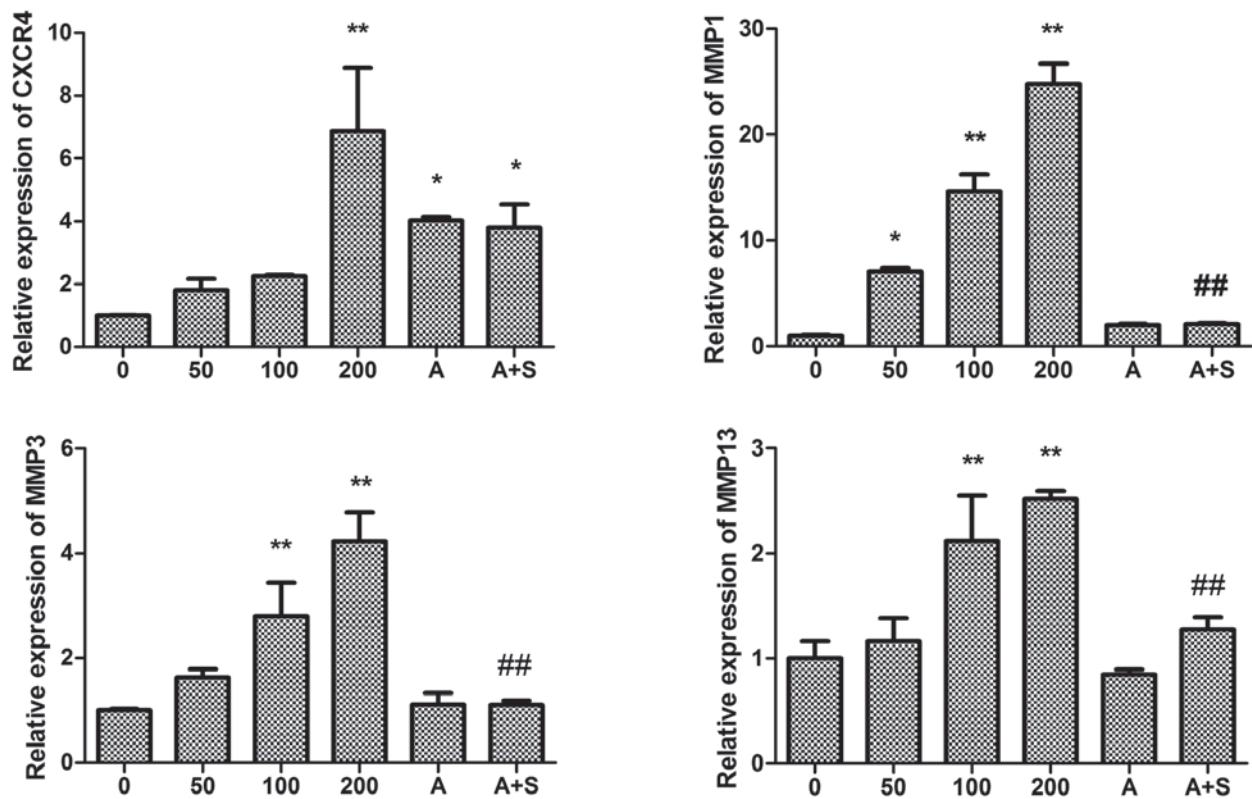


Figure 1. Effect of SDF-1 and the CXCR4-specific chemical inhibitor AMD3100 on CXCR4 and MMP-1, -3 and -13 mRNA expression in endplate chondrocytes. SDF-1 significantly increased CXCR4 and MMP-1, -3 and -13 mRNA expression. AMD3100 significantly decreased MMP-1, -3 and -13 mRNA expression. Human endplate chondrocytes were incubated with various concentrations of SDF-1 (0, 50, 100 or 200 ng/ml), AMD3100 (500 ng/ml), or SDF-1 (100 ng/ml) + AMD3100 (pretreated for 30 min prior to SDF-1 administration) for 24 h. The cell lysates were collected, and mRNA levels were determined by reverse transcription-quantitative polymerase chain reaction. The expression levels of (A) CXCR4, (B) MMP-1, (C) MMP-3, and (D) MMP-13 were determined compared with those of GAPDH. * $P < 0.05$ and ** $P < 0.01$ vs. 0 ng/ml; # $P < 0.05$ and ## $P < 0.01$ vs. 100 ng/ml. SDF-1, stromal cell-derived factor-1; A, AMD3100; A + S, AMD3100 and SDF-1; CXCR4, C-X-C chemokine receptor type 4; MMP, matrix metalloproteinase.

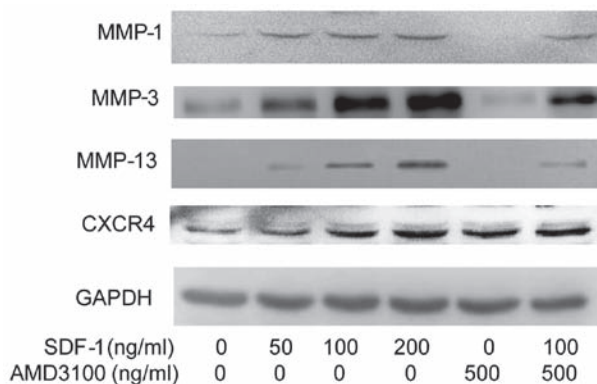


Figure 2. Detection of CXCR4 and MMP-1, -3 and -13 protein in human endplate chondrocytes. The chondrocytes were incubated with various concentrations of SDF-1, AMD3100, or SDF-1 + AMD3100 (pretreated for 30 min before SDF-1 administration) for 24 h. Cell lysates were centrifuged and the levels of CXCR4 and MMP-1, -3 and -13 in the supernatant were determined by western blot analysis. CXCR4, C-X-C chemokine receptor type 4; MMP, matrix metalloproteinase; SDF-1, stromal cell-derived factor-1.

MMP-2 and -9 protein expression in a dose-dependent manner (Figs. 4 and 5). The effects of SDF-1 on MMPs were observed at a dose of 50 ng/ml, and these effects appeared to be greater when the dose was increased. To determine whether the SDF-1/CXCR4 interaction induced increases in MMP

mRNA and protein levels, the CXCR4-specific chemical inhibitor AMD3100 was used to inhibit this interaction. The results demonstrate that AMD3100 significantly antagonized the SDF-1-induced MMP-1, -2, -3, -9 and -13 expression. These results suggest that the SDF-1-induced MMP mRNA and protein expression resulted from the interaction between SDF-1 and CXCR4.

SDF-1 accelerates degradation of the ECM in human CEP explants. Fig. 6 presents aggrecan degradation in the cartilage explant culture model treated with SDF-1 and/or the CXCR4 inhibitor AMD3100, and sham-treated controls. Proteoglycan staining with Safranin-O revealed the reduction of the ECM to the deep zones of the cartilage layers in the 200 ng/ml SDF-1 group (Fig. 6D). The AMD3100 with and without SDF-1 groups clearly exhibited less cartilage cleavage compared with that in the groups treated with SDF-1 alone.

SDF-1 ameliorates NP degeneration in vivo. In the rat tail disc degeneration model, 3 μ l PBS containing SDF-1 and/or AMD3100 was injected into the NP. Representative histological sections of the degenerated discs are shown in Fig. 7. The histological appearance of the tail disc indicated that injection with PBS solution induced NP degeneration, including an interrupted border between the AF and the NP, ECM degradation and morphological shrinkage of the NP. However,

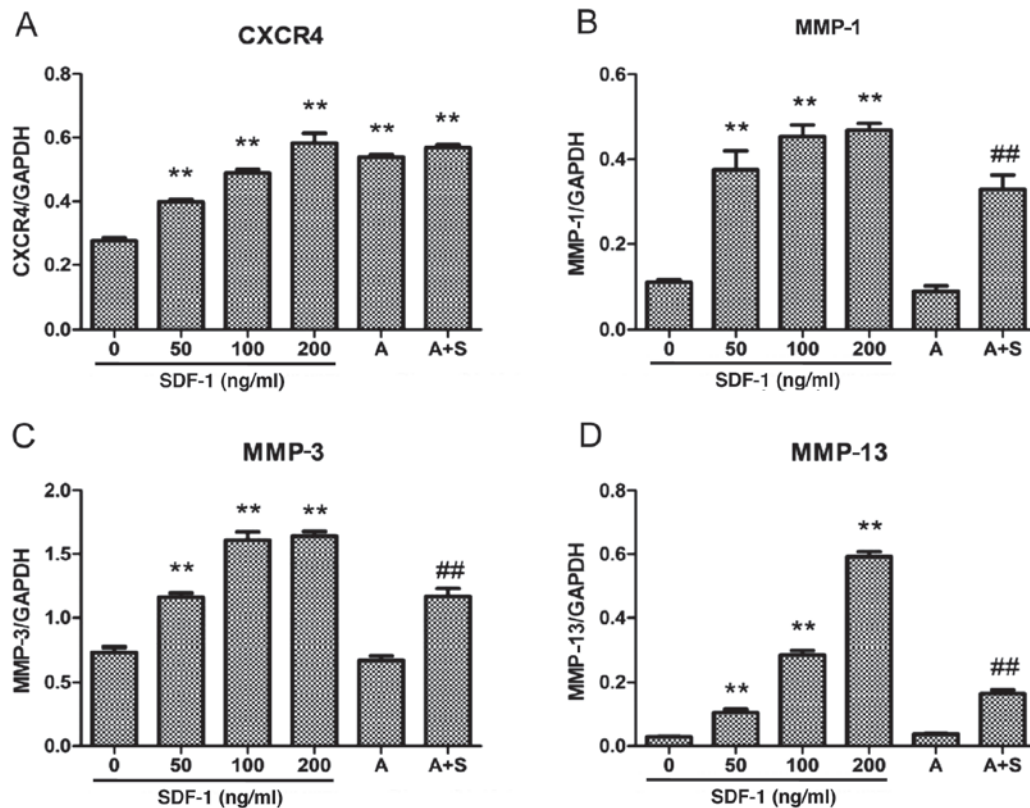


Figure 3. Effect of SDF-1 and the CXCR4-specific chemical inhibitor AMD3100 on CXCR4 and MMP-1, -3 and -13 protein expression in endplate chondrocytes. SDF-1 significantly increased CXCR4 and MMP-1, -3 and -13 protein expression. AMD3100 significantly decreased MMP-1, -3 and -13 protein expression. Human endplate chondrocytes were incubated with various concentrations of SDF-1 (0, 50, 100 or 200 ng/ml), AMD3100 (500 ng/ml), or SDF-1 (100 ng/ml) + AMD3100 (pretreated for 30 min prior to SDF-1 administration) for 24 h. The protein levels of CXCR4 and MMPs were determined by western blot analysis. The expression levels of (A) CXCR4, (B) MMP-1, (C) MMP-3 and (D) MMP-13 were determined relative to those of GAPDH: **P<0.01 vs. 0 ng/ml; ##P<0.01 vs. 100 ng/ml. SDF-1, stromal cell-derived factor-1; A, AMD3100; A + S, AMD3100 and SDF-1; CXCR4, C-X-C chemokine receptor type 4; MMP, matrix metalloproteinase.

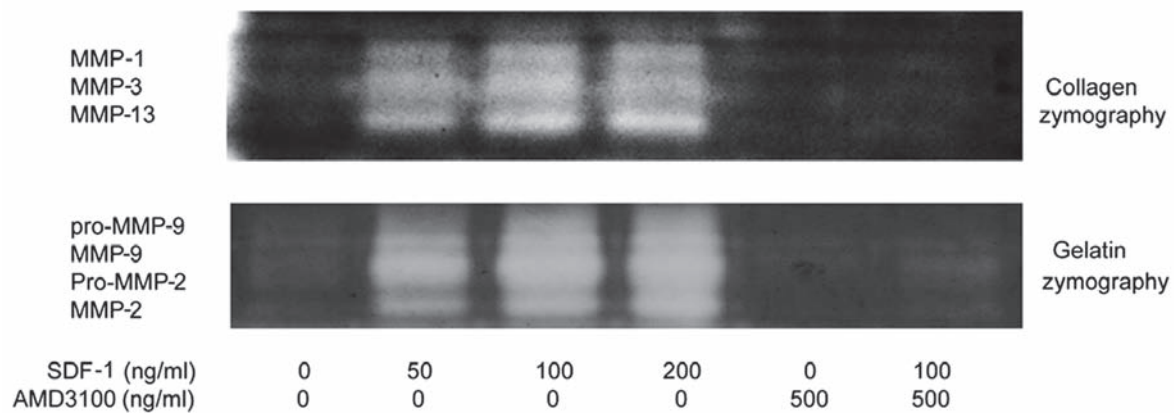


Figure 4. Detection of MMP-1, -3 and -13 in endplate chondrocytes. Human endplate chondrocytes were incubated with various concentrations of SDF-1, AMD3100, or SDF-1 + AMD3100 (pretreated for 30 min prior SDF-1 administration) for 24 h. The cell culture medium was then collected, and the activity levels of MMP-1, -2, -3, -9 and -13 were determined by zymography. MMP, matrix metalloproteinase; SDF-1, stromal cell-derived factor-1.

the injection of SDF-1 without AMD3100 ameliorated the NP degeneration compared with that in the other groups. In particular, the majority of the ECM was retained and NP cells were grouped into clusters and separated by dense areas of proteoglycan matrix in the SDF-1 group (Fig. 7D). The other three groups exhibited greater reduction of the ECM and fewer cells in the NP (Fig. 7A-C).

Discussion

In a previous study, the present research team demonstrated the upregulation of SDF-1 and its receptor CXCR4 in degenerated human and rat IVDs (6). The results of the present study suggest that SDF-1 upregulates CXCR4 expression at the mRNA and protein levels in human endplate chondrocytes isolated from the

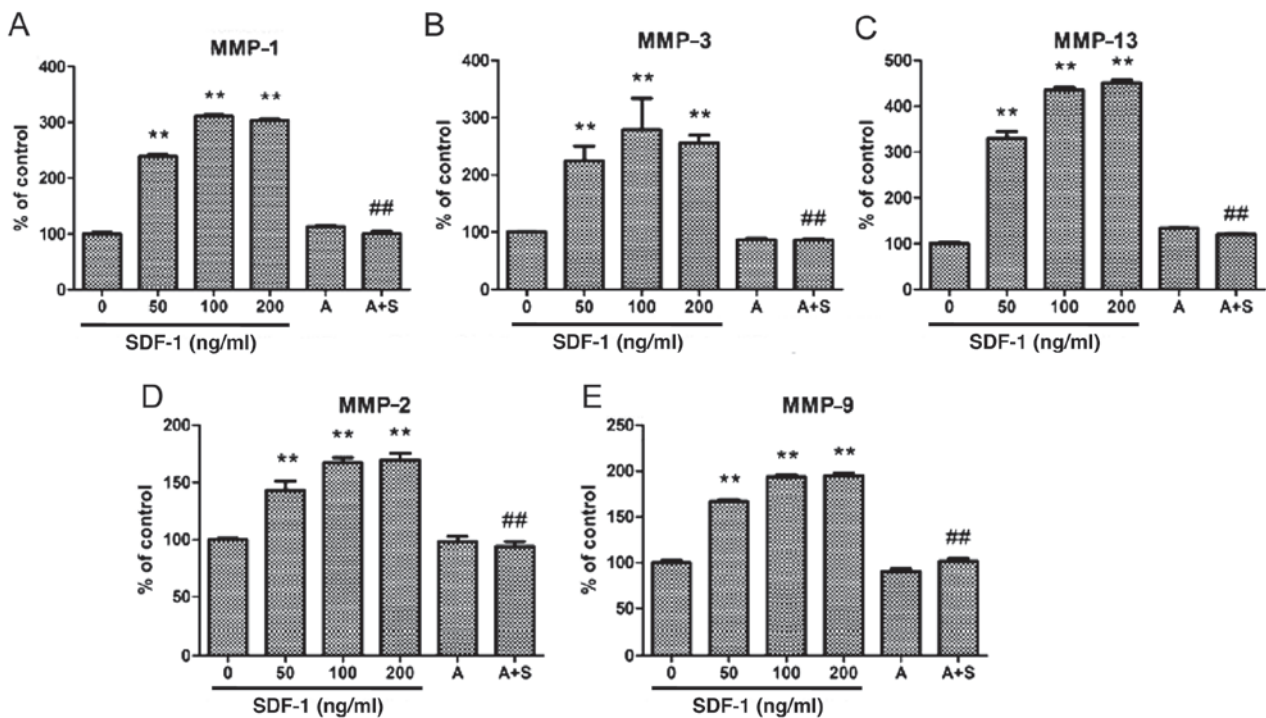


Figure 5. Zymographic analysis revealed that SDF-1 increased MMP-1, -2, -9, -3 and -13 proteolytic activity. AMD3100 significantly decreased MMP-1, -2, -9, -3 and -13 proteolytic activity. Human endplate chondrocytes were incubated with various concentrations of SDF-1 (0, 50, 100 and 200 ng/ml), AMD3100 (500 ng/ml), or SDF-1 (100 ng/ml) + AMD3100 (pretreated for 30 min before SDF-1 administration) for 24 h. The activity of (A) MMP-1, (B) MMP-2, (C) MMP-3, (D) MMP-9 and (E) MMP-13 was determined compared with the control group: ** $P < 0.01$ vs. 0 ng/ml; ## $P < 0.01$ vs. 100 ng/ml SDF-1, stromal cell-derived factor-1; A, AMD3100; A + S, AMD3100 and SDF-1; MMP, matrix metalloproteinase.

human lumbar spine. Previous studies have demonstrated that SDF-1 induces the expression of MMP-3, -9 and -13 in the cartilage cells of patients with osteoarthritis and rheumatoid arthritis (7-9). The present study reveals similar findings in human endplate chondrocytes. Furthermore, AMD3100, a CXCR4 inhibitor, resulted in a significant reduction in SDF-1-mediated MMP expression. These observations indicate that the SDF-1/CXCR4 pathway is involved in the expression of MMPs in endplate cartilage. The expression of MMPs, as matrix-destructive enzymes, at high levels in degenerative endplate cartilage is likely to result in the breakdown of ECM proteins.

Cartilage explant culture systems have been established to investigate the reactions of chondrocytes maintained in an organized structure to growth or inflammatory factors. The integrity of the tissue and chondrocytes is maintained in these *ex vivo* culture systems, allowing the cells to communicate in a manner similar to that *in vivo*, at least for a limited period of time (20). The effects of growth factor treatment may be investigated during culture; the addition of growth factors and other agents to the culture medium as single components or in combination with inhibitors enables the investigation of their specific roles during chondrocyte differentiation and the observation of alterations in cartilaginous structures (17,21,22). In a previous study by Song *et al* (22), human joint cartilage explants were efficiently transfected with small interfering RNA that specifically decreased the expression of a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-4 and -5. The results suggested that the suppression of ADAMTS-4 and -5, individually or in combination, ameliorated the degradation of aggrecan in cytokine-stimulated joint cartilage. In the present study, endplate cartilage explants were cultured under similar

conditions to joint cartilage explants. SDF-1 was added alone or in combination with the inhibitor AMD3100 to the culture medium to investigate the degradative effect of SDF-1 on aggrecan in the CEP. Proteoglycan staining using Safranin-O revealed a marked reduction of aggrecan in the SDF-1 group compared with the control group and the SDF-1 plus inhibitor group.

In addition to its role in ECM degradation, which involves the upregulation of MMP expression by chondrocytes, SDF-1 is known as a homing factor that induces the homing of stem/progenitor cells to the site of release, and therefore has therapeutic potential (23-25). The present study suggests that SDF-1 implantation has the ability to promote the regeneration of NP tissue during the degeneration process *in vivo*, despite SDF-1 inducing aggrecan degradation in endplate explant culture. Barkho *et al* (26) demonstrated that, in response to SDF-1, adult neural stem/progenitor cells differentiated into migratory cells with increased levels of MMP-3 and -9 expression, and that blocking the expression of MMP-3 or -9 in adult neural stem/progenitor cells significantly reduced chemokine-induced cell migration. Son *et al* (24) observed that MSCs homed to sites of tissue injury following signaling cues regulated by a gradient of SDF-1 in an MMP-dependent manner. Therefore, the degeneration or remodeling of ECM in endplate cartilage induced by SDF-1-associated inflammation may enhance the migration of exogenous stem cells toward the NP. In bone and cardiovascular tissues, the control of inflammation has been shown to be critical in shifting the degeneration/regeneration toward regeneration (27-29). It has been suggested that novel therapies for IVD degeneration should aim to restore the homeostatic inflammatory conditions in the disc, rather than to completely inhibit inflammation, thus enabling endogenous repair mechanisms (30).

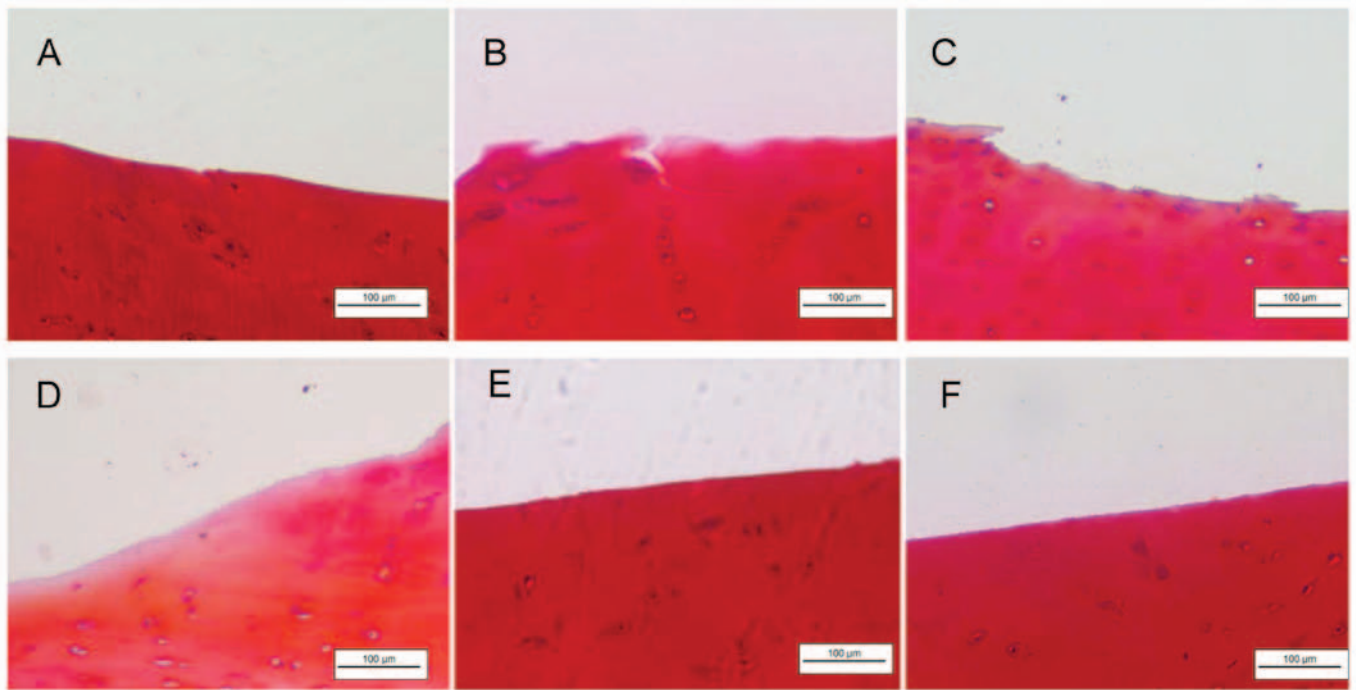


Figure 6. Safranin-O staining of sections showing aggrecan in cartilage endplate explant cultured with various factors. (A) Control group; (B) 50 ng/ml SDF-1; (C) 100 ng/ml SDF-1; (D) 200 ng/ml SDF-1; (E) 500 ng/ml AMD3100; (F) 100 ng/ml SDF-1 + 500 ng/ml AMD3100. SDF-1, stromal cell-derived factor-1.

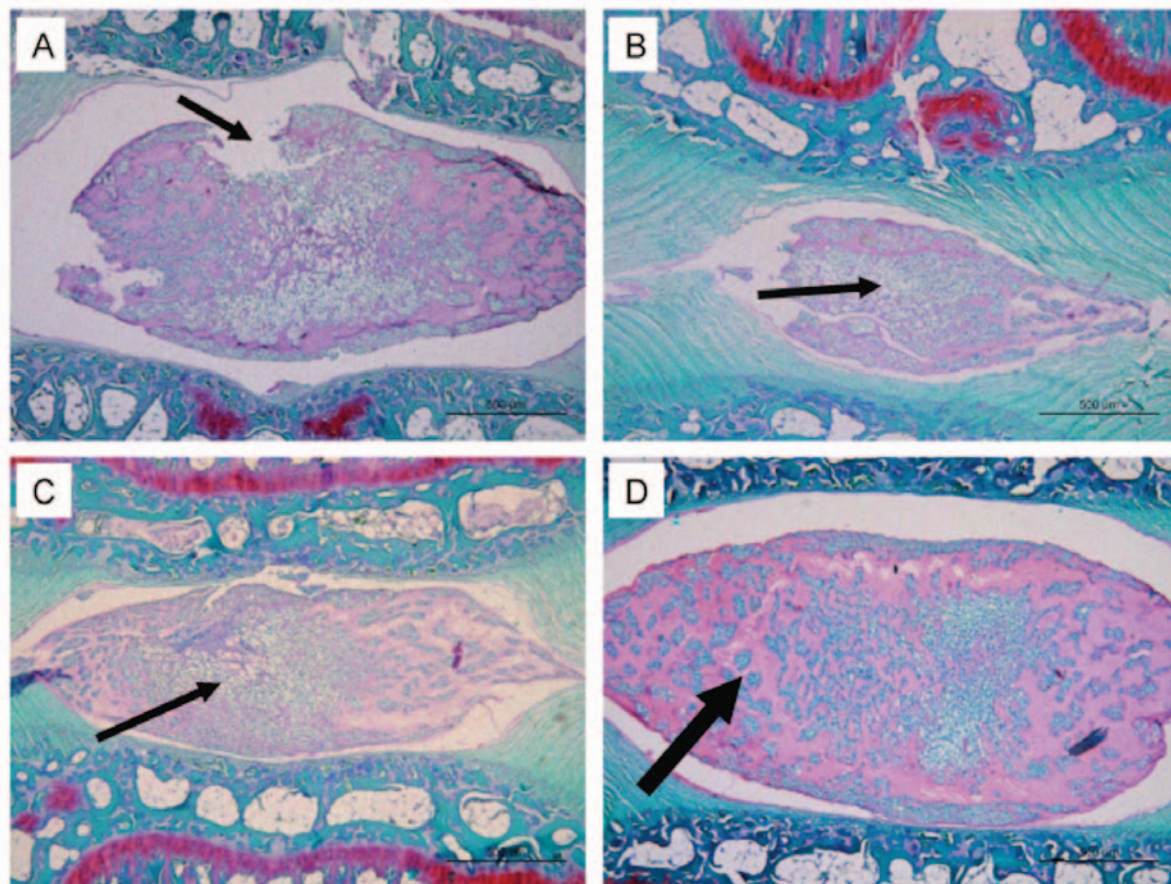


Figure 7. Safranin-O and Fast Green staining of sections showing aggrecan degradation and morphological changes in the rat NP injected with 3 µl PBS containing various factors. (A) None; (B) 500 ng/ml AMD3100; (C) 500 ng/ml AMD3100 + 200 ng/ml SDF-1; (D) 200 ng/ml SDF-1 alone. (A) Large amounts of aggrecan degradation were detected the center of the NP with some cleavage (thin black arrow) in the periphery. More proteoglycan degradation (thin black arrow) was observed in (B and C), in which AMD3100 was implanted, compared with (A). Following the injection of PBS containing SDF-1 alone, the morphology of NP was similar to that of native controls, and dense areas of proteoglycan matrix (thick black arrow) were maintained separating the NP cells. NP, nucleus pulposus; SDF-1, stromal cell-derived factor-1.

In a previous study, a recombinant adenoviral vector carrying the SDF-1 transgene was constructed and applied to transduce a novel scaffold-free living hyaline cartilage graft (SDF-t-LhCG) (31). The results indicated that the SDF-1-induced activation and recruitment of endogenous stem cells was augmented in SDF-t-LhCG implants. Due to the increased supply of endogenous stem cells recruited by SDF-1, enhanced chondrogenesis was observed in the SDF-t-LhCG implants *in situ*. In another study, full-thickness bovine chondral defects were filled with hydrogel containing recombinant human SDF-1 α , and increased cell migration followed by chondrogenic induction occurred (32). These results demonstrated that rhSDF-1 α markedly improved the recruitment of migratory chondrogenic progenitor cell to defects. Furthermore, cartilage generated in rhSDF-1 α -containing defects exhibited significantly greater interfacial strength than controls, and acquired mechanical properties comparable with those of native cartilage. Chen *et al* (33) reported that cartilage regeneration with SDF-1 effectively promoted BMSC migration and the repair of cartilage defects.

Thus, previous studies have indicated that SDF-1 is a promising therapeutic molecule that may make a significant contribution to MSC migration, and the results of the present study raise the possibility of using SDF-1 implants in the NP, and its homing action as an MSC chemoattractant, to promote IVD regeneration.

Acknowledgements

The present study was financially supported by the National Natural Science Foundation of China (NSFC; grant no. 81672246).

References

- Chan WC, Sze KL, Samartzis D, Leung VY and Chan D: Structure and biology of the intervertebral disk in health and disease. *Orthop Clin North Am* 42: 447-464, 2011.
- Li Z, Peroglio M, Alini M and Grad S: Potential and limitations of intervertebral disc endogenous repair. *Curr Stem Cell Res Ther* 10: 329-338, 2015.
- Roughley PJ: Biology of intervertebral disc aging and degeneration: Involvement of the extracellular matrix. *Spine* 29: 2691-2699, 2004.
- Goupille P, Jayson MI, Valat JP and Freemont AJ: Matrix metalloproteinases: The clue to intervertebral disc degeneration? *Spine* 23: 1612-1626, 1998.
- Pockert AJ, Richardson SM, Le Maitre CL, Lyon M, Deakin JA, Buttle DJ, Freemont AJ and Hoyland JA: Modified expression of the ADAMTS enzymes and tissue inhibitor of metalloproteinases 3 during human intervertebral disc degeneration. *Arthritis Rheum* 60: 482-491, 2009.
- Zhang H, Zhang L, Chen L, Li W, Li F and Chen Q: Stromal cell-derived factor-1 and its receptor CXCR4 are upregulated expression in degenerated intervertebral discs. *Int J Med Sci* 11: 240-245, 2014.
- Kanbe K, Takagishi K and Chen Q: Stimulation of matrix metalloproteinase 3 release from human chondrocytes by the interaction of stromal cell-derived factor 1 and CXCR4 chemokine receptor 4. *Arthritis Rheum* 46: 130-137, 2002.
- Chiu YC, Yang RS, Hsieh KH, Fong YC, Way TD, Lee TS, Wu HC, Fu WM and Tang CH: Stromal cell-derived factor-1 induces matrix metalloproteinase-13 expression in human chondrocytes. *Mol Pharmacol* 72: 695-703, 2007.
- Kanbe K, Takemura T, Takeuchi K, Chen Q, Takagishi K and Inoue K: Synovectomy reduces stromal-cell-derived factor-1 (SDF-1) which is involved in the destruction of cartilage in osteoarthritis and rheumatoid arthritis. *J Bone Joint Surg Br* 86: 296-300, 2004.
- Seta N and Kuwana M: Human circulating monocytes as multipotential progenitors. *Keio J Med* 56: 41-47, 2007.
- Krause DS: Plasticity of marrow-derived stem cells. *Gene Ther* 9: 754-758, 2002.
- Sakai D, Nishimura K, Tanaka M, Nakajima D, Grad S, Alini M, Kawada H, Ando K and Mochida J: Migration of bone marrow-derived cells for endogenous repair in a new tail-looping disc degeneration model in the mouse: A pilot study. *Spine J* 15: 1356-1365, 2015.
- Illien-Jünger S, Pattappa G, Peroglio M, Benneker LM, Stoddart MJ, Sakai D, Mochida J, Grad S and Alini M: Homing of mesenchymal stem cells in induced degenerative intervertebral discs in a whole organ culture system. *Spine* 37: 1865-1873, 2012.
- Pereira CL, Gonçalves RM, Peroglio M, Pattappa G, D'Este M, Eglon D, Barbosa MA, Alini M and Grad S: The effect of hyaluronan-based delivery of stromal cell-derived factor-1 on the recruitment of MSCs in degenerating intervertebral discs. *Biomaterials* 35: 8144-8153, 2014.
- Pfirrmann CW, Metzendorf A, Zanetti M, Hodler J and Boos N: Magnetic resonance classification of lumbar intervertebral disc degeneration. *Spine* 26: 1873-1878, 2001.
- Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25: 402-408, 2001.
- Chen P, Zhu S, Wang Y, Mu Q, Wu Y, Xia Q, Zhang X, Sun H, Tao J, Hu H, *et al*: The amelioration of cartilage degeneration by ADAMTS-5 inhibitor delivered in a hyaluronic acid hydrogel. *Biomaterials* 35: 2827-2836, 2014.
- Guide for the Care and Use of Laboratory Animals. NIH Publication No. 85-23. Revised 1985.
- Mao HJ, Chen QX, Han B, Li FC, Feng J, Shi ZL, Lin M and Wang J: The effect of injection volume on disc degeneration in a rat tail model. *Spine* 36: E1062-E1069, 2011.
- Wuelling M and Vortkamp A: Cartilage explant cultures. *Methods Mol Biol* 1130: 89-97, 2014.
- Minina E, Wenzel HM, Kreschel C, Karp S, Gaffield W, McMahon AP and Vortkamp A: BMP and Ihh/PTHrP signaling interact to coordinate chondrocyte proliferation and differentiation. *Development* 128: 4523-4534, 2001.
- Song RH, Tortorella MD, Malfait AM, Alston JT, Yang Z, Arner EC and Griggs DW: Aggrecan degradation in human articular cartilage explants is mediated by both ADAMTS-4 and ADAMTS-5. *Arthritis Rheum* 56: 575-585, 2007.
- Grunewald M, Avraham I, Dor Y, Bachar-Lustig E, Itin A, Jung S, Chimenti S, Landsman L, Abramovitch R and Keshet E: VEGF-induced adult neovascularization: Recruitment, retention, and role of accessory cells. *Cell* 124: 175-189, 2006.
- Son BR, Marquez-Curtis LA, Kucia M, Wysoczynski M, Turner AR, Ratajczak J, Ratajczak MZ and Janowska-Wieczorek A: 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* 24: 1254-1264, 2006.
- Wynn RF, Hart CA, Corradi-Perini C, O'Neill L, Evans CA, Wraith JE, Fairbairn LJ and Bellantuono I: A small proportion of mesenchymal stem cells strongly expresses functionally active CXCR4 receptor capable of promoting migration to bone marrow. *Blood* 104: 2643-2645, 2004.
- Barkho BZ, Munoz AE, Li X, Li L, Cunningham LA and Zhao X: Endogenous matrix metalloproteinase (MMP)-3 and MMP-9 promote the differentiation and migration of adult neural progenitor cells in response to chemokines. *Stem Cells* 26: 3139-3149, 2008.
- Claes L, Recknagel S and Ignatius A: Fracture healing under healthy and inflammatory conditions. *Nat Rev Rheumatol* 8: 133-143, 2012.
- Mountziaris PM, Spicer PP, Kasper FK and Mikos AG: Harnessing and modulating inflammation in strategies for bone regeneration. *Tissue Eng Part B Rev* 17: 393-402, 2011.
- Boccafroschi F, Mosca C and Cannas M: Cardiovascular biomaterials: When the inflammatory response helps to efficiently restore tissue functionality? *J Tissue Eng Regen Med* 8: 253-267, 2014.
- Molinos M, Almeida CR, Caldeira J, Cunha C, Gonçalves RM and Barbosa MA: Inflammation in intervertebral disc degeneration and regeneration. *J R Soc Interface* 12: 20141191, 2015.
- Zhang F, Leong W, Su K, Fang Y and Wang DA: A transduced living hyaline cartilage graft releasing transgenic stromal cell-derived factor-1 inducing endogenous stem cell homing in vivo. *Tissue Eng Part A* 19: 1091-1099, 2013.
- Yu Y, Trouillette MJ, Seol D, Zheng H, Buckwalter JA and Martin JA: Functional full-thickness articular cartilage repair by rhSDF-1 α loaded fibrin/HA hydrogel network via chondrogenic progenitor cells homing. *Arthritis Rheumatol* 67: 1274-1285, 2015.
- Chen P, Tao J, Zhu S, Cai Y, Mao Q, Yu D, Dai J and Ouyang H: Radially oriented collagen scaffold with SDF-1 promotes osteochondral repair by facilitating cell homing. *Biomaterials* 39: 114-123, 2015.