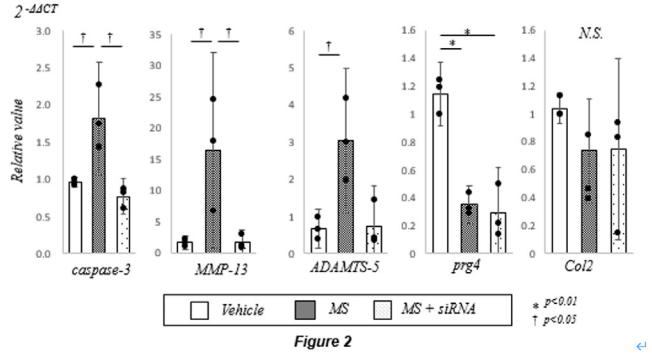

Figure 2

maintenance and chondrocyte function, but excessive mechanical stress induces cell death and cartilage degeneration. In this study, the increase in MMP-13 and Caspase-3 after stretch indicated a negative effect on chondrocyte function. On the other hand, the Piezo protein, as a mechanoreceptor channel, allows calcium to enter the cell by mechanical stretch, and Ca2+ ions induce calmodulin-mediated cell death. In the present study, we successfully knocked down Piezo2 and suppressed MMP-13 and Caspase-3. These results indicate that piezo proteins may be a potential target for the treatment of OA in the future.

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INTRAARTICULAR INJECTION OF BACTERIAL DNA FROM HUMAN OA CARTILAGE IS ASSOCIATED WITH WORSENED PTOA IN MICE

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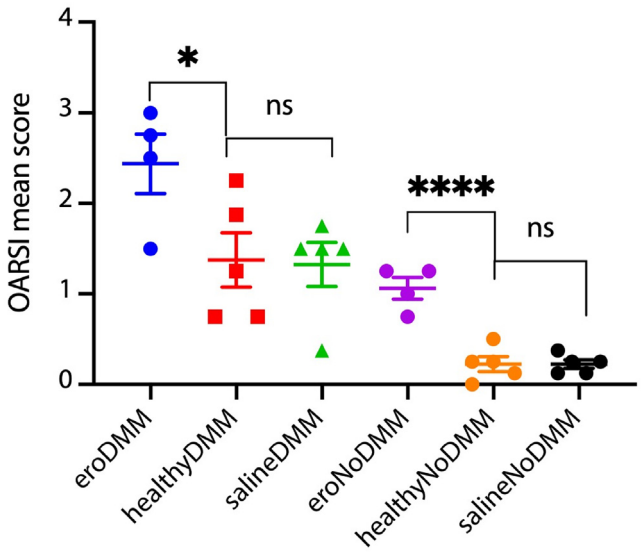
Purpose: We have previous demonstrated a bacterial DNA signature within cartilage of humans and mice and shown shifts in this signature with OA development. However, whether this bacterial DNA plays a pathogenic role in the development of OA is unclear. In this experiment, we set out to determine whether purified, amplified bacterial DNA from human OA patients accelerated histological signs of OA following intraarticular injection into germ-free B6 mice.
Methods: Human knee articular cartilage was obtained from OA-free cadaveric donors (n=12) and eroded sections of end-stage OA patients undergoing joint replacement (n=24). Bacterial DNA was separated from human DNA using an MBD2-Fc magnetic bead approach and amplified using unbiased whole-genome amplification (Qiagen REPLI-g), then cleaned, concentrated, resuspended in buffer adjusted to 0.90 w/v NaCl (normal), and decontaminated. The absence of endotoxin was confirmed using a Pierce chromogenic endotoxin assay. 16s microbiome composition analysis was performed on sample pools pre- and post-amplification. 1000ng of amplified DNA (OA-free or eroded-OA) in 2.5uL, or an equivalent volume of normal saline, were injected unilaterally into a hind knee of C57BL6n germ-free animals (n=4 eroded, n=5 healthy, n=5 saline). 14 days later, mice underwent DMM surgery in the same knee under aseptic conditions within the germ-free facility. Sterility of germ-free isolators were monitored by weekly fecal pellet bacterial plating. Eight weeks later, mice were sacrificed, knee samples fixed, embedded, sectioned, stained, and graded using the OARSI histopathologic scoring system.
Results: Whole-genome amplification did not significantly change bacterial species representation by 16s sequencing. No significant endotoxin was detected in post-amplified specimens (0.018-0.022 EU/mL). No significant differences in mean OARSI score were found comparing saline-injected with healthy human-injected animals post-DMM (1.38±0.67 vs. 1.33±0.54, mean±SEM, P=0.90). A significant increase was found in human OA-injected animals (2.43±0.66) compared to both saline-injected (P=0.03) and healthy-injected (P=0.05). Within the contralateral (non-injected, non-DMM) side, no differences were seen comparing saline and healthy-injected animals (0.22±0.18 vs. 0.21±0.33, respectively, P=0.8); however, a significant increase was



seen in human OA-injected animals (1.06±0.10) compared to both saline-injected (P=0.0002) and healthy-injected (P=0.0006) animals (Figure 1). 16S analysis of cartilage samples post-DMM is ongoing.
Conclusions: Bacterial DNA amplified from human OA patients has pro-OA effects when delivered into germ-free animals although no effects were seen when healthy human cartilage-amplified bacterial DNA was delivered; the source of this disparity is unclear although significant differences in bacterial composition exist between these two cartilage sources. Surprisingly, we also noted increases in histopathological score in the contralateral nonoperated side in OA-injected animals, suggesting a systemic effect of intraarticular injection. Future work should focus on confirmation of our findings, expansion to non-germ-free animals, and mechanistic evaluations.

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OSTEOMODULIN IS INVOLVED IN BONE AND CARTILAGE HOMEOSTASIS AND OSTEOARTHRITIS DEVELOPMENT

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Purpose: To investigate the roles of osteomodulin (OMD), a small proteoglycan known for controlling collagen fibrils organization in bone, in the development of osteoarthritis (OA) associated with subchondral bone sclerosis.
Methods: We used both loss of function and overexpressing mice for *Omd* aged 4, 8, and 16-month to study *Omd* roles in bone and cartilage metabolism and answer whether mice develop spontaneous OA. Surgical destabilization of the medial meniscus (DMM) model was performed to investigate its role in mechanically-induced OA. Additionally, we used *omd* overexpression as well as a mutant deficiency for *omd* in the zebrafish model to study *omd* effects on skeletal metabolism and cartilage development. Finally, we used *in vitro* models to further study OMD in osteoclastogenesis.
Results: *Omd* regulated bone and cartilage microarchitectures. Knock-out mice showed thinner calcified cartilage in the medial tibial plate, and their growth plate was thicker at 16-month compared to WT. Both trabecular and cortical BV/TV ratios were increased in the *Omd* knock-out mice and they showed less percentage of porosity. The trabecular number of the knock-out mice was increased while their trabecular TV was decreased. Further, their cortical bone showed increased thickness. Knock-out mice were more prone to develop cartilage lesions either spontaneously or in the DMM OA model. Interestingly, *Omd* knock-out mice developed subchondral bone sclerosis spontaneously while

overexpressing mice showed less subchondral bone sclerosis in the DMM OA model. At 16-month, *Omd*-overexpressing mice had the less trabecular number and a greater structure-model index for their trabecular bone geometry. The zebrafish model showed that the ectopic overexpression of *omd* induced developmental defects with abnormal cartilage structures. Further, we studied the development of OA in zebrafish, animal models that develop OA features in the synovial jaw joint during aging and are excellent genetic models to study OA. We showed that adult zebrafish lacking *omd* were more prone to articular cartilage degeneration. Furthermore, mutant zebrafish showed increased mature osteoclasts generation and increased TRAP staining revealing a higher osteoclast activity. Our zebrafish results are supported by our *in vitro* experiments where we demonstrated that OMD bound to RANKL and inhibited osteoclastogenesis.
Conclusions: OMD is a key factor in subchondral bone sclerosis associated with OA. This small proteoglycan participates in bone and cartilage homeostasis notably by acting on the regulation of osteoclastogenesis.

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IN SILICO PREDICTION OF CYTOKINE DRIVERS OF KNEE OSTEOARTHRITIS USING NETWORK-BASED CYTOKINE INFERENCE

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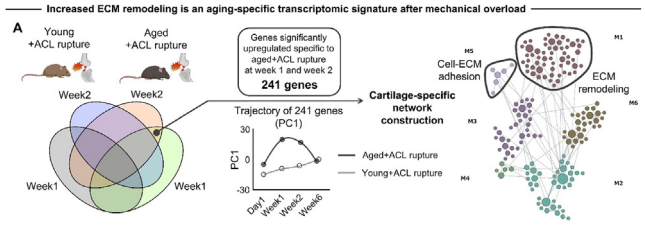
Purpose: While knee osteoarthritis (KOA) has historically been considered primarily a "wear and tear" disease, it is increasingly viewed to be a whole joint disease as a result of the complex tissue-tissue interaction across joint tissues. This complex biological process is particularly true in aged knee joint, which displays altered stress responses to traumatic injury through modulation of the immune system. However, to date, we have lacked a holistic understanding how aging and traumatic injury-induced stress interact to accelerate KOA.
To address this critical short coming, this study sought to thoroughly characterize age-related alterations in transcriptomic responses to mechanical overloading in the knee joint and identify cytokine drivers of the age-related accelerated KOA after traumatic injury.
Methods: First, to clearly demonstrate the age-related alterations in response to mechanical overloading at tissue level, we performed a systematic review of histological metrics of cartilage degeneration in knee joints after traumatic injury in both young and aged mice. Our hypothesis was that aging aggravates overload-induced cartilage degeneration in the knee joint of mice.
We next assessed the global transcriptomic changes at different time points following traumatic injury. For this purpose, we accessed the archived RNA-seq data from young (3 months) and middle-aged (15 months) mice from the study published by Sebastian et al, which yielded 2,738 genes identified across the different time points (day1, week1, week2, and week6) after anterior cruciate ligament (ACL) rupture. To assess age-related alterations in transcriptomic response to traumatic injury, we focused on differentially expressed genes that were upregulated in middle-aged, but not young, knee joint. Using cartilage-specific gene interactive network with subsequent cytokine inference at single gene level, we sought to identify disease-driver cytokines that may exert a pathological role in aged knee joint after traumatic injury.
After the identification of the possible disease-driver cytokines, we implemented a network propagation approach that was applied on the cartilage-specific gene network to verify the pathogenic role of the driver cytokine(s) identified. The results of the *in silico* network propagation approach were further crosschecked by an *in vitro* experiment with microarray analysis in which isolated murine articular chondrocytes cultured with and without the target cytokine supplementation.
Results: Systematic review for histological studies included a total of six studies that implemented histological metrics of cartilage degeneration in knee joint after mechanical overloading in both young and aged mice. As expected, meta-analysis of histological studies confirmed that aged mice displayed progressive cartilage degeneration after traumatic injury up to 8 weeks after surgery, a trend that was not observed in young mice (Figure 1).
We next assessed the age-related alterations in transcriptomic response to mechanical overloading using archived RNA-seq data. The cartilage-specific network analysis of 241 genes upregulated only in the middle-aged knee joint revealed that matrix remodeling was the biological function uniquely observed in the middle-aged knee joint after traumatic injury (Figure 2). Network-based cytokine inference and subsequent tissue-specific network propagation predicted activated Oncostatin M (OSM) and OSM receptor (OSMR), a ligand-receptor complex of known inflammatory cytokines, as a mechanistic driver of the age-related matrix remodeling. Interestingly, single-cell RNA-seq revealed that, whereas OSM is predominantly expressed in immune cells, especially neutrophils, OSMR is predominantly expressed in chondrocytes. These findings indicate a synovium-cartilage interaction in the pathogenesis of age-related accelerated KOA. As a validation of these *in silico* findings, microarray data of murine chondrocytes supplemented with OSM displayed increased matrix remodeling genes.
Conclusions: In this study, we took an unbiased approach of network-based inference of cytokine activity integrated with network

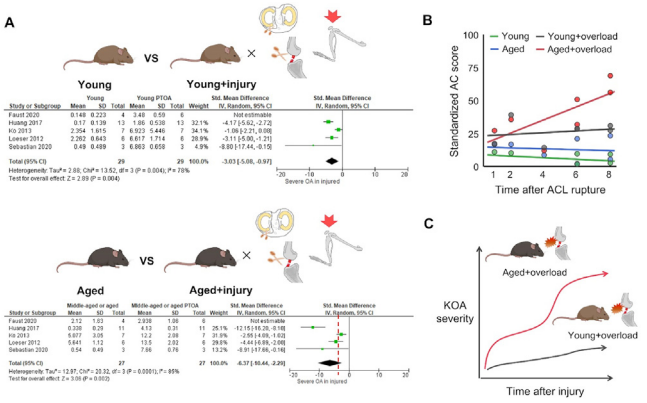
Osteoarthritis and Cartilage

Figure 1.



Osteoarthritis and Cartilage

Figure 2.



Osteoarthritis and Cartilage

Figure 3.