Elevated Levels Of BMP2 Compensate For Loss Of TGF-beta On Proteoglycan Level In Articular Cartilage During Experimental Osteoarthritis

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Disclosures:

Introduction: We have demonstrated that in aging murine articular cartilage TGF-beta signaling via Smad2/3 is drastically reduced and that loss of Smad2/3-related TGF-beta signaling predisposed cartilage for OA development. In addition, we have previously shown that inhibition of TGF-beta reduces the proteoglycan content in articular cartilage. In contrast, during OA elevated levels of BMP2 are found in chondrocytes surrounding cartilage lesions. However, it is unclear what is the effect of this BMP2 presence on the articular cartilage. Therefore, we have investigated whether elevated BMP-2 expression can counteract the loss of TGF-beta signaling during OA.

Methods: We made a unique transgenic mouse which expresses human BMP2 under control of the Col2a1 promoter but only when exposed to doxycycline (Col2a1-rtTA-BMP2). This results in a chondrocyte-specific overexpression of human BMP2 which is inducible by doxycycline. Functionality of this transgenic mouse was tested by isolating mRNA from articular cartilage, spleen and liver 72 hours after exposure to doxycycline food or standard diet. With Q-PCR we analyzed the expression of human BMP2 mRNA. In Col2a1-trTA-BMP2 mice we induced OA by destabilization of the medial meniscus (DMM-model) while treating them with doxycycline in food versus standard diet. To study the effect of loss of TGF-beta activity during OA in these young mice, we additionally intra-articularly injected an adenovirus overexpressing the TGF-beta inhibitor LAP (Ad-LAP). Ad-LAP was injected 3 days and 2 weeks after induction of DMM and doxycycline treatment started 1 day after the first Ad-LAP injection. Four weeks after induction of DMM knee joints were isolated for histology. OA was scored based on cartilage damage (adapted OARSI score, scale of 0-30) In addition, we measured proteoglycan (PG) content with digital image analysis in Safranin O stained articular cartilage of the medial tibia, which is most affected during DMM.

Results: Treatment of the Col2a1-rtTA-BMP2 transgenic mice with doxycycline clearly elevated the expression of hBMP2 mRNA in articular cartilage, but not in spleen and liver thereby confirming functionality of the transgenic animals. Doxycycline exposure in Col2a1-rtTA-BMP2 up to 8 weeks did not result in any detectable alterations in healthy articular cartilage. When OA was induced there was a clear increase in OA score (average of all DMM groups of 16.9 versus 2.5 in non-DMM groups), but this was not significantly affected by the presence of elevated chondrocyte-specific BMP2. TGF-beta inhibition with LAP did not affect the OA-score during DMM either. However, TGF-beta inhibition during DMM significantly reduced the proteoglycan content by 18% compared to DMM alone. BMP2 did not have an effect on the proteoglycan content during DMM (see figure). Nevertheless, the proteoglycan depletion that occurred by the inhibition of TGF-beta during DMM could significantly and nearly completely be counteracted by elevated chondrocyte-specific BMP2.
**Discussion:** Our data show that in healthy articular cartilage and in cartilage affected by osteoarthritis in young animals elevated levels of BMP2 did not have any detectable effects. However, when TGF-beta signaling was lost, a phenomenon occurring in aged individuals, this resulted in decreased levels of PG content in articular cartilage during OA. In this setting, elevated levels of BMP2 could compensate this loss of PG. Therefore the elevated levels of BMP2 near OA lesions could be a reparative response of the articular cartilage. Especially with ageing, when TGF-beta signaling is drastically reduced this compensatory mechanism could be of great importance as an attempt to restore damaged articular cartilage. Overall our data show that with respect to proteoglycan content elevated levels of BMP2 can compensate for the loss of TGF-beta signaling.

**Significance:** Our data indicate that the presence of elevated BMP2 during OA are a potential compensatory mechanism of the articular cartilage for the fact that TGF-beta signaling is lost. This potential attempt to repair the lost proteoglycans could be a mechanism of great importance especially in the elderly. Therefore our study provides novel insight into the mechanisms involved in OA.

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**References:** -

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