Differential Chondrogenesis in Stem Cells with a Run-X1 Inducing Molecule

Todd A. Goldstein, John Schwartz, Adam Gitlin, Daniel Grande.
Feinstein Institute of Medical Research, Manhasset, NY, USA.

Disclosures:

Introduction: Currently, Osteoarthritis (OA) has a prevalence of ~30 million people in the United States alone; amounting to an annual cost of ~185 billion dollars.1 OA is characterized by the progressive destruction of articular cartilage.1 Lesions of Articular cartilage present a major challenge for clinicians and patients alike. Currently, we only manage the symptoms and have no viable treatments to target the underlying pathology of the disease.2 Furthermore, regenerative treatments of hyaline cartilage are not consistent in achieving quality repair.3 One major obstacle to successful cartilage repair arises from the avascular nature of cartilage. This lack of blood supply renders natural regenerative healing unfeasible as nutrients and factors required for regeneration are unable to sufficiently reach the cartilage.3 There is a recognized need for an early stage treatment of cartilage lesions that can prevent disease progression into OA.5 Advancements in cellular therapy technologies now allow for various regenerative treatments of the joint’s damaged cartilage. Examples are autologous chondrocyte transplantation (ACI), microfracture chondroplasty (MFX), stem cell implantation, and tissue engineered constructs. However, even with these advancements, certain hurdles still exist.4 Recently, a small molecule 2[(1,1' biphenyl)4ylcarbamoyl]benzoic acid (Kartogenin, KG) was found to be both cartilage protective and a chondrogenesis modulator. KG’s mechanism functions by binding to filamin A, disrupting the interaction of filamin A-CBFβ and inducing chondrogenesis via regulation of the CBFβRUNX1 transcriptional program.6 However, in vivo, the molecule is cleared rapidly and would require frequent injections to maintain a therapeutic effect.

We evaluated two different stem cell populations, adipose derived stem cells (ADSC) and mesenchymal stem cells (MSC), to compare the relative chondrogenic potential of KG. We hypothesized treatment with KG would promote chondrogenic differentiation of both stem cell types. Additionally we hypothesized that local delivery of KG to a cartilage defect would improve the quality of the tissue formed.

Methods: In Vitro: Mesenchymal & Adipose derived stem cells were isolated from male SpragueDawley Rats (SDR) and cultured. Each cell line was seeded at 3x103 cells for QPCR. Each cell type was treated with 0, 10, or 100nM, of KG respectively at experimental time points 24, 48, 72, & 144 hours. Gene expression analysis of: collagen type-I, collagen type-II, SOX9, Aggrecan, Cartilage oligomeric matrix protein (COMP) was performed, and normalized by Glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

In Vivo: Kartogenin was dip coated in gelatin and coated onto a Type I fibrillar collagen scaffold (KenseyNash, Philadelphia, PA) to promote local delivery at [100nM]. Each knee of the Spague-Dawley rats used in this study were positioned supine and after sterile preparation underwent a median parapatellar arthrotomy. A rotary drill (MicroAire, Charlottesville, VA) with a 1.5 mm drill bit was used to create a circular shaped osteochondral lesion midline on the trochlea of the femur down to bleeding bone. Knees were randomized to the following groups: (1) Empty control group, (2) collagen scaffold alone, and (3) collagen scaffold coated with Kartogenin (100 nM). Rats were allowed to resume normal activity then sacrificed at 8 weeks. Knees were harvested and embedded in paraffin for histology, cut on a microtome 5 uM thick and stained with Safranin O/Fast green. The specimens were histologically analyzed and scored for evidence of healing by two blinded reviewers.

Results: In Vitro: PCR analysis yielded an increase in expression of Sox9, Aggrecan, COMP, and Collagen Type II for both ADSC and MSC cellular lines. Expression of Collagen Type I either remained the same or decreased for both samples. In MSC’s sox9’s fold change increased 1.5 to 4x, Aggrecan increased 1-2x, and Collagen Type 2 increased 1 to 1.5x(Figure 1a). In ADSC’s Sox9’s fold change ranged from 1.5-2x increase, Aggrecan increased 1-1.5x, COMP, increased from 1-2.5x, and Collagen Type 2 increased 1-3.5x(Figure 1b). In Vivo: Control samples (Fig 2A) exhibited a fibrous to fibrocartilage repair tissue that was typically depressed below the surrounding articular cartilage surface with a predominant fibrous quality, yielding an average Modified O’Driscol score of 7.5 (Fig 3). Cells were fibroblastoid in shape and the tissue hypercellular compared to the surrounding intact cartilage matrix. There was little to no SafraninO positive staining in the repair tissue indicating a lack of aggrecan present. Kartogenin treated defects (Fig. 2B) typically filled the entire defect with repair tissue congruent with the surrounding native tissue with a cellularity typical of cartilage tissue with a smooth articular surface, yielding an average Modified O’Driscol score of 20.9 (Fig 3). The repair tissue contained numerous islands of Safranin-O positive staining throughout the tissue indicating cells undergoing differentiation down the cartilage lineage.

Discussion: Our results demonstrate that Kartogenin facilitates the differentiation of MSCs and ADSCs towards a hyaline cartilage phenotype at comparable levels. MSCs still show to be more chondrogenic however their isolation is more risky/difficult. However, within the in vivo model, MDSCs underwent directed differentiation with a KG coated collagen scaffold resulting in cartilage repair. Since MSC’s do not need to be isolated and processed, this strategy has potential for use as an
adjunct for treatment of all cartilage defects currently treated by microfracture. The ability of ADSC’s to undergo similar differentiation allows for an entirely new pool of easily obtained stem cells to be utilized in future treatments. Future work will be completed studying the ability of KG to differentiate ADSC’s into functional chondrocytes in vivo along with dose control of KG local at the joint.

Significance: Articular cartilage repair remains a challenge to the orthopaedic surgeon. MFX is widely used clinically but could be improved upon by the local delivery of KG to promote chondrogenic differentiation.

Acknowledgments: We would like to thank the Department of Orthopaedic Surgery at North Shore Long Island Jewish Health System.

References:

Figure 3: Average modified O’Driscoll scale scores of eight weeks post-

Figure 2B: Illustrates a Kartogenin-treated microfracture site (4x) identified between the two arrows.
Figure 2A: Illustrates an empty collagen scaffold (4x) identified between the two arrows.

Figure 1: A) (Left) RNA fold change expression of Collagen type -2 for Mesenchymal stem cells at 10nM & 100nM Kartogenin concentration B) (Right) Same as A except treating Adipose derived stem cells.

ORS 2014 Annual Meeting
Poster No: 0338