3) weeks post-surgery; or from 4 weeks post-surgery until sacrifice at 8 weeks (group 2) (n = 8/antibody/group). At sacrifice knees were fixed, bone changes analysed by μCT, joints then decalcified and OA pathology scored (average of 2 blinded observers) on serial sagittal sections. SOST and DKK1 were immunolocalized in 3 mice of each group/antibody.

Results: Treatments were well tolerated with no adverse reactions observed; all animals completed the study. The significant effects of anti-SOST compared with IgG in operated limbs from the 3 groups are summarized in Table 1. Increased tibial cortical bone thickness and subchondral trabecular bone volume were seen by μCT in all groups with anti-SOST compared with IgG ($p<0.05$), confirming the efficacy of the SOST inhibition. However, subchondral bone plate thickness was only increased by anti-SOST in groups 1 and 2 ($p<0.05$). Similarly, histological scoring of subchondral bone sclerosis was only significantly increased with short-term SOST inhibition (groups 1 and 2). Inhibition of SOST only later in ptOA development (group 2) led to increased osteophyte size ($p<0.05$). Importantly, this later anti-SOST treatment (group 2) also increased articular cartilage structural damage ($p<0.05$). Immunohistochemistry revealed that anti-SOST antibody increased the number of SOST positive cells in calcified cartilage (x24-86%) and subchondral bone (x33-124%) in all groups. While short term SOST inhibition (groups 1 and 2) also increased DKK1 positive cells throughout the cartilage (x47-180%) and subchondral bone (x250-260%), long-term SOST blockade (group 3) reduced DKK1 positive cells in all tissues (to 30-60%).

Conclusion: Blocking SOST by systemic administration of a monoclonal antibody had detrimental effects on the progression of DMM-induced ptOA in mice. Importantly the effect was dependent on timing, being more pronounced with short-term administration and particularly when given later in disease. It is unclear whether the increase in cartilage damage (group 2), was a direct effect of anti-SOST on chondrocytes, inhibition of synovial SOST, or indirectly through changing subchondral bone. That long-term SOST-blockade did not alter joint pathology to the same extent as short-term treatment may be related to different compensatory changes in other Wnt-regulatory proteins as seen with DKK1. The temporal difference in SOST blockade in joint versus distant skeleton may reflect when SOST expression in OA is most pronounced and/or when increases in subchondral bone plate are most rapid or important in disease progression. The latter may also explain differences in our findings compared with those reported in rat ptOA, where more pronounced and prolonged bone loss occurs. Improved understanding of the regulation of Wnt signaling in different skeletal and joint tissues with time, may lead to novel therapeutic approaches in OA.

Table 1
Parameters significantly increased in anti-SOST compared to IgG treated mice (+ = $p < 0.05$, - = no change).

	Micro CT			Histology score		
	Cortical Bone	Subchondral Trabecular Bone	Subchondral Bone Plate	Subchondral Bone Plate	Osteophyte Size	Articular cartilage Erosion
Group 11-4 weeks	+	+	+	+	-	-
Group 24-8 weeks	+	+	+	+	+	+
Group 31-8 weeks	+	+	-	-	-	-

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IDENTIFICATION OF GREMLIN1 AS A CATABOLIC FACTOR INDUCED BY MECHANICAL STRESS LOADING IN ARTICULAR CHONDROCYTES

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Purpose: Excessive mechanical stress loading on articular cartilage is one of the major factors of osteoarthritis (OA); however, the molecular mechanisms of cartilage degradation by such loading remain unclear. The present study therefore analyzed gene expression profiles of articular chondrocytes under mechanical stress loading and investigated 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 onto stretch chambers at a concentration of 1.0×106 cells/chamber. After 48 h of culture on the chambers, we initially applied a uni-axial cyclic cell stretch (0.5 Hz, 10% stretch) to the articular chondrocytes for 30 min, then cultured them for an additional 24 h. cDNA microarray (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 ability was assessed by measuring the concentration of aggrecan released from cultured 3-week-old mouse femoral heads using the dimethylmethylene 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 Sox9. 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;Relafl/fl mice, rhGremlin1 did not induce the aggrecan release, while it normally induced the release in the control Relafl/fl 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 fators by rhGremlin1 were also diminished by the IKK inhibitor treatment.

Conclusion: We have identified Gremlin1 as a catabolic factor induced by the mechanical stress loaded 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.

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A ROLE FOR TGFβ-RII/MCP-5 AXIS DURING POST TRAUMATIC OSTEOARTHRITIS AND POTENTIAL ROLE OF PTHRP IN MEDIATING MCP-5 EFFECT

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Purpose: In previous studies we found that the down-regulation of the chemokine MCP5 by TGF-β Type II receptor (TβRII) signaling is required for joint development. MCP-5 role in arthritis is demonstrated by human and animal studies. MCP-5, its human homologous MCP-1 and their sole common receptor CCR2, are increased in the inflamed joints

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). By using a Tgfbr2-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 Tgfbr2-β-Gal-GFP-BAC limb buds were injected in 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 embedment for histological studies. Safranin-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 micromass condition 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 when RS504393 treatment or TβRII+ cell implants were performed at an early stage of OA development, but not at later stages (Fig. 2). In a parallel study conducted in our group we found that micro-CT analysis of DMM posterior medial tibia show an increased mineralization of subchondral bone compared to sham controls. We found that the subchondral bone mineralization in the posterior medial tibia induced by DMM was abolished when mice were treated with RS504393 at early stage compared to untreated (Fig. 3). In our in-vitro approach we demonstrated that MCP-5 has a direct inhibitory effect on PTHrP expression in TβRII+ joint progenitors (Fig.4). Consistently with TβRII expression pattern during DMM, our in vivo studies show that PTHrP mRNA expression levels in the periarticular chondrocytes increase at early stage of OA development (2 week after surgery) but such regular expression is disorganized and decreased at later stages after DMM (Fig. 5).

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.

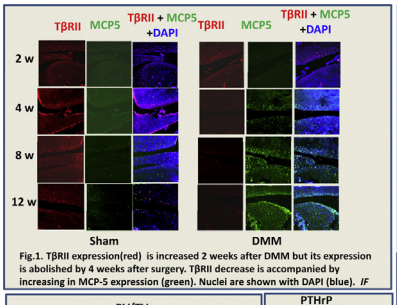

Fig.1. TβRII expression(red) is increased 2 weeks after DMM but its expression is abolished by 4 weeks after surgery. TβRII decrease is accompanied by increasing in MCP-5 expression (green). Nuclei are shown with DAPI (blue). *IF*

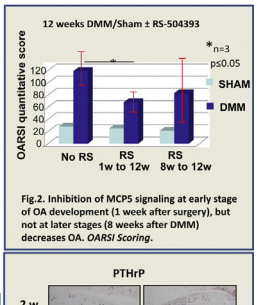

Fig.2. Inhibition of MCP5 signaling at early stage of OA development (1 week after surgery), but not at later stages (8 weeks after DMM) decreases OA. *OARSI Scoring.*

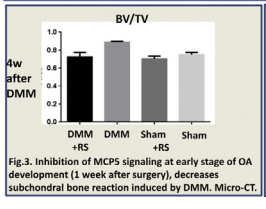

Fig.3. Inhibition of MCP5 signaling at early stage of OA development (1 week after surgery), decreases subchondral bone reaction induced by DMM. Micro-CT.

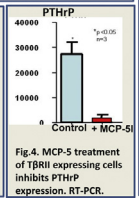

Fig.4. MCP-5 treatment of TβRII expressing cells inhibits PTHrP expression. RT-PCR.

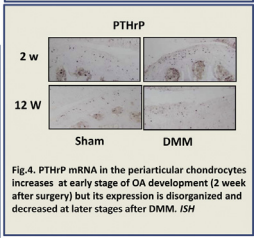

Fig.4. PTHrP mRNA in the periarticular chondrocytes increases at early stage of OA development (2 week after surgery) but its expression is disorganized and decreased at later stages after DMM. *ISH*

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BACH1 DEFICIENT MICE REDUCE SEVERITY OF AGE-RELATED AND EXPERIMENTAL OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA) is a highly prevalent disease in clinical practice. It had been reported that various factors such as aging, trauma and pro-inflammatory cytokines are involved in OA pathogenesis. Oxidative stress is elevated in joint tissues including articular cartilage in aging and trauma, and it cause activating catabolic factors such as inflammatory cytokines. Antioxidant enzymes such as Heme oxygenase-1 (HO-1) are an important to prevent an accumulation of oxidative stress, and play an important role in maintenance of cellular homeostasis. However, the role of HO-1 in OA development remain unclear. Bach1 is a transcriptional repressor of HO-1, thus Bach1-/- mice is upregulated HO-1 in various tissues. The objective of this study was to define the function of HO-1 in OA pathogenesis using Bach1-/- mice by two different animal models of OA: an aging model, a surgical model.

Methods: To clarify the expression of HO-1 with aging, the knee joints of newborn, 3, 12 and 22 month-old (m-o) wild-type mice were assessed by immunohistochemistry using HO-1 antibody (ab52947). To elucidate whether HO-1 involved in OA pathogenesis, we used two different animal model of OA; an aging models and a surgical models. As the aging model, we prepared 22 m-o Bach1-/- mice (n=16, 22 knees) and wild-type mice (n=8, 14 knees). Experimental OA was induced in 12 week-old (w-o) Bach1-/- mice (n=13) by transection of the medial meniscotibial ligament and the medial collateral ligament in the right knees and sacrificed 12 weeks later. Wild-type mice (n=11) were used as a control. Each knee was stained with Safranin O, and the modified OARSI and Mankin's scoring systems were used to validate the histological changes of joint tissues. To define systemic inflammation in Bach1-/- mice, the levels of proinflammatory cytokines in serum were measured. Furthermore, to elucidate whether reduction of OA severity in Bach1-/- mice associated with cellular homeostasis, we examined LC-3, autophagy marker and the expression of antioxidases such as SOD2 in knee joints by immunohistochemistry, and also examined the relation between HO-1 and Autophagy or SOD2 in articular chondrocytes using HO-1 siRNA (siHO-1).

Results: The knee joints from wild-type mice at neonatal, 1 to 22 m-o showed an aging-related significant reduction in HO-1 positive cells. Whereas articular cartilage and meniscus in Bach1-/- mice showed significantly more HO-1 positive cells than 22 m-o wild-type mice. Bach1-/- mice reduced the development of an age-related OA-like pathology compared with the wild-type mice at 22 m-o. Histological scores indicated that Bach1-/- mice were significantly decreased in the severity of the OA-like changes. Bach1 -/- mice inhibited not only degradation of cartilage but also OA-like changes of other joint tissues such as meniscus degeneration, osteophyte formation and synovitis. In OA surgical model, Bach -/- mice reduced the severity of OA-like changes than wild-type mice as well as an aging model. In aged-mice (22 m-o), proinflammatory cytokines in wild-type mice were significantly increased compared with that in young mice (10 w-o), however, these cytokines in Bach1-/- mice did not significantly change between young- and aged-mice. In aged-mice, IL-1b, IL-2, IFNg and TNFa in Bach1-/- mice were significantly reduced compared with that in wild-type mice. The number of LC3-positive chondrocytes in Bach1-/- mice was significantly higher than wild-type mice. SOD2 positive chondrocytes were also higher in Bach1-/- mice than wild-type mice. Although the expression of SOD2 by siHO-1 was reduced in Bach1-/- chondrocytes, LC-3 was not directly regulated by HO-1.

Conclusions: Our present study showed that Bach1 -/- mice reduce the severity of aging-related and experimental OA-like changes. The activation of autophagy and the expression of SOD2 were maintained in articular cartilage of aged-Bach1 -/- mice. Thus, control of HO-1 might be an effective treatment for OA prevention.