Blockade of osteocyte pyroptosis drives OA cartilage degeneration promoting remodeling of subchondral bone

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Disclosures: Yuki Ogawa: None. M Alaa Terkawi: None. Ryota Suzuki: None. Masaya Nakajo: None. Yuki Fujie: None. Taiki Tokuhiro: None. Taku Ebata: None. Endo Tsutomu: None. Daisuke Takahashi: None. Norimasa Iwasaki: None.

INTRODUCTION: In osteoarthritis (OA) progression, impaired bone metabolism leads to dramatic alterations in the microarchitecture of the subchondral bone (SCB) [1]. However, the detailed molecular mechanisms underlying these changes remain unclear. Recent studies have reported interactions between the subchondral bone and articular cartilage in OA [2]. Our earlier work demonstrated that degenerated chondrocytes induce GSDMD-mediated pyroptosis in osteocytes within subchondral bone. Nevertheless, the effects of osteocyte pyroptosis OA progression remain unknown. Thus, the objective of this study is to investigate the impact of osteocyte pyroptosis in subchondral bone on OA progression using osteocyte specific Gsdmd knockout (KO) mouse.

METHODS:
Primary osteocytes isolated from mice were seeded on collagen type I-coated plates and stimulated with lipopolysaccharide (10 ng/ml) for 3 hours, followed by aluminum hydroxide (20 µg/ml) for an additional 3 hours to induce pyroptosis. The pyroptotic osteocytes were then co-cultured with murine chondrocytes using a 1.0 µm Transwell insert for 24 hours. After co-culture, chondrocytes were collected, and the effects of osteocyte pyroptosis on chondrocytes were analyzed by Western blotting. Osteocyte-specific Gsdmd knockout (KO) mice were generated using the Cre-loxP recombination system. Eight-week-old male mice underwent anterior cruciate ligament transection (ACLT) to induce an instability OA model. Knee joints were collected at 2, 4, and 6 weeks post-surgery for histological evaluation and µCT evaluation. In addition, osteocytes isolated from WT and KO mice were subjected to the aforementioned pyroptosis induction protocol, and differential responses were analyzed using bulk RNA sequencing. Statistical analysis was performed using unpaired t test and one-way ANOVA (GraphPad Software Inc.). The significance level was set at $p < 0.05$.

RESULTS: In OA chondrocytes co-cultured with pyroptotic osteocytes, the expression of catabolic factors, including MMP3 and ERK1/2, was significantly higher compared with OA chondrocytes co-cultured with unstimulated osteocytes (Figure 1). In the OA model, cartilage degeneration was more severe in KO mice than in WT mice, accompanied by an increased number of TRAP-positive cells in the subchondral bone. The number of empty lacunae, indicating bone cell death, was significantly higher in WT mice. µCT analysis revealed that both BV/TV and vBMD were significantly higher in KO mice compared with WT mice (Figure 2). GO and pathway enrichment analyses of the upregulated genes revealed significant enrichment in immune-related processes. Notably, these genes were also significantly enriched in the NOD-like receptor and NF-κB signaling pathways, both of which play central roles in the activation of inflammation, and also osteoclast differentiation pathway (Figure 3).

DISCUSSION: This study demonstrated that osteocyte pyroptosis induces functional changes in chondrocytes and promotes catabolic activity. However, recent findings highlight Gsdmd has more diverse functions, rather than inflammation initiation, particularly in homeostasis and physiology of normal tissue [3][4]. consist with these reports, in our study, suppression of osteocyte pyroptosis in KO mice resulted in accelerated OA progression and increased subchondral bone turnover. Furthermore, Gsdmd-deficient osteocytes exhibited activation of alternative signaling pathways upon stimulation, leading to increased production of inflammatory and bone remodeling–related factors, thereby contributing to pathological changes in the subchondral bone. These findings suggest that osteocyte pyroptosis in the subchondral bone may play a protective role in suppressing OA progression.

SIGNIFICANCE/CLINICAL RELEVANCE: Our findings suggest that targeting GSDMD in treatment of OA should be considered with caution due to potential side effects of pro-inflammatory response.

REFERNCES: [1] Zhang M, et al. Osteoarthritis Cartilage. 2009 [2] Yuan XL, et al. Osteoarthritis Cartilage. 2014 [3] C. G. Weindel, et al. Trends Cell Biol. 2023 [4] P. Devant et al. Cell Rep.2023

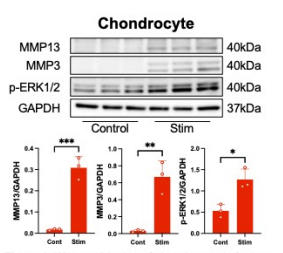

Figure 1. Western blotting of chondrocytes after co-cultured with pyroptosis osteocytes. Catabolic factors significantly increased in stimulation group.

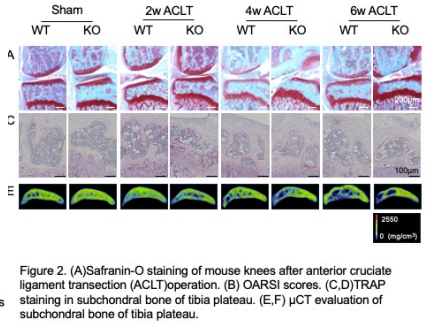

Figure 2. (A)Safranin-O staining of mouse knees after anterior cruciate ligament transection (ACLT)operation. (B) OARSI scores. (C,D)TRAP staining in subchondral bone of tibia plateau. (E,F) µCT evaluation of subchondral bone of tibia plateau.

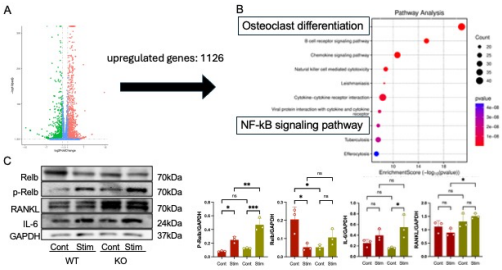

Figure 3. (A)Volcano plot of Bulk RNA seq compared with Gsdmd-deficient and wild type osteocyte after pyroptosis induction. (B) KEGG pathway enrichment analysis of upregulated gene in Gsdmd-deficient osteocytes. (C) Western blotting of osteocytes after pyroptosis induction.