Excessive mechanical stress loading on articular cartilage is one of the major factors of osteoarthritis (OA); however, the molecular functions of the candidate gene.

Methods: To load mechanical stress on cultured chondrocytes, we used a programmable cell stretcher system, STB-140 (STREX). Primary articular chondrocytes from 5-day-old mouse knee joints were seeded using the dimethylmethane blue dye-binding assay. We used mouse primary articular chondrocytes and mouse chondrogenic cell line ATDC5 for further in vitro functional analyses.

Results: Microarray analyses revealed that 2,076 genes were increased more than twice by the mechanical stress loading. Among them, we focused on Gremlin1, which is one of the most highly expressed genes. Gremlin1 is known to be a secreted protein that regulates limb development as a BMP antagonist during skeletal development, although its function in articular cartilage is unknown. Immunohistochemistry revealed that Gremlin1 protein was localized in cytoplasm of mouse normal knee articular chondrocytes, and the expression was enhanced in the cells of the experimental mouse OA model. In the organ culture of mouse femoral heads, recombinant human (rh) Gremlin1 treatment increased aggrecan release into the medium in a dose-dependent manner. In primary culture of mouse articular chondrocytes, rhGremlin1 treatment induced the expressions of catabolic factors including Mmp13 and Adamts5, and suppressed those of anabolic factors including Col2a1, aggrecan and Sox5. Lentiviral overexpression of Gremlin1 in ATDC5 cells also induced the catabolic factor expressions and suppressed the anabolic factor expressions. In organ culture of mouse femoral heads, rhBMP-7 treatment decreased the aggrecan release, and this anti-catabolic effect was abolished by the co-treatment of rhGremlin1. To further identify other signals related to the catabolic effect of Gremlin1 under the mechanical stress, we performed pathway analyses and gene ontology analyses using the microarray data and Genespring software, and found the NF-κB signal to be the most potent candidate pathway associated with both mechanical stress loading and Gremlin1. To know the involvement of the NF-κB signal with the catabolic effect by Gremlin1, we used conditional knockout mice of Rela, a representative transcription factor of the NF-κB signal. In the organ culture of femoral heads from Col2a1-Cre;Relaf/f/f mice, rhGremlin1 did not induce the aggrecan release, while it normally induced the release in the control Rela/f/f femoral heads. We further confirmed that suppression of the NF-κB signal by an IKK inhibitor (BMS-345541) treatment also diminished the increase of aggrecan release. Induction of catabolic factors and suppression of anabolic factors by rhGremlin1 were also diminished by the IKK inhibitor treatment.

Conclusion: We have identified Gremlin1 as a catabolic factor induced by the mechanical stress load to articular chondrocytes, not only through the BMP signal but also through the NF-κB signal. Gremlin1 may provide a novel therapeutic target of OA.

Table 1

| Parameters significantly increased in anti-SOST compared to IgG treated mice | (SurePrint G3, Agilent Expression Array) analyses were performed using samples before and after the mechanical loading. The gene expressions were confirmed by real-time RT-PCR and immunohistochemistry. The catabolic activity was assessed by measuring the concentration of aggrecan released from cultured 3-week-old mouse femoral heads using the dimethylmethane blue dye-binding assay. We used mouse primary articular chondrocytes and mouse chondrogenic cell line ATDC5 for further in vitro functional analyses.

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of patients with arthritis and in rodent models of arthritis. We found that increasing MCP5 expression levels correlate with osteoarthritis (OA) severity in a murine model of OA (destabilization of medial meniscus, DMM). Using a Tgβ2-β-Gal-GFP-BAC mouse, containing β-gal and GFP reporters for TβRII expression, we have recently characterized the TβRII expressing cells (TβRII+) as joint progenitors. Our previous studies also demonstrated that intra-articular implants of isolated TβRII+ cells into DMM knees were able to abolish MCP-5 up-regulation. Several studies have supported the hypothesis that TGF-β and PTHrP act in a common signaling cascade to inhibit the development of a calcifying phenotype in articular chondrocytes. Our studies are aimed at determining whether blockade of MCP-5 signaling during DMM affected OA progression and whether PTHrP could mediate such signaling.

Methods. We followed two in-vivo approaches: 1) mice subjected to DMM were treated with an antagonist of the MCP5 receptor CCR2 (RS504393 4mg/Kg/day in drinking water) 1 and 8 weeks after DMM; 2) FACs sorted TβRII− cells from Tgβ2-β-Gal-GFP-BAC limb buds were injected into the intra-articular space of DMM knees 1 and 8 weeks after DMM (25X103 cells/15μl PBS) to inhibit MCP-5 expression in articular cartilage. In both approaches, mice were euthanized 4 and 12-weeks after DMM. Knees were subjected to micro-CT analysis followed by decalcification and paraffin embedding for histological studies. Safra-nin-O/Fast green staining was performed for OA grading following the OARSI scoring system for mouse. Adjacent sections were subjected to double-immunofluorescence (IF) studies for TβRII and MCP-5 expressions and in-situ hybridization (ISH) studies for PTHrP mRNA. In our in-vitro approach, we isolated TβRII− cells and cultured them in micro-plates mixed with or without MCP-5 (20 ng/ml) for 3 days. cDNA was isolated and subjected to quantitative RT-PCR.

Results. TβRII expression is increased 2 weeks after DMM but its expression is abolished by 4 weeks after surgery. TβRII decrease is accompanied by increasing in MCP-5 expression (Fig.1). Inhibition of MCP5 signaling by both approaches was able to decrease OA lesions and in-situ hybridization (ISH) studies for PTHrP mRNA. In our in-vitro approach, we isolated TβRII− cells and cultured them in micro-culture condition with or without MCP-5 (20 ng/ml) for 3 days. cDNA was isolated and subjected to quantitative RT-PCR.

Conclusions. Our findings show that progression of OA in early stage is associated with derangement of TβRII-progenitors that leads to an increase of MCP-5 and suggest that PTHrP might mediate MCP-5 effect allowing articular chondrocytes to progress toward OA degeneration. Our results open new perspective for OA treatment.