In this study, we evaluate the RNAi effect against MMP13 in preventing cartilage degradation by directly injecting chemically modulated siRNAs to surgically induced murine OA knee joints. Our hypothesis was that effective RNAi could be achieved by injection of highly concentrated siRNA in the knee joint, resulting in reduced development of osteoarthritis.

**Methods:** Knee OAs were surgically induced to C57BL/6 mice by destabilization of the medial meniscus (DMM) method, by transecting the medial meniscotibial ligament under general anesthesia. To determine the timing of protease expressions in the murine DMM induced OA model, messenger RNA (mRNA) of MMP13 from knee cartilage were analyzed at 3 days, 1 week, 2 weeks, 4 weeks, and 6 weeks after surgery by quantitative polymerase chain reaction (qPCR).

One week after DMM induction, chemically modulated siRNAs were injected in the knee joint. Two different content of injection were evaluated. Treatment groups consisted of a group which MMP13 siRNA was injected, and another group in which control siRNA was injected. The control siRNA was confirmed not to affect MMP13 in vitro. The RNAi effects were assessed by quantifying MMP13 mRNA in knee cartilage at 48 hours after siRNA injection. Histological assessment of articular cartilage at 8 weeks post DMM operation was conducted to assess the effect of siRNA injection at one week after DMM in osteoarthritis progression, according to the OARSI recommendation for OA knee scoring in mice.

**Results:** Surgically induced OAs were successfully achieved in the DMM model of mice. MMP13 expressions elevated from the third post operative day, and peaked in a week after DMM surgery (Fig. 1). Expression level of MMP13 mRNA was significantly decreased at 48 hours after injection of MMP13 siRNA to the knee joint, compared to negative control siRNA injection (Fig. 2). Significant improvement was observed in the histological score of MMP siRNA treated group, compared to the control siRNA injected group.

**Conclusions:** Direct injection of siRNAs targeting MMP13 at the early phase of OA development has the potential to inhibit protease expression and inhibits cartilage degradation in vivo.

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**Figure 1:** MMP13 mRNA expression levels after DMM induction

**Figure 2:** MMP13 mRNA expression 48 hours post siRNA injection