J Musculoskelet Neuronal Interact 2008; 8(4):316-317

38th International Sun Valley Workshop
August 3-6, 2008
ASBMR/Harold M. Frost Award Presentations

Hylonome

Blocking VEGF as a potential approach to improve cartilage healing after osteoarthritis

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Keywords: Muscle Derived Stem Cells, Osteoarthritis, VEGF, BMP4

Background

Osteoarthritis (OA), a chronic degenerative joint disorder with worldwide impact, is characterized by articular cartilage destruction and osteophyte formation. Since articular cartilage is a tissue type that is poorly supplied by blood vessels, nerves, and the lymphatic system, it has a very limited capacity for repair after injury. Although several therapies have been used to treat OA, no widely accepted treatment methods have been established, with the exception of arthroplasty. Stem cells are attractive sources because of their superior capacity for self-renewal, proliferation, and survival following environmental stress. Several studies have suggested that stem cells can undergo chondrogenesis and repair articular cartilage in experimental cartilage injury models, including studies using muscle-derived stem cells (MDSCs)[1-4]. We have already reported that bone morphogenetic protein 4 (BMP4)-transduced MDSCs improves cartilage regeneration in the *in vitro* pellet culture and the *in vivo* cartilage defect model[2]. Based on those results, this series of experiments was designed to clarify the therapeutic efficacy of BMP4 transduced MDSCs for OA.

The control of angiogenesis during the chondrogenic differentiation of stem cells is one of the most important issues surrounding the application of stem cells to cartilage repair. Among angiogenesis-modulating factors, vascular endothelial growth factor (VEGF) is an important mediator of angiogenesis. Therefore, in the current study, we used a gain- and loss-of-function approach based on tissue engineering techniques to assess the role of VEGF in MDSC-mediated cartilage repair.

Methods

Mouse MDSCs were obtained from three-week old male wild type mice using a modified preplate technique[5]. The effect of VEGF on chondrogenesis using a chemical induced OA model in 10-week-old female nude rats was tested using genetically engineered MDSCs. In this model, MDSCs, transduced with a retroviral vector to express BMP4-GFP (BMP4-MDSCs), were co-injected in the joint capsule with MDSCs transduced to express either VEGF-LacZ (VEGF-MDSCs) or sFlt1-LacZ (a VEGF antagonist) (sFlt1-MDSCs) to test gain- and loss-of VEGF function (5×10^5 cells total). The effect of VEGF and blocking VEGF on the *in vitro* chondrogenic ability of MDSCs was also tested using a mixed pellet co-culture system followed by gross and histological analyses (transduced-MDSCs and OA chondrocytes) (2×10^5 cells total).

Results

In vivo examination of articular cartilage regeneration showed macroscopically and histologically that sFlt1-MDSCs improved, and VEGF-MDSCs prevented, the BMP4-MDSC regeneration of articular cartilage compared to the BMP4-MDSCs alone, with higher histological score in the sFlt1/BMP4-MDSC group than the other groups at week 12 ($p<0.05$). Double immunohistochemistry (IHC) of collagen type 2 (col2) and GFP or β-galactosidase (β-gal) at week 4 demonstrated that sFlt1-MDSCs improved,and VEGF-MDSCs prevented, chondrogenic differentiation and intrin-

The authors have no conflict of interest.

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Accepted 11 August 2008

sic chondrogenesis compared to BMP4-MDSCs alone ($p<0.05$). TUNEL stain and BrdU assay at week 4 demonstrated that sFlt1-MDSCs, unlike VEGF-MDSCs, lead to less apoptosis and more proliferation compared to BMP4-MDSCs alone ($p<0.05$). *In vitro* mixed co-culture showed that the BMP4MDSCs produced significantly larger pellets with hyaline cartilage-like matrix production than all other groups ($p<0.05$), which was also confirmed by the chondrogenic differentiation capacity of MDSCs using quantitative double IHC of col2 and GFP or β-gal. Fluorescent *in situ* hybridization showed no fusion between OA chondrocytes and MDSCs and higher intrinsic chondrogenesis in the BMP4-MDSC group ($p<0.05$).

Conclusions

Gene-based therapy using sFlt1 and BMP4-transduced MDSCs enhanced intrinsic chondrogenesis and chondrogenic differentiation of MDSCs via BMP4 secretion, and contributed to an appropriate environment which led to decreased apoptosis of chondrocytes by blocking VEGF, resulting in cartilage regeneration and healing in the OA model.

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