Mmp13 is a Critical Target Gene during the Development of Osteoarthritis

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Introduction:
Osteoarthritis (OA) is a common joint degenerative disease affecting more than 25% of people over the age of 18. The pathogenesis of OA is poorly understood and currently there is no effective disease-modifying treatment for OA. Several lines of evidence suggest that the TGF-β1-Smad pathway plays a critical role in OA development [1, 2]. Recent human genetic studies have demonstrated that mutations in the Smad3 gene are associated with high incidence of hip and knee OA in patients [3]. However, the downstream target gene(s) for this signaling pathway during OA development remain unknown.

MMP13, also known as collagenase 3, is the primary collagenase that preferentially cleaves type II collagen in articular cartilage [4]. Expression of the constitutively active Mmp13 gene leads to an OA-like phenotype in mice [5]. In this study, we hypothesize that Mmp13 is a critical target gene which may be involved in the TGF-β signaling pathway during OA development.

Methods:
Experimental Animals: 1) To generate TGF-β receptor conditional knockout (cKO), Tgfb2creER² mice and Mmp13 cKO, Mmp13Tgβ1/2 conditional, mice, Col2a1CreER² mice were bred with Tgfb2creER² and Mmp13Tgβ1/2 mice separately. 2) To generate Tgfb2;Mmp13 double knockout (dKO) mice, Tgfb2creER² mice were bred with Mmp13Tgβ1/2 mice.Tamoxifen was administered to 2-week-old mice (1mg/10g body weight, i.p., x5 days).

Meniscal-Ligamentous Injury (MLI) Surgery: MLI surgery was performed on 10-week-old Mmp13Tgβ1/2 mice which were then sacrificed at 4, 8, 12, or 16 weeks after surgery. MLI surgery was also performed on 10-week-old wild-type mice and an Mmp13 inhibitor was injected thereafter every other day until 12 weeks after surgery. Hindlimbs were harvested for histology, histomorphometry and immunohistochemistry (IHC) analysis.

Cre-recombination Efficiency: To determine if the Col2a1CreERT2 transgene could target floxed genes specifically in articular cartilage, Col2a1CreER² transgenic mice were bred with Rosa26 or Rosa-tomato reporter mice. Tamoxifen (TM, 1mg/10g body weight/day, i.p.) was administered. The mice were sacrificed at 1 month of age. Cre-recombination efficiency was evaluated by X-Gal staining or observed under a fluorescent microscope.

Histology and Immunohistochemistry Analysis: Hindlimbs were harvested and fixed in 10% NB-formalin for 3 days and de-calcified for 14 days in 14% EDTA and then paraffin-embedded. 3-µm sections were cut and stained with Alicant blue/H&E. Histomorphometric analysis was performed using Osteometrics software (Decatur, GA). Immunohistochemistry staining was performed using TGFβ2, Col2 and Col10 antibodies.

Quantitative Real-Time PCR: Total RNA was extracted from primary articular chondrocytes which were isolated from 1-month-old Tgfb2 cKO mice and their Cre-negative littermates. cDNA was synthesized from 1µg of RNA using the iScript cDNA synthesis kit (Bio-Rad). Real-time RT-PCR analysis was performed using murine specific primers including the major collagenase Mmp13, aggregases Adamts5/8, as well as several chondrocyte marker genes such as Sox9, Runx2, Alp, Col2, Col10, etc.

Luciferase and ChiP Assay: A plasmid containing the 3.4-kb human Mmp13 promoter fragment was transfected into RSC chondrocytic cells and the luciferase assay was performed to check the effect of TGF-β signaling on Mmp13 transcription. The ChiP assay was performed to determine if Runx2 could specifically bind to the Mmp13 promoter in RCS cells.

Statistical Analysis: Results are presented as the mean ± standard deviation. Statistical analyses include Student’s t-tests and two-way ANOVA. p<0.05 was considered statistically significant.

Results:
X-Gal staining revealed a Cre-recombination efficiency of greater than 80% in articular cartilage. Analysis of Col2a1CreERT2;tm1G/M (Rosa-tomato) mice by fluorescent microscopy further demonstrated the efficient Cre-recombination in articular chondrocytes during the postnatal stage.

Mmp13 (16-fold increase) and Adamts5/8 (4- and 6-fold increases, respectively) mRNA expression were significantly up-regulated in articular chondrocytes of Tgfb2 cKO mice. Indeed, the expression of Tgfb2 was significantly increased in articular chondrocytes derived from Tgfb2 cKO mice. The expression of fibrosis-related genes, such as type I collagen (Col1) and type III collagen (Col3), were also increased in Tgfb2-deficient chondrocytes.

In 3-month-old Tgfb2 cKO mice, a severe OA-like phenotype was observed, including chondrocyte hypertrophy in the articular cartilage with dramatically-increased col10 expression, tears and clefts in the articular cartilage surface, severe loss of articular cartilage tissue and osteo-cartilaginous formation. Histomorphometric analysis confirmed that articular cartilage area on both the tibia and femur were significantly reduced by deletion of the Tgfb2 promoter. In this study, we also found that inhibition of TGF-β signaling up-regulates Mmp13 expression in a Runx2-dependent manner. While TGF-β treatment significantly down-regulated Mmp13 expression by 9-fold, mutation of the Runx2 binding site in the proximal region of the Mmp13 promoter strongly reversed the inhibitory effect of TGF-β on Mmp13 promoter activity. These results indicate that TGF-β signaling represses Mmp13 transcription through Runx2. The ChiP assay further demonstrated that the transcription factor Runx2 could specifically bind to the highly conserved Runx2 binding site (5'-PyGPYGGTPy-3') in the Mmp13 promoter in RCS cells. To further determine the role of Runx2 in TGF-β signaling and the regulation of Mmp13 expression, we transfected Tgfb2 siRNA into RCS cells with or without Runx2 siRNA and found that the up-regulation of Mmp13 expression by TGF-β signaling inhibition (Tgfb2 siRNA) was significantly reversed by Runx2 siRNA.

Conclusion:
In this study, we demonstrated that Mmp13 is a critical target gene of TGF-β signaling in articular chondrocytes, which may be a potential target for therapeutic intervention for OA.

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References: