Inflammatory chondrocytes induce cell death signaling pathway in osteocytes leading to progression of osteoarthritis

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INTRODUCTION: The progression of osteoarthritis (OA) includes dramatical changes in microstructure of subchondral bone (SCB) due to impairment of bone metabolism. However, despite of attempts for understanding mechanism behind these changes, the detailed molecular mechanism of how the bone metabolic changes in subchondral bone occurred have not been clarified. There is accumulating evidence highlighting the importance of interaction between chondrocytes in cartilage and osteoblast and osteoclast in SCB in progression of OA [1][2]. Osteocytes are the most abundant type of cell in mature bone tissue with long-lived surviving that play essential function as sensors that respond to mechanical, biological, the purpose of this study is directed to explore the interaction between chondrocytes and osteocytes as a step toward understanding the pathological changes in SCB during OA.

METHODS: The procedures for the animal experiments were approved by the Institute of Animal Care and Use Committee of the Hokkaido University Graduate School of Medicine. In vitro, co-cultured model of murine chondrocytes and osteocytes was developed to study the interaction between the cells. Primary articular chondrocytes were isolated from cartilage of 5-6-day-old C57BL/6J mice and seed in a transwell insert with a diameter of 1.0 μm. Cells were stimulated with 10 ng/ml of IL-1β for 6h and then washed by PBS. MLO-Y4 (mouse osteocyte cell line; kindly provided by Professor Lynda F. Bonewald, Indiana Center for Musculoskeletal Health) were cultured on 24-well plate and co-cultured with the stimulated chondrocytes for 24hrs and 48hrs. Osteocyte responses were evaluated with Crystal Violet staining, Western blotting, qRT-PCR (these were reported last year please refer to abstract number as ORS2022; Ab nu 0443) and in additional RNA-seq. In vivo, the knees of OA model mice, anterior cruciate ligament tear (ACLT) model and collagenase injection model (CIOA), were compared to control models. 8-week-old C57BL/6J mice was operated on ACL tearing as the ACLT model and 12-week-old C57BL/6J mice was injected of collagenase VII 6μl to the left knee joint as the CIOA model. For both mouse models, the mice knees ware collected 6 weeks later operation or injection and subjected to HE, gasdermin D (GSDMD) staining and TRAP staining. The number of osteocytes in SCB, the thickness of SCB, and the number of GSDMD positive cells and TRAP positive surface of bone were measured. In addition, human OA knees which had undergone total knee arthroplasty were collected and stained GSDMD and caspase1 (Casp1). Statistical analysis was performed using unpaired t test and one-way ANOVA(GraphPad Software Inc.). The significant level was set at $p < 0.05$.

RESULTS: Our earlier study demonstrated that inflammatory chondrocytes impaired the function of chondrocytes in vitro. To fully understand this molecular mechanism, RNA-seq analysis of osteocytes after being cultured with inflammatory chondrocytes was performed. Results demonstrated that up-regulated genes were most significantly enriched in NOD like receptor signaling pathway, inflammation and cell death terms (Figure 1A). Importantly, stimulated osteocytes exhibited an elevation in the expression of pyroptosis-related molecules such as Casp1 and GSDMD. Likewise, western blot analysis showed an increase in protein level of cleaved Casp 1 and GSDMD (Figure 1B). In consistent with our in vitro data, SCB of OA knee was thinner with reduced number of osteocytes and increased number of GSDMD positive osteocytes and osteoclasts as compared to control in both OA models (Figure 2A). In consistent with mouse results, GSDMD-positive osteocytes were detected in SCB of human OA joint (Figure 2B).

DISCUSSION: The results of this study demonstrated that OA chondrocytes induced inflammatory cell death program in osteocytes that promotes inflammation in bone microenvironment leading that impairs bone metabolism in SCB, thereby promoting OA. Development of inflammation in bone microenvironment has been reported to be associated with increase in number of bone-resorbing osteoclasts and bone loss. The increased turnover of bone metabolism and vascularity resulting in decreased SCB thickness have been observed in early OA [3][4]. Moreover, loss of osteocytes in bone microenvironment is one of major features of osteoporosis associated with decreased bone quality and volume. Collectively, our findings provide a possible explanation of the pathological changes in SCB in OA and shed light to a new therapeutic target. Targeting the interaction between chondrocytes and osteocytes may lead to new therapeutic approach for treatment of OA.

SIGNIFICANCE/CLINICAL RELEVANCE: Development of catabolism in chondrocyte may induce pyroptosis of osteocyte in OA leading to thinner SCB associated with progression of OA. Targeting the interaction between chondrocytes and osteocytes might be a novel strategy for management of OA.

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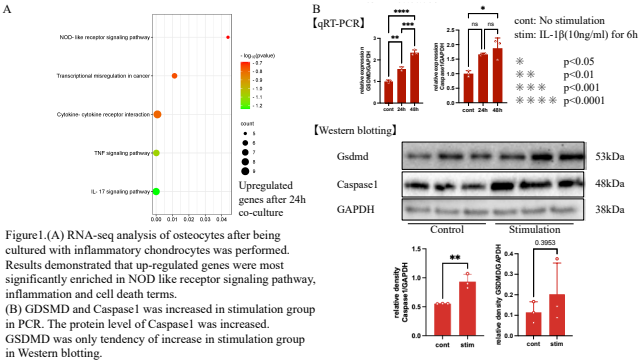

Figure1.(A) RNA-seq analysis of osteocytes after being cultured with inflammatory chondrocytes was performed. Results demonstrated that up-regulated genes were most significantly enriched in NOD like receptor signaling pathway, inflammation and cell death terms.
(B) GDSMD and Caspase1 was increased in stimulation group in PCR. The protein level of Caspase1 was increased. GSDMD was only tendency of increase in stimulation group in Western blotting.

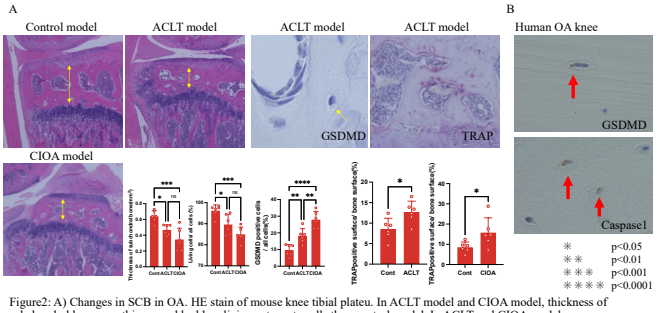

Figure2: A) Changes in SCB in OA. HE stain of mouse knee tibial plateu. In ACLT model and CIOA model, thickness of subchondral bone was thinner and had less living osteocyte cells than control model. In ACLT and CIOA model, Proportion of Gsdmd positive cells was more than control model and TRAP positive surface percentage.

B) Human OA knee Tibial plateu. GSDMD positive osteocytes and Caspase1 positive osteocyte were detected in SCB of human OA joint .