Acid-sensing receptor GPR68 mediates inflammatory and structural responses to joint acidosis in osteoarthritis

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DISCLOSURES: None

INTRODUCTION: Osteoarthritis (OA) is the most prevalent joint disease worldwide and a leading cause of disability. It is characterized by cartilage degeneration, synovial inflammation, and subchondral bone remodeling. While mechanical and inflammatory factors are well recognized, the role of joint acidosis, a sustained reduction in intra-articular pH remains poorly understood. Clinical studies have consistently reported reduced synovial fluid pH in patients with advanced OA, but the mechanistic link between extracellular acidification and joint pathology is largely unknown. GPR68 (OGR1) is a proton-sensing G protein coupled receptor that responds within the pathological pH range of OA joints. It has been implicated in inflammatory signaling in other tissues, but its role in OA progression is unclear. We hypothesized that GPR68 transduces extracellular acid stress into inflammatory and catabolic responses, thereby contributing to OA progression. Here, we integrate in vitro studies, in vivo DMM surgery, transcriptomic and proteomic analyses, and pharmacological modulation to investigate the role of GPR68 in acid-sensing and OA pathogenesis.

METHODS: The study was approved by the IACUC and IRB ethics committee. Primary human articular chondrocytes were stimulated with IL1β under neutral (pH 7.4) or acidic (pH 6.4) conditions. GPR68 function was modulated by siRNA knockdown or by Ogerin, a selective positive allosteric modulator. Inflammatory and catabolic gene expressions were analyzed by bulk RNA-sequencing, SYBR Green® assays and Western-immunoblotting analyses. GPR68 activation was assessed using a genetically encoded fluorescent biosensor which was designed by inserting a circularly permuted GFP into third intracellular loop of receptor, yielding a transient fluorescence in response to proton stimulation. To determine the role of Gpr68 inhibition on OA progression *in vivo*, we performed DMM surgery to simulate clinical post-traumatic OA (PTOA). Global Gpr68 knockout (Gpr68-/-) and littermate wild type (WT) control mice underwent sham or DMM surgery. Only male mice (N=7 mice per group) were used to minimize variability and establish proof-of-concept; future studies will include both sexes to examine sex-specific effects. Joints at 8-week post-surgery were analyzed by histology, OARSI scoring, and Safranin-O/Fast Green staining. Synovial fluid from wild-type and Gpr68-/- DMM joints was subjected to Luminex-based multiplex proteomic analysis for 22 cytokines. Statistical analyses were performed using one-way ANOVA with $p<0.05$ representing statistical significance.

RESULTS: To investigate the role of acidosis in inflammation and catabolism, we established an in vitro model by culturing human chondrocytes in acidic medium (pH 6.4) with or without IL-1β. Pan-transcriptome analysis revealed that extracellular acidification markedly potentiated IL-1β–induced gene expression, with enhanced upregulation of cytokines, chemokines, and matrix-degrading enzymes (**Fig. 1A**). These data indicate that low pH sensitizes chondrocytes to inflammatory stimuli. We next asked whether similar acid-linked signatures were evident in vivo. Transcriptomic analysis of DMM joints demonstrated induction of acid-base regulators (SLC16A1, CA2), inflammatory mediators (IL1B, PTGS2, MMP13), and hypoxia-responsive genes (HIF1A), suggesting coordinated acidosis-associated reprogramming during OA progression. To assess receptor responsiveness, we used a fluorescent biosensor for GPR68. Confocal imaging confirmed robust activation of GPR68 upon pH reduction from 7.4 to 6.4 in human chondrocytes, validating it as a functional acid sensor (**Fig. 1B**). Next, we assessed the specific contribution of GPR68 on acidosis enhanced inflammatory response in human chondrocytes. Knockdown of GPR68 amplified IL-1β-driven inflammatory chemokine and cytokine (CCL2, CXCL2, CCL5, PTGS2 and MMP13) expression under acidic conditions, while pharmacological potentiation with Ogerin suppressed inflammatory gene induction, showing that GPR68 restrains acid-driven inflammation. Finally, in vivo DMM surgery in Gpr68-/- mice revealed more severe OA progression, with higher OARSI scores, greater proteoglycan loss, subchondral sclerosis, and osteophyte formation compared to wild-type controls (**Fig. 1C**). Synovial fluid proteomics showed elevated inflammatory mediators (CXCL2, CXCL6, CCL7, IL-6, GM-CSF) in knockout joints, indicating loss of GPR68 promotes an amplified inflammatory microenvironment. Together, these results demonstrate that GPR68 functions as a protective acid sensor, modulating inflammatory and structural responses to joint acidosis.

DISCUSSION: This study provides the first integrated evidence that GPR68 directly links extracellular acidosis to inflammatory and structural changes in OA. By combining in vitro, in vivo, and omics approaches, we show that low pH is not simply a byproduct of cartilage degradation, but an active signal amplified through GPR68. The in vitro finding that acidic pH potentiates IL-1β responses highlights the synergy between metabolic stress and cytokine signaling, consistent with prior observations that pH stress alters chondrocyte metabolism and protease activity. Transcriptomic signatures in DMM joints further reinforce the biological relevance of acidosis, with induction of transporters, metabolic enzymes, and hypoxia-related genes pointing to systemic adaptation of the OA joint to an acidic niche. Crucially, our biosensor and functional studies confirm that GPR68 operates within the OA-relevant pH range and actively restrains inflammation. Loss of GPR68 by knockdown or genetic deletion removes this regulatory brake, leading to enhanced inflammatory gene expression and worsening OA pathology. Conversely, pharmacological potentiation with Ogerin demonstrates that GPR68 can be harnessed to dampen acid-driven inflammatory responses, offering translational promise. This positions GPR68 not merely as a passive sensor, but as a central checkpoint in how joints respond to sustained acidosis. Together, these findings reframe acidosis as an active driver of OA progression and identify GPR68 as a key molecular mediator. Beyond mechanistic insight, our data points to the therapeutic potential of targeting proton-sensing pathways to modify OA, a direction that has not been explored clinically.

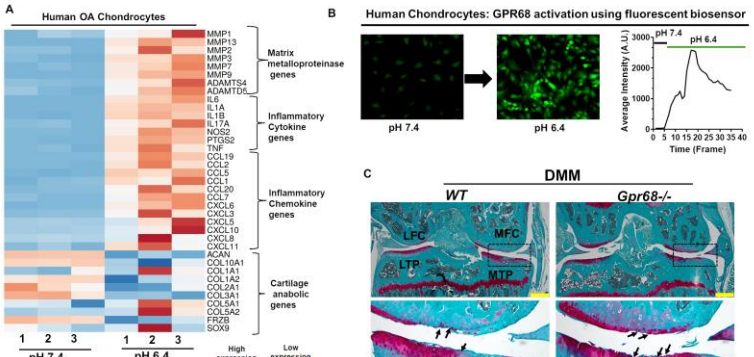

Figure 1. GPR68 mediates chondrocyte and joint responses to acidosis in OA. (A) Acidic pH (6.4) potentiated IL-1β–induced inflammatory and catabolic gene expression in human OA chondrocytes; (**B**) Fluorescent biosensor assay showing GPR68 activation in chondrocytes upon pH reduction (7.4 → 6.4); (**C**) Safranin-O/Fast Green staining revealed greater cartilage degeneration in Gpr68$^{-/-}$ mice versus WT after DMM.

SIGNIFICANCE: Our work establishes GPR68 as a critical mediator of acid-sensing in OA, linking extracellular pH decline to inflammation, cytokine production, and structural degeneration. This study introduces the first in vivo evidence that GPR68 deficiency exacerbates OA, while its potentiation mitigates acid-driven inflammation in human chondrocytes. From a translational perspective, GPR68 represents a promising therapeutic target for OA. Selective agonists or potentiators may restore homeostatic acid-sensing and dampen inflammation, offering a novel strategy beyond conventional anti-inflammatory or mechanical approaches.

ACKNOWLEDGEMENTS: This work was supported in part by NIH (R03TR003669-01A1), VA-CReATE motion pilot grant, and Emory Orthopaedic seed grant to NMK.