The Effect Of Stromal Cell-derived Factor 1 On Vascular Endothelial Growth Factor (VEGF) Expression In Cartilage Related Cells

Shuya Wang¹, Hongjun Zheng, PhD², Cheng Zhou, MS², Yin Yu, MS², Yifang Mei, PhD¹, James A. Martin, PhD².

¹The First Affiliated Hospital of Harbin Medical University, Harbin, China, ²Orthopaedics and Rehabilitation, University of Iowa, Iowa city, IA, USA.

Disclosures:

Introduction: Osteoarthritis (OA) is a chronic joint disorder which causes pain, stiffness, reduced motion, and disability. It is characterized by slow progressive degeneration of articular cartilage with joint-space narrowing, subchondral bone alteration, and synovitis. The exact etiology of OA is still not well understood. It was reported that vasculatures are crucial for skeletal development during all the stages, the embryonic phase, postnatal growth and bone remodeling. Abnormal blood vessel formations at the cartilage-subchondral bone interface may contribute to the progression of OA[1]. Vascular endothelial growth factor (VEGF) is a major regulator of vasculogenesis and angiogenesis. It plays a vital role in regeneration of tissue[2] showing that VEGF can affect chondrocytic proliferation, apoptosis and metabolism, which leads to release of metalloproteinases (MMPs), as well as other mediators that contribute to cartilage matrix degradation [3-5]. Stromal cell-derived factor 1 (SDF-1) is a chemokine isolated from bone marrow stromal cells. In normal human CD34+ cells and megakaryoblasts, SDF-1 can stimulate production of VEGF. Dramatic increase of SDF-1 was observed in synovial fluid of OA patients, suggesting that SDF-1 may impact the progression of OA. In our previous studies, it showed chondrogenic progenitor cells (CPCs) on the surface of cartilage have higher expression of SDF-1 comparing to normal chondrocytes. Based on these findings we hypothesized that CPCs have greater potential to contribute to intra-articular VEGF than chondrocytes or other joint cells. To test this we compared the effect of SDF-1 and AMD3100, an inhibitor of the SDF-1 receptor, on the expression of VEGF in normal chondrocytes (NCs), CPCs and synoviocytes.

Methods: Cell isolation and cultivation: CPCs were harvested from bovine cartilage explants. Briefly, scratching injuries were used to stimulate the activation and proliferation of CPCs; CPCs were isolated by trypsinized 7-10 days after injuries. The underlying cartilages were digested with protease (0.4% for 1.5hrs) and collagenase (0.02% for 16 hrs) to isolate NCs, synoviocytes were harvested from the same bovine knee joint.

Cell stimulation: Cells were seeded in 6-well plate (0.3×10⁶ cells/cm²). After two-day culture, followed by 24hrs starvation in serum free medium, cells were treated with IL-1 β (10ng/ml), SDF-1 β (20ng/ml, 50ng/ml and 100ng/ml) for 24hrs, respectively. To examine the SDF-1/CXCL12 signaling pathway involved in SDF-1 β treatment, cells were pretreated with AMD3100 (200ng/ml) (SDF-1 receptor CXCR4 specific inhibitor) for 2hrs before addition of SDF-1 β.

Real-time polymerase chain reaction (RT-PCR): Total RNA from CPCs, NCs and synoviocytes was extracted with the RNeasy Mini Kit. Two steps RT-PCR analysis was carried out by using TaqMan reverse transcription reagents and SYBR green PCR Master Mix.

Statistical analysis: All data were presented as mean ± standard error. Significant differences among groups were assessed by one-way analysis of variance. Statistical analyses were performed using SPSS software. The difference was considered significant if the P-value was <0.05.

Results: Results of RT-PCR shows that SDF-1 β (20ng/ml & 50ng/ml) can significantly increase VEGF expression in CPCs (2.05 folds & 3.64 folds, respectively) (Figure 1 B). Lower concentration of SDF-1 β (20ng/ml) can also increase the expression of VEGF in synoviocytes (Figure 1 C). However, higher concentration of SDF-1(100 ng/ml) has no effect on VEGF expression in both CPCs and synoviocytes. The effect of SDF-1 on VEGF expression is impaired by SDF-1 receptor inhibitor AMD3100. While the results exhibited that SDF-1 β has no effect on VEGF expression in NCs (Figure 1 A).
Figure 1. VEGF expression analysis.
A: For NCs there is no difference among different treatment. B: For CPCs SDF-1 β20ng/ml and SDF-1 β50ng/ml could dramatically increase the expression of VEGF. Pre-treated with AMD3100 reduced SDF-1 β (20ng/ml & 50ng/ml)-increased VEGF expression.
C: For synoviocytes SDF-1 β20ng/ml increase VEGF expression.*p<0.05 as compared with basal level. #p<0.05 as compared with SDF-1 β-treated group.

Discussion: It has been thought that chondrocytes are the singular cell source of articular cartilage. However it is reported that repair tissue from human articular cartilage during the late stages of OA harbors a unique progenitor cell type, termed chondrogenic progenitor cells (CPCs). The results of this study confirmed that SDF-1β 20ng/ml and 50ng/ml can result in up-regulation of VEGF expression in CPCs and AMD3100 can weaken this effect. It was reported that SDF-1 may cause apoptosis of chondrocytes [6], so we speculate that high-concentration of SDF-1 β may also lead to the damage of CPCs and that may be the reason that there is no difference for NCs among different treatments. VEGF, as a potent mitogen and angiogenic factor, has been considered as a key component in the vascularization of bone tissues, along with high expression both in the lower hypertrophic and mineralized zones of the cartilage and vasculogenesis, all of which are important steps toward ossification, ultimately leading to OA. SDF-1 is elevated in the synovial fluid of OA patients, contributing to the high expression of VEGF in CPCs. So we confirm that SDF-1-increased VEGF expression in CPCs and synoviocytes which may play a role in the pathogenesis of OA.

Significance: This study compared the effect of SDF-1-increased VEGF expression among NCs, CPCs and synoviocytes. SDF-1 has a prominent effect on VEGF expression in CPCs. This may provide a new target therapy of OA.

Acknowledgments:

References:

ORS 2014 Annual Meeting
Poster No: 1252