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Attenuation of subchondral bone abnormal changes in osteoarthritis by inhibition of SDF-1 signaling

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SUMMARY

Background: Current conservative treatments for osteoarthritis (OA) are largely symptoms control therapies. Further understanding on the pathological mechanisms of OA is crucial for new pharmacological intervention.
Objective: In this study, we investigated the role of Stromal cell-derived factor-1(SDF-1) in regulating subchondral bone changes during the progression of OA.
Methods: Clinical samples of different stages of OA severity were analyzed by histology staining, micro-CT, enzyme-linked immunosorbent assay (ELISA) and western blotting, to compare SDF-1 level in subchondral bone. The effects of SDF-1 on human mesenchymal stem cells (MSCs) osteogenic differentiation were evaluated. *In vivo* assessment was performed in an anterior cruciate ligament transaction plus medial meniscus resection in the SD rats. The OA rats received continuous infusion of AMD3100 (SDF-1 receptor blocker) in osmotic mini-pump implanted subcutaneously for 6 weeks. These rats were then terminated and subjected to the same *in vitro* assessments as human OA samples.
Results: SDF-1 level was significantly elevated in the subchondral bone of human OA samples. In the cell studies, the results showed SDF-1 plays an important role in osteogenic differentiation of MSCs. In the OA animal studies, there were less cartilage damage in the AMD3100-treated group; microCT results showed that the subchondral bone formation was significantly reduced and so did the number of positive Nestin or Osterix cells in the subchondral bone region.
Conclusions: Higher level of SDF-1 may induce the subchondral bone abnormal changes in OA and inhibition of SDF-1 signaling could be a potential therapeutic approach for OA.

Introduction

Osteoarthritis (OA) is one of the leading cause of physical disability affecting nearly 80% of the individuals aged 75 years or over[1]. Current pharmacologic therapies mainly target at symptoms controlling and their efficacy in altering the progression of OA are disappointing. Further understanding of the pathological mechanisms of OA development is crucial for the design of the new pharmacological intervention.

Various inflammatory cytokines including interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and NF-κB signal, attribute to

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the development and progress of OA[2–8]. Articular cartilage degeneration is the main feature in OA and many factors such as matrix metalloproteinase 13 (MMP13)[9], collagen fragments (C2C)[10] as well as nerve growth factor (NGF)[11] are involved. The homeostasis and integrity of articular cartilage rely on its biochemical and biomechanical interplays with the subchondral bone and the surrounding soft tissues[12]. Subchondral bone provides mechanical support for the articular cartilage and constantly undergoes bone remodeling[13]. Bone marrow lesions are closely associated the severity of cartilage damage and pain in OA[14]. However, the relationship between the abnormal formation of subchondral bone and progression of OA still remains largely unknown.

The role of stromal derived factor-1 (SDF-1) in the pathogenesis of OA or rheumatoid arthritis (RA) has drawn increasing attention in recent years[4,15–17]. A dramatic elevation of SDF-1 is found in the knee synovium from RA and OA patients[4]. SDF-1 has been shown that it could regulate chondrocyte catabolic activities by stimulating the release of MMP-3 and MMP-13[4]. Synovectomy could significantly reduce the concentrations of SDF-1, MMP-9, and MMP-13 in blood serum[15]. In animal study, blockage of SDF-1 signaling pathway using AMD3100, a CXCR4 antagonist of the SDF-1 receptor, attenuated the cartilage degeneration[18]. SDF-1 is a well-known factor that regulates the mesenchymal stem cells (MSCs) function[19–21] by mediating bone morphogenetic protein (BMP) and osteogenic differentiation of MSCs[22,23]. Furthermore, in 2006 Lisignoli G and colleagues demonstrated that SDF-1 could significantly induce proliferation and collagen type I expression in osteoblasts from OA patients[17]. This study implies that SDF-1 may play a role in the abnormal changes in subchondral bone with OA. SDF-1 influences cartilage degeneration through stimulating the release of MMPs from chondrocytes, however, whether SDF-1 contributes to subchondral bone changes in OA is still not fully understood. Therefore, the objective of this study is to investigate the role of SDF-1 in subchondral bone changes during OA development.

Materials and methods

Clinical sample collection

This study was approved by the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee and informed consent was obtained from each donor. All experiments were carried out in accordance with the research guidelines of the Chinese University of Hong Kong. The clinical specimens were obtained from patients with OA at the time of total knee arthroplasty surgery (n = 12; 8 women and 4 men; age 65.8 ± 6.5 years, range 49–76 years). Various regions of the knee joint were harvested and the samples were immediately placed into sterile DMEM culture medium and transported to the laboratory for further processing. Samples were divided into two parts; OA group where cartilage was severely damaged or fibrillated with OARSI score of 15–18; and relatively normal (RN) group, where the cartilage is non-fibrillated with OARSI scores of 0–3. The cartilage and subchondral bone explants were cut from OA or normal sites of the OA joint [Fig. 1(a)] were cut into the size of 1.5 × 0.5 cm full-thickness and subjected to future experiments.

Bone marrow MSCs culture

Human fetal bone marrow stem cells (hBM-MSCs) were obtained from the Stem Cell Bank at the Prince of Wales Hospital of the Chinese University of Hong Kong. Ethical approval was obtained from the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee (ethical approval code: CRE-2011.383). Informed written consent form was approved by the Clinical Research Ethics Committee and signed by the donor before sample collection. All experiments were carried out in accordance with the research guidelines of the Chinese University of Hong Kong. These cells were cultured in the complete alpha-minimum essential medium (α-MEM, Manassas, Virginia, USA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin–neomycin (complete culture medium; all from Invitrogen Corporation, Carlsbad, CA, USA) in a 5% CO_2 humidified incubator at 37°C.

In MSCs osteogenic induction, the MSCs were trypsinized and seeded in 6-well plate at a concentration of 1 × 10^5 cells per well. These cells were incubated in the α-MEM for two or 3 days, the medium was then replaced by osteogenic induction medium (OIM) which contains 100 nmol/L dexamethasone, 10 mmol/L beta-glycerophosphate and 0.05 mmol/Ll-ascorbic acid-2-phosphate.

To block the SDF-1 signaling in primary MSCs, cells were incubated with AMD3100, a CXCR4 antagonist, which selectively binds to CXCR4 and prevents the binding of SDF-1[24]. The working concentration of AMD3100 (Sigma, StLouis, MO, USA) in this study was 400 μM, which has been shown to effectively inhibit SDF-1 signaling without toxicity[22–24].

Animal experiments

All experiments were approved by the Animal Research Ethics Committee, the Chinese University of Hong Kong. 16-week old Male Sprague–Dawley (SD) rats, with the weight of 450–500 g, were used in this study. All rats received anterior cruciate ligament transaction plus medial meniscus resection (ACLT + MMx) at the right knee as previously described[25]. In brief, each rat was anesthetized by administrating 0.2% (vol/vol) xylazine and 1% (vol/vol)

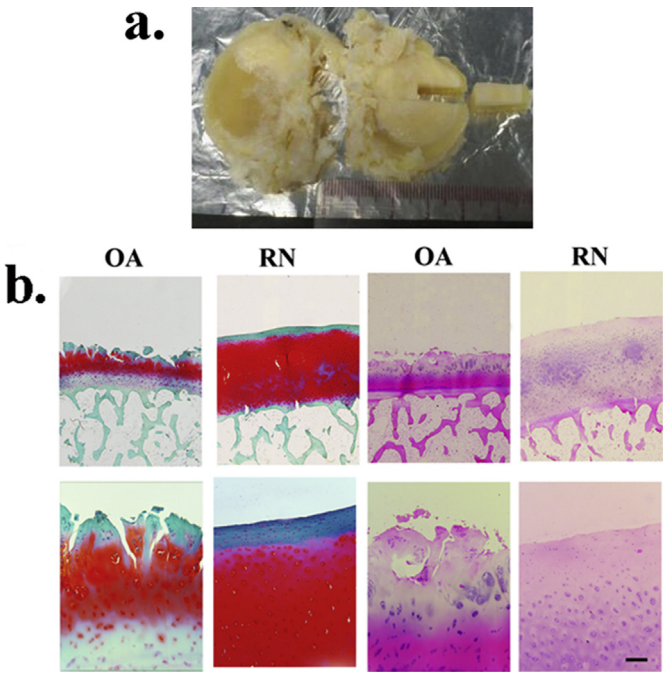

Fig. 1. (a) Gross appearance of tibial plateau of the samples obtained from patients at the time of total joint arthroplasty. On the OA part, the cartilage was severely fibrillated or damaged and from the more affected compartment; and on the RN part, the cartilage was RN or nonfibrillated which was from the uninvolved compartment. (b) Safranin-O/fast green and H&E staining of sagittal sections of the subchondral tibia in the OA and RN compartments of the same OA patient. Scale bar, 200 μm (in top), 50 μm (in bottom).