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

Sei Fujii, Shinya Ishizuka, Hiroki Obi, Takefumi Sakaguchi, Jun Fukui, Shiro Imagama
Department of Orthopedics, Nagoya University Graduate School of Medicine, Aichi, Japan

Email of Presenting Author: sei.fujii.nagoya@gmail.com

**Disclosures:** Sei Fujii(N), Shinya Ishizuka(N), Hiroki Obi(N), Takefumi Sakaguchi(N), Jun Fukui(N), Shiro Imagama(N)

**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 s

**DISCUSSION:** In this study, Ad-ZsGreen-HAS2 was successfully transduced and HAS2 was overexpressed in chondrocytes, meniscus cells and synoviocytes. As a result, Ad-ZsGreen-HAS2 injection showed suppressive effect on OA progression in DMM OA model mouse. 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:**

**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 = 100μm.

Figure 2: Histological 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).