

PPAR γ Promoter Demethylation Rescues Articular Chondrocytes from Catabolic State in Hip FAI

Tomoyuki Kamenaga¹, Jie Shen, Robert H Brophy², Regis J O'Keefe³, John C Clohisey², Cecilia Pascual-Garrido⁴

¹Tomoyuki Kamenaga, ²Washington University Orthopedics, ³Washington University School of Medicine, ⁴Washington University

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 (PPAR γ) is reported to be widely expressed in healthy articular chondrocytes (ACs) and is essential for cartilage homeostasis. A recent transcriptome study identifies PPAR γ as a key target linking aberrant DNA methylation to OA progression. This study aimed to investigate the PPAR γ expression and methylation status at the PPAR γ 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 (IL1 β) 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*, *PPAR γ* , *MMP13*, and *COL10A1*. Methylation specific PCR (MSP) was performed to assess the methylation status at *PPAR γ* 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 PPAR γ 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 PPAR γ 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 $p<0.001$, early vs late $p=0.008$, Figure 2B). Next, we performed MSP analysis to investigate the contribution of these abnormal DNA methylation to the PPAR γ suppression in OA. In MSP analysis, human PPAR γ promoter are featured by typical CpG islands (Figure 3A). As the OA progresses, hypermethylation at PPAR γ promoter (ND: 9%, Early-FAI: 34%, Late FAI-OA: 48%) was observed (Figure 3B). IL1 β stimulus increased PPAR γ 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 PPAR γ 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 PPAR γ suppression induced by IL1 β (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 PPAR γ expression and methylation status of PPAR γ promoter site in human hip FAI disease progression. PPAR γ expression was gradually suppressed via promoter hypermethylation during OA progression in hip FAI. Moreover, in the current study, the pro-inflammatory stimulus with IL1 β further increased catabolic markers and enhanced PPAR γ suppression via promoter excessive hypermethylation in human hip ACs. Notably, our MSP analysis confirmed the DNA demethylating agent prevent the IL1 β induced progressive PPAR γ promoter hypermethylation and effectively alleviate PPAR γ suppression and cartilage degenerative damage, suggesting that the regulation of PPAR γ promoter methylation could be a therapeutic target to rescue ACs from catabolic state in hip FAI disease.

Figure 1. PPAR γ is gradually suppressed in articular cartilage during hip FAI-OA progression.

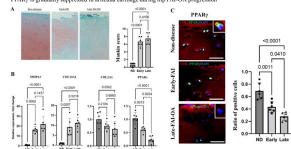


Figure 2. Aberrant expression of DNMTs in articular cartilage as the hip OA disease progresses.

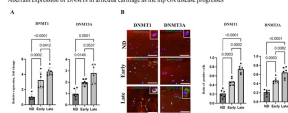


Figure 3. DNMT 3A alterations cause PPAR γ promoter hypermethylation and PPAR γ suppression.

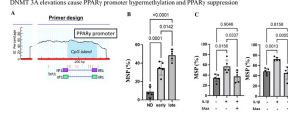


Figure 4. DNA demethylating agent effectively reduces IL1 β induced OA damage and PPAR γ suppression in human hip OA ACs.

