Autonomous TGF-β1 Suppression Improves BMP-dependent Cell-based Articular Cartilage Repair in a Rat model

Ping Guo1, Xueqin Gao1, Lara Pferdehirt1, Aiping Lu2, Matthieu Huard2, Naoki Nakayama2, Farshid Guilak2* and Johnny Huard2*

1Center for Regenerative & Personalized Medicine, Steadman Philippon Research Institute, Vail, Colorado, USA.
2Department of Orthopaedic Surgery, Washington University, St. Louis, Missouri, USA. *Corresponding author

Introduction: Articular cartilage is an important avascular tissue that, once damaged, does not spontaneously heal and often leads to osteoarthritis (OA). One of the strategies to improve such repair outcomes has been to enhance chondrogenic signaling in vivo, using transforming growth factor beta (TGF-β) and bone morphogenetic proteins (BMP). However, BMP over dosage leads to ectopic ossification, hypertrophic differentiation of chondrocytes and osteophyte formation, and TGF-β alone results in synovial hyperplasia and joint fibrosis, leading to cartilage degeneration and osteophyte formation. To eliminate the deleterious effect of TGF-β, we created a "smart" cell-based drug delivery system using CRISPR-Cas9 to genetically engineer muscle-derived stem cells (MDSCs) capable of sensing TGF-β and producing an antiinflammatory, antiangiogenic product in vivo, without changing the expression of myogenic and stem cell markers, cell proliferation or myogenic differentiation [1]. MDSCs can repair AC damage when transduced with retroviral-BMP4 [2]. In this study, we tested whether Smart MDSCs producing decorin (Dec-MDSC) could improve cartilage repair when co-transplanted with BMP4-expressing MDSCs. We hypothesized that TGF-β1-deletion of decorin from Dec-MDSCs will facilitate cell-based cartilage repair in synergy with BMP4.

Methods: Genome-engineered muscle-derived stem cells for autoregulated anti-inflammation and antiangiogenesis. As previously described, the MDSCs were genetically knocked in of decorin open reading frame (ORF) into the Smad7 locus under the Smad7 promoter. The luciferase ORF was knocked in as a control [1]. Dec-MDSC and BMP4-MDSC chondrogenesis in vitro. Four groups of cells were used for chondrogenesis experiments: 1: BMP4-MDSC, 2: BMP4-MDSC + Smart Dec-MDSC, 3: BMP4-MDSC+ Luc-MDSC, and 4: Smart Dec-MDSC. All cells were culture expanded in proliferation medium. The BMP4 secretion level of BMP4-MDSC is approximately 19ng/10^6 cells/24hrs, measured by ELISA as previously reported [3]. 3D chondrogenic pellets culture was performed as previously reported [4]. 1.25x10^5 cells were used for each pellet. For the BMP4-MDSC group, only BMP4-MDSC is used. For group 2 and 3, 6.125x10^4 BMP4-MDSC and Smart BMP4-MDSC or Luc-MDSC were mixed. For group 4, 1.25x10^5 Dec-MDSCs were used. 3-4 replicate pellets were prepared for each group. Pellets were cultured at 24 hr-intervals for 14 days for the MDSC group and 21 days for the BMP4-MDSC group, then rinsed one time with PBS and gross imaged. Pellets sizes were measured using Image J using a reference ruler as scale. Pellets were further embedded in NEG freezing medium. Cryosections at 8µm were cut for Alcian blue and Safranin O staining. In vivo transplantation using osteochondral defect model: Eight-week-old male NIH Nude rats (NIHRN admission to the U.S. National Cancer Institute) were used for the experiments. Animal protocol was approved by the Colorado State University Animal Care and Use Committee. Briefly, the rats were anesthetized with 2-3% isoflurane, cylindrical osteochondral defects of 1.5 mm diameter and 1.0 mm depth were created in the center of the trochlear groove of distal femur in the right knee using a 1.8 mm trephine drill. The left knee was used as the control.

Cell transplantation. Seven rats per group were assigned for the cell transplantation experiment, and PBS was used as the control. 6 groups of male rats were transplanted with BMP4-MDSC, Luc-MDSC, Dec-MDSC, Luc-MDSC + 1/5 BMP4-MDSC and Dec-MDSC + 1/5 BMP4-MDSC. Chondrogenic pellets were made with different MDSCs (1.25x10^6 total cells) and cultured for three days and transplanted to the osteochondral defect made with trephine drill, and immediately sealed with fibrin sealant hydrogel (Tissel Fibrin Sealant). Six weeks post cell pellets transplantation, rats were sacrificed, and the injured knee joints were harvested, and the distal femur were dissected and fixed for 6 days in NBF. MicroCT imaging was performed using Viva-CT 80 (Scanco Medical) for the entire distal femur using 30μm voxel size, 70KV and 114μA. After MicroCT scanning, the distal femur was decalcified (5% formic acid for 14 days), processed, paraffin embedded, and sectioned for histological analysis. Alcian blue and safranin O stain were performed using H&E protocol to examine the cartilage matrix.

Results: Chondrogenesis results. The pellets sizes measurements indicated the sizes of BMP4-MDSC + Smart Dec-MDSC cells pellets were similar as BMP4-MDSC group. The Smart Dec-MDSC cells group showed a relatively smaller pellet size (P<0.073). No significant differences were found between any other groups (Fig. A1 and D). Alcian blue staining demonstrated BMP4-MDSC, BMP4-MDSC + Smart Dec-MDSC and BMP4-MDSC + Luc-MDSC groups formed compact pellets with few scattered Alcian blue positive cells, while Smart Dec-MDSC group showed loose pellets with few Alcian blue positive cells detected in any pellets. No difference between BMP4-MDSC + Smart Dec-MDSC group and BMP4-MDSC group and BMP4-MDSC + Luc-MDSC group (Fig. 1B). Safranin O staining showed minimum orange red staining in all groups. No significant differences between BMP4-MDSC + Smart Dec-MDSCs and BMP4-MDSC group and BMP4-MDSC group and BMP4-MDSC group were observed (Fig. 1C). Smart cells enhanced BMP4-MDSC chondrogenesis in vivo without ectopic bone formation. Six weeks post cell transplantation, MicroCT results revealed that the osteochondral defect healed in BMP4-MDSCs group, but ectopic bone formation was observed. However, Dec-MDSC+BMP4-MDSCs group defect nearly healed without ectopic bone or osteophyte formation. In other groups, the defects showed incomplete defect healing (Fig. 2A). The gross views of the rat osteochondral defect repair in each group are shown in Fig. 2B. ICRS macro score quantification indicated that both BMP4-MDSCs and BMP4-MDSC + Dec-MDSCs significantly enhanced cartilage repair compared to PBS group (Fig. 2C, P=0.0072 and 0.0188). However, the BMP4-MDSC group showed ectopic bone formation and cartilage overgrowth, while Dec-MDSCs+BMP4 MDSCs showed significantly better cartilage repair without any ectopic bone formation. Other cell groups showed no significant improvement in cartilage repair than PBS control. Alcian blue and Safranin O staining results showed BMP4-MDSC group with strong blue staining, but the overgrowth cartilage was not well integrated. The Dec-MDSC+BMP4-MDSC group showed cartilage healing with natural cartilage curvature despite less intense blue staining in comparison to PBS group. Other groups showed superficial cartilage healing with large cysts beneath. Further histology analysis and histology scoring are ongoing. OARSI histology score indicated BMP4-MDSCs and Dec-MDSC+BMP4-MDSC groups showed significantly lower histology scores (better healing) for both groups compared to PBS group while other groups showed no significant improvement compared to PBS group (Fig. 3B).

Discussion: Using self-regulating suppression of TGF-β signaling in a time and location dependent manner represents a new strategy to enhance cartilage repair. Despite in vitro results did not show significant beneficial effects of Dec-MDSCs likely due to MDSCs growing too fast and the chondrogenic medium are the same in each group and has no excess TGF-β1. However, Dec-MDSCs promote cartilage repair of BMP4-MDSC without incurring ectopic bone formation by using BMP4-MDSCs alone as indicated by microCT, gross images and cartilage matrix staining and histology score evaluation. These results indicated that the Dec-MDSCs has the capacity of autonomously regulated TGF-β function after cartilage damage in vivo. The regulated TGF-β1-inhibition benefited the BMP-dependent cell-based cartilage repair by suppressing ectopic bone formation and promoting well integrated cartilage repair only in six weeks compared to controls in rats. Further longer time point will be needed to demonstrate more complete cartilage healing using this strategy. In conclusion, we demonstrated that autoregulated delivery of a TGF-β1 inhibitor, decorin, secreted from Dec-MDSCs can facilitate cell-based cartilage repair mediated by BMP4-MDSCs.

Significance: The autonomous TGF-β1 suppression using Dec-MDSC created by CRISPR/Cas9 technology represents a novel and innovative cell-based therapy for cartilage repair.

Acknowledgements: We thank Dr. Ehrhart and Laura for their help on this project. This study is supported by NIH (R21 AR079075).

ORS 2024 Annual Meeting Paper No. 196