

Effects of Hyaluronan synthase 2 (HAS2) gene transfer in a mouse model of knee osteoarthritis

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INTRODUCTION: Using *in vitro* models, we previously reported that Hyaluronan synthase 2 (HAS2) overexpression enhanced endogenous hyaluronic acid (HA) production and significantly suppressed interleukin-1 beta induced MMP-13 expression in bovine and human osteoarthritic (OA) chondrocytes. [1]. The purpose of this study was to confirm whether HAS2 overexpression in chondrocytes, synoviocytes or other cells in knee joint has a suppressive effect on OA progression in destabilization of the medial meniscus (DMM) OA model mouse.

METHODS: For HAS2 overexpression, the adenoviral vector Ad-ZsGreen-HAS2, utilized in previous studies, was amplified and purified. Ad-ZsGreen-HAS2 was injected into the knee joints of male mice (C57BL/6J) aged 12 weeks. Green fluorescence microscopy was performed to determine the optimal titer and the timing of virus injection. Male mice underwent sham surgery or DMM at 12 weeks of age. At 13 weeks of age, we administered Ad-ZsGreen-LacZ (control) or Ad-ZsGreen-HAS2 into the knee joint (n=68). At 4 or 8 weeks following surgery, sections of knee joint tissue were prepared and stained with Safranin O-Fast Green. The progression of OA was evaluated using the Osteoarthritis Research Society International (OARSI) score, based on the method of Pritzker et al [2]. The OARSI score was compared to the total score (0-130) of five slices of the medial tibial plateau in the coronal section of the knee. The data was collected and statistical analysis was performed with Mann-Whitney U test. P values of less than 0.05 were considered statistically significant.

RESULTS: Appropriate Ad-ZsGreen-HAS2 transduction was observed at titers of 10^{10} IFU/ml, at both 2 and 4 weeks after injection. ZsGreen fluorescent protein expression was observed in cartilage, meniscus and synovium. (Fig. 1A). OA change was observed at 8 weeks after DMM surgery. Figure 2 demonstrates the histological evaluation of knee osteoarthritis, based on the sum of OARSI scores, at 4 and 8 weeks after sham or DMM surgery with Ad-ZsGreen-LacZ (n=9) or Ad-ZsGreen-HAS2 (n=8) viral transduction. OARSI scores were significantly higher in DMM mice compared with sham mice both 4 and 8 weeks after the surgery. DMM mice injected with Ad-ZsGreen-HAS2 demonstrated lower levels of cartilage degeneration (Fig. 1B) and OARSI scores (Fig. 2) at 8 weeks as compared to DMM mice with control virus (p=0.019).

DISCUSSION: In this study, Ad-ZsGreen-HAS2 was successfully transduced and HAS2 was overexpressed in chondrocytes, meniscus cells and synoviocytes. As a result, Ad-ZsGreenHAS2 injection showed suppressive effect on OA progression in DMM OA mouse model. Drawing from our prior research, we hypothesize that HAS2 transduction enhances the endogenous HA production, exerting a protective effect on chondrocytes and potentially serving as a viable treatment option for knee OA. In future experiments, the increase of HA should be evaluated by HA binding protein staining or HA gel assay, and also the expression of OA related catabolic genes, MMP13 and ADAMTS-4 should be evaluated by immunohistochemical staining.

SIGNIFICANCE/CLINICAL RELEVANCE: It is possible to introduce the HAS2 gene to chondrocytes, meniscus cells and synoviocytes in knee joints using adenoviral vectors, offering a potential treatment option for knee OA.

REFERENCES:

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- [2] Pritzker, K. P. H. *et al.* Osteoarthritis cartilage histopathology: Grading and staging. *Osteoarthritis Cartilage* **14**, 13–29 (2006)

IMAGES:

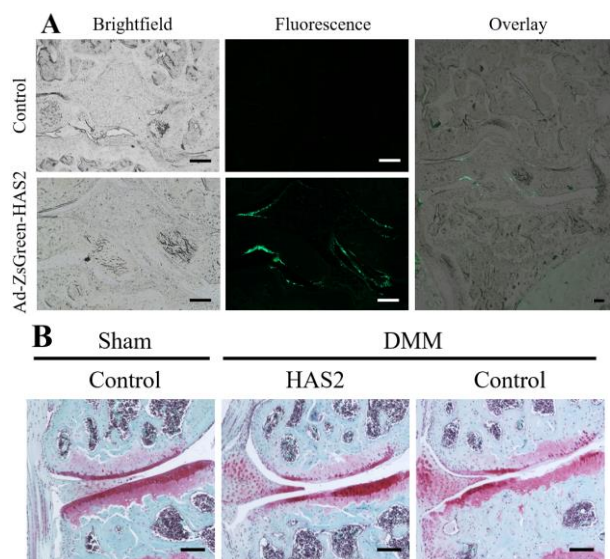


Figure 1: (A) Representative images of male C57BL/6 knee joints at 4 weeks after injection of control or Ad-ZsGreen-HAS2. (B) Representative images of Safranin O-Fast Green stained sections of the medial compartment of male C57BL/6 knee joints at 8 weeks after sham or DMM surgery. Bars = 100 μ m.

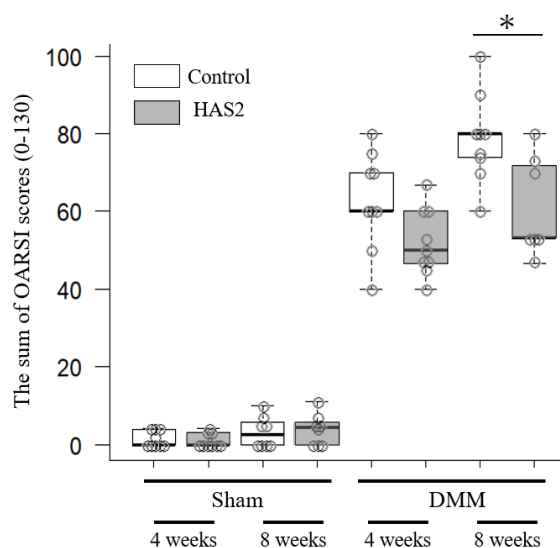


Figure 2: Histogramical evaluation of knee osteoarthritis using the sum of OARSI scores. Mice treated with control or Ad-ZsGreen-HAS2 were evaluated at 4 and 8 weeks after sham surgery or DMM surgery. Data are shown with the Tukey boxplot with an overlay of individual scores as open circles. *p < 0.05 (Mann-Whitney U test).