knee joints with TGF-beta alone or TGF-beta + BMP-2 three times on alternate days (200 ng/knee) and measured proteoglycan (PG) content in cartilage.
In addition, we evaluated the significance of a shift in Smad balance in human OA cartilage by exposure of the explants to TGF-beta or a combination of TGF-beta + ALK5 inhibitor SB-505124. RNA was isolated for Q-PCR analysis.
Results: Overexpression of BMP-2 in murine knee joints, thereby stimulating the Smad1/5/8 signaling route, decreased Smad2/3P expression in cartilage and increased staining of VDIPEN and NITEGE epitopes (MMP- and ADAMTS-mediated cartilage breakdown respectively). In vitro, chondrocyte exposure to BMP-2 resulted in up regulation of aggrecan, collagen type II, collagen type X and MMP13 expression. Thus, elevating Smad1/5/8 signaling is associated with concomitant reduction of Smad2/3 signaling and OA-like alterations.
After in vivo exposure to TGF-beta, a mild decrease in PG content (8%) in tibial cartilage was found. However, combining TGF-beta with BMP-2 resulted in 21% decrease in PG content. PG staining was lost in the deep zones of tibial cartilage, just above the tide-mark.
To investigate the effect of reduced Smad2/3 activation on overall Smad signaling we blocked ALK5 activity (Smad2/3 route) in TGF-beta activated chondrocytes with SB-505124. Besides the expected decrease in Smad2/3P, Smad1/5/8P was enhanced, thereby contributing to a shift in Smad signaling balance.
Human OA cartilage exposure to TGF-beta inhibited MMP-13 expression. This inhibition was totally blocked by SB-505124. This indicates that in chondrocytes inhibition of Smad2/3P activation enhances MMP-13 expression.
Conclusions: Our data indicate that the Smad2/3 and Smad1/5/8 balance determines chondrocyte function. Either up regulation of the Smad1/5/8 pathway or down regulation of the Smad2/3 route, both we have observed in OA cartilage, appears associated with increased chondrocyte MMP-13 expression. This fits with published observations that Smad1/5/8 stimulates while Smad2/3 signaling blocks chondrocyte terminal differentiation.
These results indicate that factors changing the balance to dominant Smad1/5/8 signaling, for instance changes in TGF beta superfamily ligands or receptors, will affect chondrocyte differentiation and MMP-13 expression and might play a role in OA development.

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THE CHROMATIN MODIFYING ENZYME SIRT1 REPRESSES THE ARTHRITIS-ASSOCIATED MMP13 EXPRESSION IN HUMAN ARTICULAR CHONDROCYTES

E.-J. Lee, M. Dvir-Ginzberg, O. Gabay, V. Gagarina, D.J. Hall
NIH, Bethesda, MD

Purpose: Osteoarthritis (OA) is a degenerative joint disease of the articular cartilage resulting in the depletion of collagens and proteoglycans due in part to accelerated turnover and inadequate repair. Maintenance of healthy articular cartilage in human adults relies on the optimal expression of a number of unique extracellular matrix genes, such as aggrecan and collagen type II, by the chondrocytes in this tissue. MMP13 is a proteolytic enzyme that degrades the extracellular matrix, including the cartilage-specific component, type II collagen. MMP13 is thought to play a critical role in cartilage degradation in OA. SirT1 is an NAD-dependent histone deacetylase that lengthens organism life span by reducing the severity of age-related diseases. Since OA is an age-associated disease, it is pertinent to explore the role SirT1 plays in MMP expression in human adult chondrocytes from OA patients. Here we show that MMP13 expression and activity is downregulated by SirT1 in human chondrocytes.
Methods: 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/β-catenin signaling pathway is involved in IL-1beta-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.

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P188 ALONE OR IN COMBINATION WITH OP-1 IS EFFECTIVE IN REDUCING CARTILAGE DEGENERATION AND PROMOTING CELL SURVIVAL IN THE MODEL OF ACUTE TRAUMA TO HUMAN ANKLE CARTILAGE: THE MECHANISM OF ACTION

S. Bajaj, K. Sampath, C. Pascual-Garrido, F. Hirschfeld, A.A. Hakimiyan, L. Rappoport, M.A. Wimmer, T.R. Oegema Jr., **S. Chubinskaya**
Rush Univ. Med. Ctr., Chicago, IL

Purpose: There is a clear need to recognize the risk of OA following joint injury, and to develop and implement strategies to prevent posttraumatic OA. The main **objectives** of this continuing study were to investigate the effect and understand the mechanism of action of P188 surfactant alone or in combination with osteogenic protein-1 (OP-1) on cartilage degeneration and cell survival in acute trauma to human ankle cartilage.
Methods: We developed a model of open joint acute trauma in which injury is created to human ankle cartilage *ex vivo* by the application of a 4 mm cylindrical impactor with stress of 30 MPa. This type of injury is limited to damage on the articular surface. To investigate the effect of therapeutic interventions, sixteen normal tali from human organ donors were impacted using a 4mm indenter