

## The Effects of Hyaluronan synthase 2 (HAS2) gene transfection in a mouse model of knee osteoarthritis

Keita Shimizu, Shinya Ishizuka, Sei Fujii, Takefumi Sakaguchi, Takafumi Mizuno, Kazuki Yoshida, Ryosuke Sugimoto, and Shiro Imagama  
 Department of Orthopedics, Nagoya University Graduate School of Medicine, Aichi, Japan  
 Email of Presenting Author: withoutaste2822@gmail.com

**Disclosures:** Keita Shimizu (N), Shinya Ishizuka (N), Sei Fujii (N), Takefumi Sakaguchi (N), Takafumi Mizuno (N), Kazuki Yoshida (N), Ryosuke Sugimoto (N), and Shiro Imagama (N)

**Introduction:** We previously reported that overexpression of hyaluronan synthase 2 (HAS2) promotes endogenous hyaluronan (HA) production in bovine chondrocytes and human osteoarthritis (OA) chondrocytes, and significantly suppresses interleukin-1 beta (IL-1  $\beta$ )-induced matrix metalloproteinase-13 (MMP-13) expression. [1] This study aimed to confirm whether overexpression of HAS2 in knee cartilage cells, synovial cells, or other cells has an inhibitory effect on the progression of osteoarthritis (OA) in the surgical destabilization of the medial meniscus (DMM) mouse model.

**Methods:** The adenovirus vector Ad-ZsGreen-HAS2 used in previous studies was used. Male mice were subjected to sham surgery or DMM at 12 weeks of age. At 13 weeks of age, Ad-ZsGreen-LacZ (control group) or Ad-ZsGreen-HAS2 was injected into the knee joint (n=68). We chose male mice because males have more severe OA after DMM than females.[2] OA progression was evaluated using the Osteoarthritis Research Society International (OARSI) score and Safranin O-Fast Green staining of knee joint tissue section at 4 or 8 weeks after surgery, and immunohistochemistry stained with anti-MMP-13 antibody[3]. The number of positive-stained cells in chondrocytes, synoviocytes, and meniscal cells was counted and analyzed. The data was statistically analyzed using the Mann-Whitney U test. A p-value of less than 0.05 was considered statistically significant.

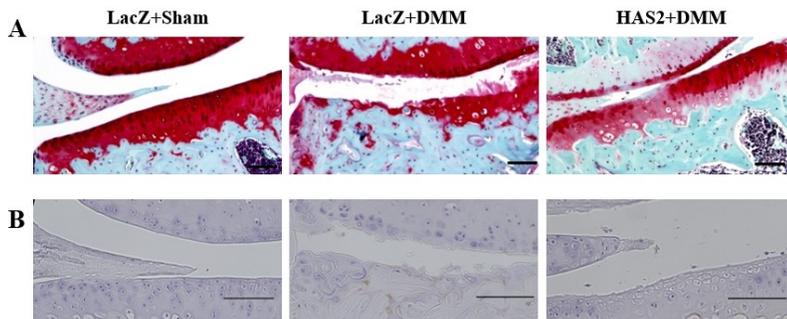
**Results:** OARSI scores were significantly higher in DMM mice compared to sham mice at both 4 and 8 weeks post-surgery. DMM mice treated with Ad-ZsGreen-HAS2 had significantly lower cartilage degeneration (Figure 1A) and OARSI scores at 8 weeks compared to DMM mice treated with the control virus(Figure 2). The number of MMP13-positive cells was significantly higher in DMM mice than sham mice. DMM mice with Ad-ZsGreen-HAS2 had significantly fewer positive cells than those transduced with Ad-ZsGreen-LacZ, the control virus (Figure 1B).

**Discussion:** Our study previously showed Ad-ZsGreen-HAS2 transduction into the knee joint results in overexpression of HAS2 in chondrocytes, meniscal cells, and synovial cells. Present study showed HAS2 overexpression in those cells inhibited OA progression in the DMM OA mouse model. This study suggests potential efficacy as a therapeutic option for knee OA. As it is still unclear whether the inhibitory effect is HA dependent or not, future study is necessary.

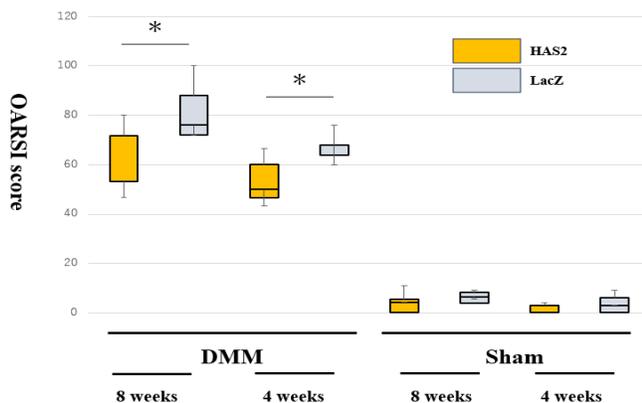
**Significance/Clinical Significance:** Using an adenovirus vector, the HAS2 gene was transferred into chondrocytes, meniscal cells, and synovial cells of the knee joint, confirming protective effects on chondrocytes. This demonstrates promising potential as a future treatment for knee OA.

### References:

- [1] Ishizuka, S. et al. Overexpression of hyaluronan synthase 2 (HAS2) suppresses chondrocyte degradation activity via a mechanism independent of extracellular hyaluronan. *J. Biol. Chem.* 294, 13562–13579 (2019)
- [2] H.-L. Ma et al. Osteoarthritis severity is sex dependent in a surgical mouse model. *Osteoarthritis Cartilage* 15, 695-700 (2007)
- [3] Pritzker, K. P. H. et al. Osteoarthritis cartilage histopathology: Grading and staging. *Osteoarthritis Cartilage* 14, 13–29 (2006)



**Figure 1 :** (A) Representative images of the medial compartment of knee joint tissue sections stained with Safranin O-Fast Green 8 weeks after DMM surgery and sham surgery. (B) Representative images of the medial compartment of knee joint tissue sections stained with anti-MMP13 antibody 8 weeks after DMM surgery and sham surgery. Immunopositive cells (brown staining) were observed predominantly in the DMM mice with LacZ. Bars=100  $\mu$  m.



**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 is shown with box plots. \*p < 0.05 (Mann-Whitney U test).