Human chondrocytes (hChs) were isolated from the knees of osteoarthritic patients undergoing total knee arthroplasty, supplied by the National Disease Research Interchange (NDRI), Philadelphia, PA. Cells were cultured in monolayer. Chondrocyte transfections were carried out using the Amaxa Nucleofector technology. Cells were processed for protein or RNA extraction followed by immunoblotting or RT-PCR according to standard procedures, using the indicated antibodies and primer pairs.

Results: MMP13 levels were assessed in OA human chondrocytes stably expressing SirT1. Immunoblot assays confirmed SirT1 overexpression and an increase in SirT1 activity in cells expressing wild type SirT1 indicates that the ectopic SirT1 is active within these chondrocytes. The SirT1-expressing cells display significantly reduced MMP13 RNA and protein levels compared to control cell lines. The activity of MMP13 was also decreased in SirT1-overexpressing cells. These results demonstrate that MMP13 gene expression is suppressed by SirT1. Treatment of chondrocytes with the SirT1 inhibitor nicotinamide (NAM), led to a significant increase in MMP13 mRNA levels and MMP13 protein levels. When SirT1 protein levels were also reduced by SirT1 siRNA, it led to increased MMP13 expression. Treatment of cells with IL1beta led to induction of MMP13, yet SirT1 was partially able to block this IL1beta-mediated induction.

To explore the basis by which SirT1 represses MMP13 transcription, we focused on the Lef1 transcription factor, a component of the Wnt-signaling pathway. Lef1 has been demonstrated to positively regulate MMP13 transcription in chondrocytes. We find that SirT1 represses Lef1 levels and as a consequence represses transcription from Lef1 target promoters.

Conclusions: We find that longevity factor SirT1 is a potent repressor of MMP13 gene expression, an enzyme involved in joint destruction in OA, an age-associated disease. The repression of MMP13 occurs even in the presence of the cytokine IL1beta. MMP13 is a critical player in joint destruction in OA. The underlying mechanism by which SirT1 represses MMP13 appears to involve the Lef1 transcription factor a known regulator of MMP13 transcription. That Lef1 is repressed by SirT1 is consistent with recent data indicating that Wnt/b-catenin signaling pathway is involved in IL1beta-mediated cartilage degeneration. Taken together, these data show that SirT1 represses the expression of genes known to play a critical role in the onset of cartilage pathology.