Cartilage Autophagy Dysregulation during OA Progression in Hip Femoroacetabular Impingement

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INTRODUCTION: Femoroacetabular impingement (FAI) is the leading cause of hip osteoarthritis (OA). Peroxisome proliferator-activated receptor gamma (PPARγ) is reported to have a protective effect on articular cartilage [1]. Autophagy is essential for maintaining cellular homeostasis [2]. We have previously observed suppression of PPARγ expression and dysregulation of autophagy markers with the progression of human hip OA [3]. This study aims to investigate the effect of PPARγ on autophagy in hip articular cartilage in human tissue.

METHODS: Full-thickness cartilage explants were collected from the femoral head-neck junction of patients with hip FAI who underwent hip arthroscopy for the treatment of hip cam-FAI (FAI) and patients with end-stage FAI-related OA (OA) who underwent total hip replacement (THR). As a non-disease (ND) group, healthy samples were procured from fresh allografts. A 4-mm biopsy punch was used to harvest explants and incubated at 37 °C and 5% CO2 for 24 hours. Subsequently, we cultured the explants in untreated conditions, under catabolic stimulus with interleukin-1β (IL1β) with or without PPARγ agonist (Rosiglitazone) or inhibitor (T0070907) for 48 hours. After culture, sections were stained with safranin O and fast green for histological analysis, and cartilage degeneration was graded based on the Mankin score. Immunofluorescence staining assessed autophagy-related marker genes (LC3B and p62). RNA was extracted from the explants, and gene expressions were analyzed in three groups via qPCR for the following specific markers: GAPDH, LC3B, p62, and MMP-13. The comparisons between groups were performed using ANOVA, with Šídák's correction applied for multiple post hoc comparisons as appropriate. Differences were considered significant at $p < 0.05$ (corrected). Data expressed as mean ± SD for parametric test. Human cartilage specimens were obtained with approval from the Institutional Review Board (IRB) of Washington University School of Medicine, in accordance with the Declaration of Helsinki and relevant national regulations. Written informed consent was obtained from all patients prior to sample collection. Cartilage samples were collected from a total of 21 patients (7 with cam-type FAI, 7 with end-stage FAI-related OA, and 7 non-disease donors), including both male and female donors.

RESULTS: Cartilage degeneration was observed with an increased Mankin score following IL1β stimulation and treatment with PPARγ inhibitor (control vs. IL1β, p<0.001; control vs. PPARγ inhibitor, p=0.01) (Fig.1a). Conversely, catabolism was reduced when the cartilage was treated with a PPARγ agonist (control vs PPARγ agonist, p=0.03, Fig.1b). Imunofluorescence showed an increase in LC3B-positive cells following PPARγ agonist treatment (control vs. PPARγ agonist, ND, p=0.0428; OA, p=0.0431), while IL1β and PPARγ inhibitor reduced LC3B-positive cells (control vs. IL1β, ND, p=0.0017; OA, p=0.0028; control vs. PPARγ inhibitor, ND, p=0.0004; OA, p=0.0992, Fig.2a,b). Similarly, the number of p62-positive cells were reduced by PPARγ agonist (control vs. PPARγ agonist, ND, p=0.0109; OA, p=0.0015), while IL1β and PPARγ inhibitor increased p62-positive cells (control vs. IL1β, ND, p<0.0001; OA, p=0.0127; control vs. PPARγ inhibitor, ND, p=0.0027; OA, p<0.0001, Fig.2c,d). qPCR showed MMP-13 expression was significantly decreased with PPARγ agonist treatment (control vs. PPARγ agonist, ND, p=0.014; OA, p=0.001) and increased following IL1β stimulation and PPARγ inhibition (control vs. IL1β, ND, p=0.048; OA, p=0.011; control vs. PPARγ inhibitor, ND, p=0.002; OA, p=0.045). Co-treatment with PPARγ agonist suppressed the IL1β-induced upregulation of MMP-13 (IL1β vs. IL1β+PPARγ agonist, ND, p=0.003; FAI, p=0.016; OA, p=0.033), whereas the addition of PPARγ inhibitor reversed this effect (IL1β+PPARγ agonist vs. IL1β+PPARγ agonist+PPARγ inhibitor, ND, p=0.031; FAI, p=0.016; OA, p=0.004, Figure 3a). In parallel, LC3B expression was significantly increased after PPARγ agonist treatment (control vs. PPARγ agonist, ND, p=0.049; OA, p=0.024) and decreased following IL1β stimulation and PPARγ inhibition (control vs. IL1β, ND, p<0.001; OA, p=0.044; control vs. PPARγ inhibitor, ND, p=0.006; OA, p=0.008, Figure 3b). The IL1β-induced suppression of LC3B was rescued by PPARγ agonist (IL1β vs. IL1β+PPARγ agonist, ND, p=0.049; OA, p=0.05). p62 expression showed the opposite trend, decreased with PPARγ agonist and increased with IL1β or PPARγ inhibitor. The rescue effect was also confirmed in p62 expression following PPARγ agonist treatment (IL1β vs. IL1β+PPARγ agonist, ND, p=0.043; OA, p=0.027, Figure 3c).

DISCUSSION: This study demonstrates that PPARγ receptor activation in human hip cartilage suppresses catabolic activity and promotes autophagy-related gene expression. PPARγ agonist treatment reduced MMP-13 levels and reversed IL1β-induced catabolic changes. These findings support the role of PPARγ in maintaining cartilage homeostasis via autophagy modulation. In conclusion, our findings support the hypothesis that PPARγ plays a protective role in hip cartilage by enhancing autophagy and reducing catabolic signaling. These results suggest that targeting the DNMT3A–PPARγ–autophagy axis may represent a promising therapeutic strategy to prevent or slow OA progression in patients with FAI. Further in vivo studies and clinical correlation are warranted to validate this approach.

SIGNIFICANCE: This study highlights a novel mechanism by which PPARγ modulates autophagy and catabolic activity in human hip cartilage. These findings suggest that targeting the PPARγ-autophagy axis may offer a promising therapeutic strategy to preserve cartilage integrity and delay the onset of hip osteoarthritis in patients with femoroacetabular impingement.

REFERENCES: **1.** Zhu Y et al. *Proc Natl Acad Sci USA* 1995. **2.** Mancias JD et al. *J Mol Biol* 2016. **3.** Pascual-Garrido C et al. *J Arthroplasty* 2022.

Figure 1: Histology (Safranin-O/fast green staining) and Mankin score in healthy cartilage explants.

Figure 2: Relative gene expressions of cartilage explants in each group (GAPDH, LC3B, p62, and MMP-13).

Figure 3: Immunofluorescence staining of cartilage explants in each group (GADPH, LC3B, and p62).

Figure 1.

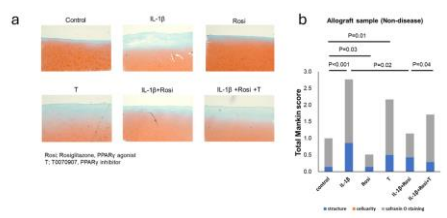

Figure 2.

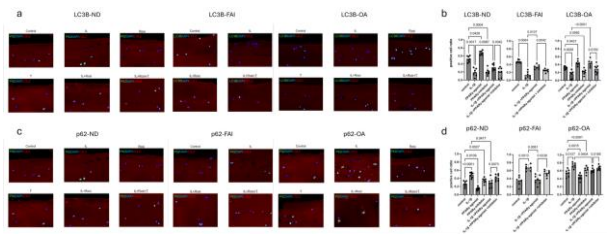

Figure 3.