**Autophagy Activation by Rapamycin Reduces Severity of Experimental Osteoarthritis**

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**INTRODUCTION**
Osteoarthritis (OA) is affects all joint tissues but cell loss and extracellular matrix degradation in articular cartilage are among the earliest changes. Autophagy is an essential cellular homeostasis mechanism for the removal of dysfunctional intracellular macromolecules and organelles (1). Previous findings indicated deficient autophagy in aging and OA cartilage (2). The Mammalian Target of Rapamycin (mTOR) is a key inhibitor of autophagy. In this study we determined whether inhibition of the mTOR pathway is associated with disease-modifying activity in experimental OA.

**METHODS**
Experimental OA in mice. Experimental OA was induced in 2 months old C57Bl/6J mice male by transection of the medial meniscotibial ligament and the medial collateral ligament in the right knee. The left knee was not subjected to surgery and used as control. The animals were euthanized 10 weeks after the knee surgery.

Rapamycin treatment. Two independent experiments were performed. Each experiment included a total of 18 mice (9 mice treated with rapamycin (Rapa) and 9 mice receiving vehicle). Rapa was obtained from LC Laboratories (Woburn, MA). Mice received daily intraperitoneal injections of Rapa at 1 mg/kg body weight/dose in a total injection volume of 0.3 ml in PBS and vehicle animals received DMSO (0.4%) in a total injection volume of 0.3 ml of PBS for 10 weeks.

**Histological analysis of mouse knee joints.** Knee joints from mice were fixed, decalcified and paraffin embedded. Safranin-O stained sections were examined for histopathological changes using a semiquantitative scoring system (3).

Cellularity. Knee joint sections were stained with Hematoxylin and Eosin. Three pictures were taken under 40x magnification, representing the center of the femoral condyle that is not covered by the menisci as well as the medial and lateral femoral condyles. Total number of cells in each section was counted.

**Immunohistochemistry (IHC) and immunofluorescence (IF):** Sections were stained with anti-phospho-rbS6, to detect changes in phosphorylation of S6K1, a target of mTOR. The aggrecan-degrading enzyme ADAMTS5 was also detected by IHC. To monitor autophagy activation, sections were stained with antibody to LC3 and analyzed by IF.

**Quantification and localization of positive cells:** ADAMTS5 positive cells were counted as was explained for cellularity (see above). The total number of ADAMTS5-positive cells was counted in each section.

**Statistical analysis:** Statistically significant differences between two groups were determined with t tests. The results are reported as mean ± standard deviation. P values of less than 0.05 were considered significant.

**RESULTS**
Systemic administration of rapamycin modulates mTOR signaling and autophagy in mouse knee joints. Rapamycin (Rapa) treatment suppressed rpS6 phosphorylation in articular cartilage and menisci in the knee joints compared to vehicle treated mice (Figure 1A). Furthermore, we found an increase in LC3 expression after Rapa treatment. This increase was correlated with an increase in LC3 puncta, indicating strong activation of autophagy in articular cartilage (Figure 1B).

Rapamycin reduces severity of experimental OA. Mouse knee joints in the vehicle group exhibited significant cartilage degeneration, with proteoglycan depletion, loss of surface lamina and fibrillations. Rapa treatment decreased the severity of these OA-like changes (Figure 2A). Comparison of the combined scores from the 2 experiments showed a significant decrease of cartilage pathology (P < 0.01) by approximately 50% after rapa treatment compared to vehicle control (Figure 2B).

Rapamycin maintains cartilage cellularity and reduces ADAMTS5 expression in experimental OA. Rapa treatment attenuated the reduction in cartilage cellularity in knees with experimental OA. This difference was significant (P < 0.001) compared to vehicle treated mice (Figure 3A). Furthermore, ADAMTS5 was expressed by a lower number of chondrocytes in Rapa treated mice compared with vehicle-treated mice with experimental OA (Figure 3B). This result was significant (P < 0.001) compared to vehicle treated knee joints (Figure 3C).

**DISCUSSION**
These results suggest that rapamycin reduces severity of experimental OA. This is associated with autophagy activation, reduced loss of cartilage cells and lower ADAMTS5 expression. Pharmacological inhibition of mTOR by rapamycin may be a potentially effective therapeutic approach for the treatment of OA.

**SIGNIFICANCE**
This study establishes proof-of concept that enhancement of cellular homeostasis mechanisms such as autophagy can attenuate the severity of instability associated and rapidly progressive OA in mice. These results suggest the possibility that similar approaches may be effective in preventing aging associated changes in autophagy (2) and reduce the risk for OA development.

**REFERENCES**