AAV2-hFGF18 Increases Cartilage Thickness and Promotes Hyaline Cartilage Anabolism

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INTRODUCTION: Disease modifying treatments for Osteoarthritis (OA) remain elusive. While several therapeutic approaches targeting inflammation or direct extracellular matrix (ECM) replacement have been investigated, they appear ineffective at reversing articular cartilage loss, either due to the synovial pharmacokinetics or lack of mechanistic therapeutic activity. Recent discovery of the chondrogenic properties of rhFGF18 are encouraging, however, they are complicated by the required frequency of intra-articular administration caused by synovial joint pharmacokinetics. Gene therapy is a promising therapeutic modality capable of a high degree of localization, long-term durability, and potential disease modification. In this study, we aimed to compare rhFGF18 protein injections and AAV2-delivered hFGF18 by analyzing effects on cartilage anabolism via primary human chondrocyte proliferation, gene expression, as well as articular and meniscal cartilage generation *in vivo*.

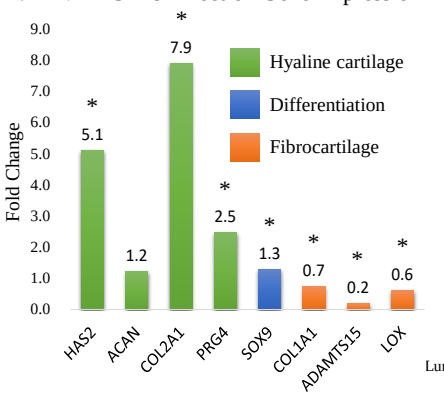
METHODS: Chondrogenic properties of AAV2-hFGF18 were compared to HEK-expressed rhFGF18 protein and *E. coli*-expressed truncated, rhFGF18 analog *in vitro*, relative to AAV2-GFP and PBS controls. Cytocompatibility was measured by characterizing dose-dependent treatment effects on total nuclear cell counts over 168h in culture. Gene expression analysis was performed using RNA-seq on chondrocytes subjected to AAV2-hFGF18 at MOI 10, rhFGF18 protein at 1,000 ng/mL, and PBS. Durability of gene expression in the joint following intra-articular injection was confirmed *in vivo* by bioluminescence imaging of rats injected using AAV2-nLuc. *In vivo* cartilage anabolism was evaluated measuring thickness of the tibial and meniscal cartilage (white zone of the anterior horn of the lateral meniscus) in male Sprague-Dawley rats (300-375g), following administration of AAV2-hFGF18, rhFGF18 protein, and AAV2-GFP. Statistical analysis was performed in Minitab 21.1 and included ANOVA and Tukey's HSD post-hoc testing at p_{crit}=0.05; RNA-seq was analyzed by DESeq2, at p_{crit}=0.01. This study was approved by IACUC.

RESULTS: Cytocompatibility of the AAV2-delivery vector was confirmed up to an MOI of 50k for chondrocytes and 500k for primary human synoviocytes, demonstrating no statistically significant, dose-dependent toxicity over the evaluated range. In parallel, transduction efficiency reached 58±16% in synoviocytes and 97±3% in chondrocytes at an MOI of 50k after 168h in culture. The HEK- and *E. coli*-expressed rhFGF18 both induced chondrocyte proliferation over a 48h period when delivered at doses ranging between 1 and 10k ng/mL, resulting in cell count increases of 38-110% and 36-85% respectively and demonstrating a statistically significant dose-response. Similarly, treatment of chondrocytes with AAV2-hFGF18 at MOIs of 100 and 1,000 induced proliferative increases of 36 and 84% respectively, relative to an 8% increase in the PBS control. AAV2-hFGF18 transduced synoviocytes were also able to induce chondrocyte proliferation in transwell culture by up to 134% (MOI of 500k) relative to 135% for the rhFGF18 analog treatment (1,000 ng/mL) and 6% for AAV2-GFP control (MOI 500k). RNA-seq revealed that rhFGF18 protein analog and AAV2-hFGF18 treatment arms upregulated hyaline cartilage associated genes including COL2A1 and HAS2 (Fig 1), while downregulating fibrocartilage associated COL1A1. In addition, AAV2-hFGF18, but not rhFGF18 analog, was able to upregulate SOX9, a chondrocyte differentiation marker, and PRG4, an important articular cartilage surface protein, while downregulating the aggrecan-targeting protease ADAMTS15. This activity translates to statistically significant weight-normalized cartilage thickness increases *in vivo* on the tibial plateau and meniscal tip following a single intra-articular injection of AAV2-hFGF18 (12.5% and 18.9% respectively relative to the AAV2-GFP control) and a regimen of 6 twice-weekly injections of rhFGF18 analog (9.4% and 17.9% respectively relative to the AAV2-GFP control). Moreover, due to the observed durability (Fig 2), AAV2-hFGF18 offers a potential safety advantage over the multi-injection protein treatment at the 1- and 2-month timepoints, as evidenced by reduced joint swelling over the study period.

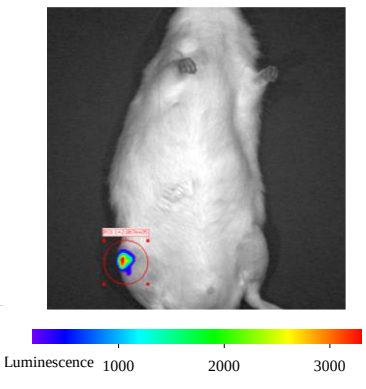
DISCUSSION: Previous preclinical and clinical studies have demonstrated chondrogenic properties of rhFGF18 administered via successive weekly injections. Our results demonstrate similar mechanistic effects of AAV-delivered hFGF18, with potential advantages in the *de novo* production of hyaline cartilage, transcriptomic profile changes, and the required injection frequency. We have confirmed a wide safety window for the AAV2 vector, as evidenced by a broad cytocompatibility in chondrocytes and synoviocytes. In parallel, we demonstrated that AAV2-hFGF18 treatment was able to induce similar patterns of gene expression to the rhFGF18 analog at doses 5,000x smaller than the upper limit of the evaluated cytocompatibility range. The durability of the gene therapy, together with the chondroproliferative activity of hFGF18, and upregulation of hyaline cartilage ECM associated genes may play a central role in driving the observed increase in cartilage tissue. In conclusion, our study suggests that AAV2-delivered hFGF18 may present a promising strategy for the restoration of articular cartilage by promoting hyaline cartilage-related gene expression, increasing anatomically relevant extracellular matrix production, and chondrocyte proliferation (Fig 3).

SIGNIFICANCE/CLINICAL RELEVANCE: While disease modifying treatments for osteoarthritis remain elusive, results of the recent, multi-center, placebo controlled clinical trial evaluating the rhFGF18 protein show promise for cartilage regeneration. The present study provides the foundation for a potential gene augmentation approach to treating chondrodegenerative diseases, based on the anabolic properties of hFGF18, while offering a more streamlined treatment paradigm without pharmacokinetic limitations of repeat protein injections.

1. AAV2-FGF18 Effect on Gene Expression



2. nLuc Bioluminescence at 4 months



3. Cartilage Thickness Measurements