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. Autophagy is essential for maintaining cellular homeostasis. We have previously observed suppression of PPARγ expression and dysregulation of autophagy markers with the progression of human hip OA. This study aims to investigate the effect of PPARγ on autophagy in hip articular cartilage in human tissue.

METHODS:

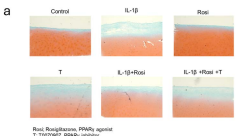
Full-thickness cartilage explants were collected from the femoral head-neck junction of seven patients with hip FAI who underwent hip arthroscopy for the treatment of hip cam-FAI (FAI) and seven patients with end-stage FAI-related OA (OA) who underwent total hip replacement (THR). As a non-disease (ND) group, seven 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.

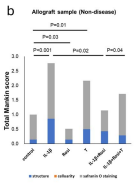
RESULTS:

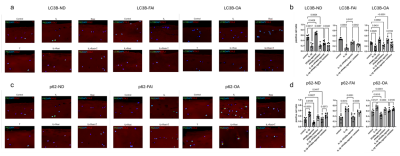
Cartilage degeneration was observed with an increased Mankin score following IL1β stimulation and treatment with PPARγ inhibitor (control vs. IL1β, $p<0.001$; control vs. PPARγ inhibitor, $p=0.01$) (Fig.1a). Conversely, catabolism was reduced when the cartilage was treated with a PPARγ agonist (control 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 redeced 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).

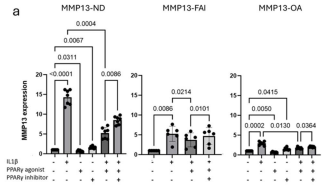
DISCUSSION AND CONCLUSION:

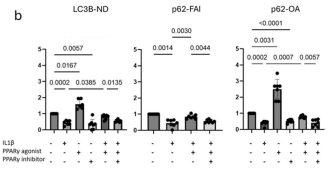
This study demonstrates that PPARγ receptor activation in human hip cartilage suppresses catabolic activity and promotes autophagy-related gene expression. PPARγ agonist treatment reduced MMP-13 levels and reversed IL1β-induced catabolic changes. These findings support the role of PPARγ in maintaining cartilage homeostasis via autophagy modulation. Further studies are needed to assess if regulation of this pathway could potentially slow down progression of hip OA in hip FAI.

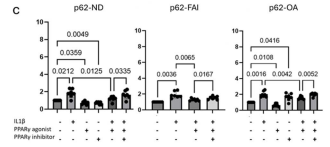

a
Control
IL-1β
Rosi
T
IL-1β +Rosi
IL-1β +Rosi +T
Rosi, Rosiglitazone, PPARγ agonist
T: TG070667, PPARγ inhibitor


b
Allograft sample (Non-disease)
P=0.01
P=0.03
P=0.001
P=0.02
P=0.04
Total Mankin score
normal
IL-1β
Rosi
T
IL-1β+Rosi
IL-1β+Rosi+T
structure
cellularity
safranin O staining


a
LC3B-ND
LC3B-FAI
LC3B-OA
b
LC3B-ND
LC3B-FAI
LC3B-OA
c
p62-ND
p62-FAI
p62-OA
d
p62-ND
p62-FAI
p62-OA


a
MMP13-ND
MMP13-FAI
MMP13-OA
MMP13 expression
0.0004
0.0067
0.0311
<0.0001
0.0086
0.0214
0.0086
0.0101
0.0415
0.0050
0.0002
0.0130
0.0364
IL1β
PPARγ agonist
PPARγ inhibitor


b
LC3B-ND
p62-FAI
p62-OA
0.0057
0.0167
0.0002
0.0385
0.0135
0.0030
0.0014
0.0044
<0.0001
0.0031
0.0002
0.0007
0.0057
IL1β
PPARγ agonist
PPARγ inhibitor


c
p62-ND
p62-FAI
p62-OA
0.0049
0.0359
0.0212
0.0125
0.0335
0.0065
0.0036
0.0167
0.0416
0.0108
0.0016
0.0042
0.0052
IL1β
PPARγ agonist
PPARγ inhibitor