INTRODUCTION  Intra-articular injection of hyaluronan (HA) for symptomatic relief from knee osteoarthritis (OA) has become widely accepted; however the mechanism(s) of its effect on joint tissue pathology remain unclear. In this regard, a beneficial effect of intra-articular HA on objective measures of human gait has recently been reported in a preliminary study [1]. In addition we have shown [2] that prophyllactic HA injection can prevent gait changes and also protect against cartilage erosion in the murine TTR (TGfβ1-injection and Treadmill Running) model of OA. To further examine the potential mechanism(s) of HA-mediated protection we have studied the effect of prophyllactic HA injection on gene expression in separate joint tissues (articular surfaces and meniscal/synovial samples) dissected from mice in the destructive phase (at 2 weeks of treadmill running) of the TTR model. In addition we compared the effects of HA injection with the effects of Adamts5 knockonout on gene expression, and we have more rigorously examined the effects of HA on joint histopathology in the TTR model.

METHODS  Osteoarthritis model: Male mice (wild types or Adamts5-/- in C57BL/6) were bred in-house and all protocols were approved by Rush University Medical Center Animal Care Committee. OA was induced by unilateral intra-articular injection of active TGfβ1 (200 ng in 0.1% (w/v) BSA, 2X over 48h) followed by daily enforced uphill treadmill running (17 degree gradient at 32 cm/sec for 20 min) for 2 weeks[3]. Ten ul of HA (Supartz, 10mg/ml) or saline was injected through the patellar ligament 24 hours after the 2nd TGfβ1 injection. Experimental groups were: 1) OA (Histology n=4, QPCR n=8); 2) OA+HA (Histology n=4, QPCR n=8); 3) OA+Saline (Histology n=4).

Histopathology: Femora were prepared intact in 10% neutral buffered formalin for 48h, decalcified in 0.5M EDTA for 14 days, processed and embedded in paraffin. Whole-joint sagittal sections (5 um) were obtained from ~10 equidistant locations spanning the entire lateral and medial compartments. Sections were deparaffinized and stained with SafraninO/Fast Green. Quantitative PCR: Articular surfaces and meniscus/synovium were harvested separately as described [3], placed in liquid nitrogen and pulverized in a Bessman Tissue Pulverizer. RNA was prepared using the PerfectPure RNA Kit for Fibrous Tissue (5PRIME). Taqman-based and QPCR was as described [4] with the following primer sets: aggrecan, Mn05541794_m1; col2a1, Mn10094656; m1; col10a1, Mn00487041_m1; with both GAPDH and ETS as normalization genes using the 2^-delta-delta method.

RESULTS   Prophyllactic HA induced the expression of aggrecan (~3.5-fold, p<.0001), col2a1 (~3-fold, p<.0001) and col10a1 (~4-fold, p<.0001) in the articular surfaces, relative to mice with untreated OA (Fig.1). The same gene induction was seen in the meniscal/synovial preparations from the same mice (data not shown). Notably, a similar induction of chondrogenic genes was observed in the same joint tissues from Adamts5-/- mice at 2weeks in the TTR model of OA(Fig.1) This is consistent with the finding [3] that mice lacking ADAMTS5 mount a chondrogenic response, including cartilage aggrecan deposition, in both the TTR and DMM models of OA. The prochondrogenic effects of HA injection were supported by histological evaluation of the knee joints (Fig.2) showing the formation of periarticular aggrecan-rich chondroid structures, which were not generated by prophyllactic saline injection in this model.

DISCUSSION  Suggested mechanisms for the beneficial clinical effects of intra-articular HA for some OA patients include 1) analgesia 2) anti-inflammation and 3) chondrogenesis. The present study supports the idea that chondrogenesis is a likely outcome of HA injection in human OA. In this regard it is important to note that the induction of collagen X synthesis by hyaluronan represents a chondrogenic response in the mouse, since it is a normal component of mouse cartilage throughout development, growth and aging [5].We speculate that this chondrogenesis results from complexation of ADAMTS5 by HA, such as seen in both human OA cartilages [6], and equine ligament pathology [7]. In conclusion, since the chondrogenic effect of HA injection mimics the effect of Adamts5 knockout, it is likely that it will also diminish the fibrogenic effects of joint injury on resident chondrocytes and chondro-

Prophylactic injection of hyaluronan activates chondrogenic genes and induces periarticular cartilage deposition in murine knee osteoarthritis

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