PPARγ Promoter Demethylation Rescues Articular Chondrocytes from Catabolic State in Hip FAI

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INTRODUCTION:

Recent advance in osteoarthritis (OA) research shows OA progression is associated with abnormal epigenetic alterations of many OA-susceptible genes. Femoroacetabular impingement (FAI) is known as a leading cause of hip OA and offers a unique opportunity to study the early molecular mechanism of hip OA disease. Peroxisome proliferator-activated receptor-gamma (PPARγ) is reported to be widely expressed in healthy articular chondrocytes (ACs) and is essential for cartilage homeostasis. A recent transcriptome study identifies PPARγ as a key target linking aberrant DNA methylation to OA progression. This study aimed to investigate the PPARγ expression and methylation status at the PPARγ promoter during OA progression in hip FAI.

METHODS: Full-thickness cartilage samples were collected from the impingement zone. Twelve patients underwent hip preservation surgery for cam FAI (early FAI) and eleven patients underwent total hip replacement for advanced OA secondary to cam FAI (late FAI-OA) were included. As a non-disease (ND) group, six healthy samples were procured from hip joint cadavers. The explants were cultured in unstimulated conditions or catabolic stimulus (IL1β) with or without DNA demethylating agent, 5-Aza-2'-deoxycytidine (5Aza). Histological analysis was performed with safranin O/fast green. Gene expression was analyzed via qPCR for *GAPDH, DNMT1, DNMT3A, PPARγ, MMP13*, and *COL10A1*. Methylation specific PCR (MSP) was performed to assess the methylation status at *PPARγ* promoter site.

RESULTS: The early FAI group included younger subjects (mean=34.4 years) than the late FAI OA group (mean=56.2 years). Cartilage samples in early and late FAI-OA displayed a histological OA phenotype, quantified by Mankin score (Figure 1A). ACs in early and late FAI-OA displayed increased expression of catabolism and chondrocyte hypertrophy markers (*MMP13*: ND vs early *p<0.001*, ND vs late *p<0.001*, *COL10A1*: ND vs early *p<0.001*, ND vs late *p<0.001*, Figure. 1B). Contrary, expression of PPARγ gradually declined as the disease progressed (ND vs early *p=0.001*, early vs late *p=0.003*, Figure. 1B). IF analysis also displayed a gradual decrease in the ratio of the PPARγ positive cells during disease progression (ND vs early *p=0.001*, early vs late *p=0.041*, Figure 1C). As a first step to assess the epigenetic mechanisms of aberrant DNA methylation in OA progression, we examined the cartilage expression of *DNMT1 and DNMT3A*. We found that in RT-PCR, overexpression of *DNMT1* and *DNMT3A* levels was observed with disease progression (*DNMT1*: ND vs early *p<0.001*, early vs late *p=0.041*, *DNMT3A*: ND vs early *p=0.015*, early vs late *p=0.053,* Figure 2A). IF analysis confirmed overexpression of DNMT1 and DNMT3A in ACs in late FAI-OA (*DNMT1*: ND vs early *p<0.001*, early vs late *p<0.001*, *DNMT3A*: ND vs early *<0.001*, early vs late *p=0.008,* Figure 2B). Next, we performed MSP analysis to investigate the contribution of these abnormal DNA methylation to the PPARγ suppression in OA. In MSP analysis, human PPARγ promoter are featured by typical CpG islands (Figure 3A). As the OA progresses, hypermethylation at PPARγ promoter (ND: 9%, Early-FAI: 34%, Late FAI-OA: 48%) was observed (Figure 3B). IL1β stimulus increased PPARγ promoter methylation for early (34% to 57%, *p=0.016*, Figure 3C) and late stages (48% to 79%, *p=0.001*, Figure 3C) resulting in suppression of PPARγ expression *(P=0.073*, Figure 4B). Contrary, treatment with 5Aza (a DNA demethylator) brought the level of methylation back to 37% for early FAI (*p=0.033,* Figure 3C) and 46% for late FAI-OA *(p<0.001,* Figure 3C) and reversed the PPARγ suppression induced by IL1β (early-FAI *p=0.009,* Figure 4C and late FAI-OA ACs *p<0.001* Figure 4C).

DISCUSSION AND CONCLUSION:

This study is the first to assess PPARγ expression and methylation status of PPAR γ promoter site in human hip FAI disease progression. PPARγ expression was gradually suppressed via promoter hypermethylation during OA progression in hip FAI. Moreover, in the current study, the pro-inflammatory stimulus with IL1β further increased catabolic markers and enhanced PPARγ suppression via promoter excessive hypermethylation in human hip ACs. Notably, our MSP analysis confirmed the DNA demethylating agent prevent the IL1β induced progressive PPARγ promoter hypermethylation and effectively alleviate PPARγ suppression and cartilage degenerative damage, suggesting that the regulation of PPARγ promoter methylation could be a therapeutic target to rescue ACs from catabolic state in hip FAI disease.