Enhancing regenerative potential of articular cartilage with a novel regulator of gp130 signaling

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INTRODUCTION: Cartilage injury often leads to osteoarthritis (OA), characterized by degradation of joints currently affecting hundreds of million of people worldwide. The regenerative capacity of adult articular cartilage is minimal and none of the existing therapies can generate long lasting articular cartilage tissue. Thus, there is a pressures need for novel strategies in this filed. Our previous data have shown that signaling through gp130 directs articular chondrocyte activation and that a novel small molecule gp130 agonist RCGD423 capable of modulating both pro-inflammatory and regenerative responses. Current study dissects reparative potential of RCGD423 in rat model of cartilage injury and osteoarthritis.
METHODS: Medial meniscal tear and focal injury rat model of osteoarthritis. Statistical methods: Student t-test, one-way ANOVA followed by the Newman-Keuls test. All animal experiments were conducted in accordance and under the supervision of the University of Southern California Department of Animal Resources.

RESULTS: The pathogenesis of OA often begins from an injury to articular cartilage, which establishes chronic, low-grade inflammation that promotes matrix degradation over time and eventual destruction of cartilage (1). Currently, there are no approved agents that slow or interrupt this process. Based on the functional and molecular profiles of chondrocytes stimulated by RCGD423, we hypothesized that this small molecule could act to prevent cartilage loss following injury. To directly address whether RCGD423 could ameliorate cartilage degeneration, we adopted a rat medial meniscal tear (MMT) model, in which 30-50% of the meniscus is removed. This results in joint destabilization and cartilage degeneration that mimics the pathology observed in human OA (Figure 1A). First, we developed and tested a method to achieve sustained release of RCGD423 *in situ*, similar to what would likely be applicable in a clinical situation. As a delivery vehicle, we employed FDA-approved poly(lactic-co-glycolic) acid (PLGA) microspheres loaded with R 423. Direct evaluation of off-loading kinetics of RCGD423 from PLGA microspheres showed that 20 μg of the compound could sustain a 0.1-0.3 μM concentration for at least 12 days *in vitro*; in the context of a rat joint with a volume of 150-200 μL, these concentrations would be increased 5- to 7-fold, although off-loading from PLGA microspheres can be accelerated *in vivo* vs. *in vitro (2)*. Based on these data, we designed a 6 week experiment in which operated animals would receive an intra-articular injection of 4 μg RCGD423 loaded onto microspheres, or empty microspheres as a control, at the time of surgery and another three weeks later. Micro computed tomography (micro CT) demonstrated an almost total loss of cut meniscus (Figure 1B). We then used the OARSI histological scoring system (3) to quantify the extent of cartilage damage in all animals. Tissue sections were stained with Safranin O to detect proteoglycans (Figure 1C); these results demonstrated dramatic loss of cartilage on the tibial plateau in control animals, while in RCGD423-treated animals there was little to no cartilage degradation or structural changes. Quantification of all animals (n=8) for control and experimental groups by a blinded observer revealed a strongly significant protection of cartilage by RCGD423 as evidenced by consistently lower OARSI scores (Figure 1D). At the molecular level, control animals showed high levels of cartilage degrading proteins, while these were mostly absent in RCGD423-treated rats (Figure 1E). Taken together, these results define RCGD423 as a potential agent with disease-modifying activity in a rat model of osteoarthritis.

DISCUSSION: Our findings strongly suggest that activation of endogenous reparative response represents an attractive approach for articular cartilage regeneration. Novel small molecule RCGD423 promotes atypical homodimeric signaling, driving transient increases in MYC and active STAT3 while suppressing IL-6-mediated inflammation. We have previously shown that RCGD423 functions as a direct gp130 agonist in articular cartilage and that activation of elements of this pathway in adult chondrocytes drives the adoption a fetal-like functional profile. Current study strongly showed direct disease modifying activity of RCGD423 in rat model of osteoartrjtoss. Future studies are needed to further assess translational potential of this novel regulator of cartilage regeneration and repair.

SIGNIFICANCE: These results identify a novel strategy for regeneration of articular cartilage and possibly other tissues regulated by gp130 signaling.

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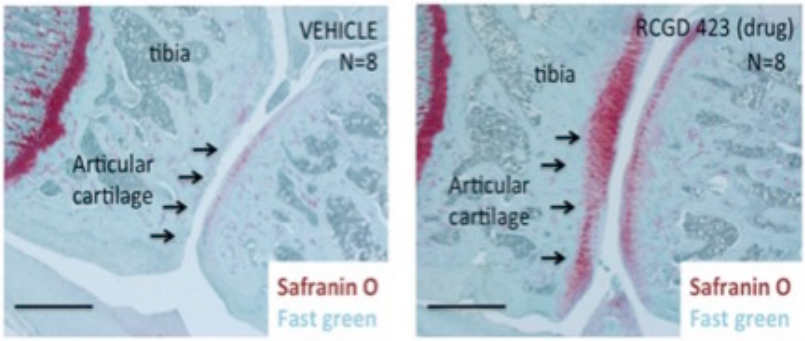

Figure. 1: RCGD423 prevents articular cartilage degradation in a rat model of osteoarthritis.