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2022 Hip Society Award

Otto Aufranc Award: Identification of Key Molecular Players in the Progression of Hip Osteoarthritis Through Transcriptomes and Epigenetics

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ABSTRACT

Background: This study aimed: (1) to compare the transcriptome profile of articular cartilage in cam-FAI (early stage) to advanced OA secondary to cam-FAI (late stage) and (2) to investigate epigenetic changes through the expression of DNA methylation enzymes DNMT3B, DNMT1, and DNMT3A and peroxisome proliferator-activated receptor gamma (PPARγ) in human cartilage samples during the progression of hip OA.

Methods: Full-thickness cartilage samples were collected from the anterolateral head-neck junction (impingement zone) of 22 patients (9 early-FAI and 13 late-FAI). RNA sequencing and in vitro cartilage cultures with histological analysis and immunohistochemistry staining for PPARγ and DNMT3B were performed. Target gene validation was confirmed with RT-PCR.

Results: Fifty genes and 42 pathways were identified differentially between early and late-FAI (fold change <-1.5 or >1.5, $P < .01$). PPARγ and DNMT3B were gradually suppressed with disease progression. Contrarily, disease progression induced expression of DNMT1/3A.

Conclusion: By comparing comprehensive gene expression in early and late stage hip degeneration at the whole-genome level, distinct transcriptome profiles for early and late stage disease were identified along with key molecular contributors to the progression of hip OA. Preservation of endogenous PPARγ may have therapeutic potential to delay or prevent hip OA.

Hip Femoroacetabular Impingement (FAI) is considered a pre-osteoarthritis (OA) disease with an etiologic role in up to 50% of hip OA. Understanding molecular changes in FAI may unravel the early molecular mechanisms of hip OA. These early mechanisms of disease likely lead to chronic joint inflammation that plays a critical role in OA progression. An inflammatory cascade has been identified in the impingement zone cartilage of hips with FAI, suggesting that the impingement area is metabolically active and a potential structural precursor to hip OA [1,2]. However, triggers for inflammation and the mechanism by which chronic inflammation contributes to the progression of hip OA remain unknown.

Previous transcriptome analysis has identified several pathways activated in hip OA [3,4]. There are no prior studies investigating molecular changes during the early disease stage. Thus, we believe that study of hips with FAI may identify pathological pathways during early disease and provide insight into OA development. A previous comparison of gene expression changes in hip OA to that of patients with no signs of OA (control nondiseased cartilage) identified a number of novel pathological pathways during late-stage OA disease [3]. Moreover, recent advances in OA research show that OA development is associated with aberrant epigenetic alterations of many OA-susceptible genes [5]. Chronic inflammation can lead to epigenetic changes, including aberrant expression of DNA Methyltransferases (DNMTs), resulting in hypermethylation or hypomethylation of DNA and leading to activation or suppression

Identification of key molecular players in the progression of hip osteoarthritis through transcriptomes and epigenetics.

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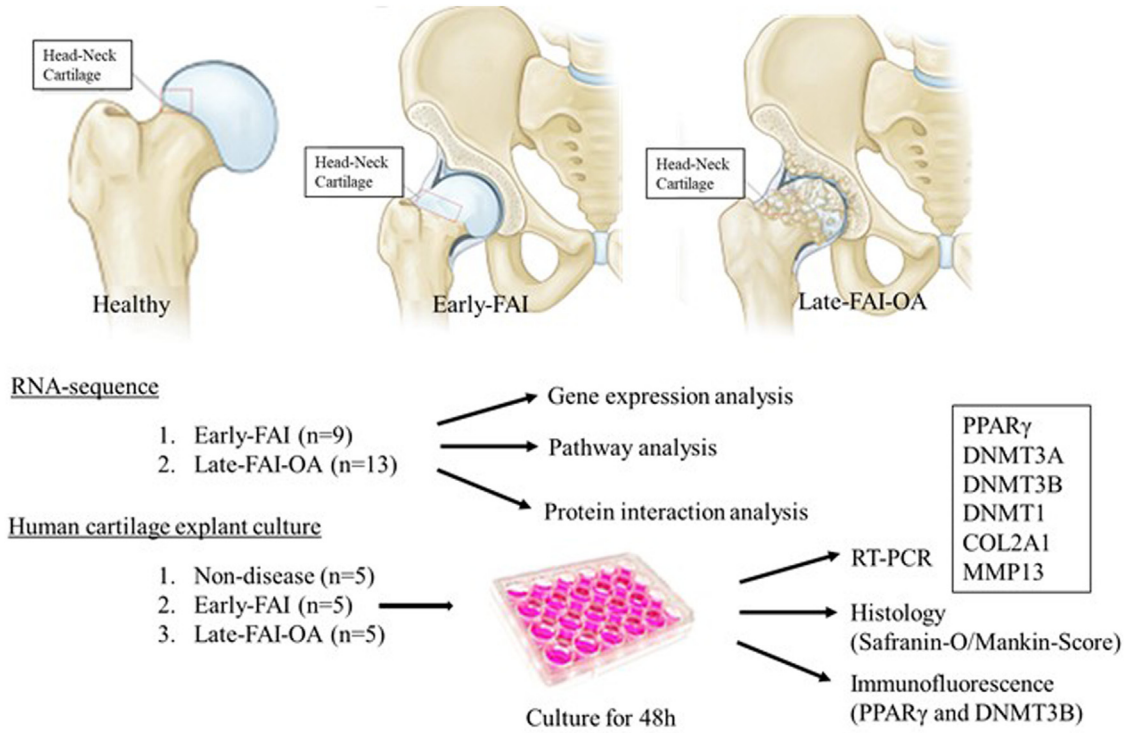

Fig. 1. Study design. FAI, femoroacetabular impingement. OA, osteoarthritis. RT-PCR, real-time polymerase chain reaction. PPARγ; Peroxisome proliferator-activated receptor-gamma. DNMT; DNA Methyltransferase.

of OA genes. DNA methylation is normally catalyzed by DNMT1, DNMT3a, and DNMT3b. Previous studies showed that Dnmt3b loss of function results in DNA hypomethylation and activation of catabolic genes and is associated with an OA-like pathology [6]. Contrarily, hypermethylation may result in the suppression of critical genes that support cartilage homeostasis, thus contributing to the progression of OA [7]. Peroxisome proliferator-activated receptor-gamma (PPARγ) is widely expressed in chondrocytes and is essential for cartilage homeostasis [7]. We and others have found that PPARγ is reportedly reduced in the OA joint [8–11]. PPARγ inhibits several inflammatory and catabolic events in OA pathogenesis [12,13], suggesting a protective role for PPARγ in preventing and treating OA. Previously, PPARγ promoter hypermethylation, specifically by DNMT1/DNMT3a, has been linked to PPARγ suppression [4,14,15]. Thus, it is possible that aberrant DNA methylation contributes to PPARγ suppression and hip OA progression [16]. Despite current knowledge of the effects of PPARγ suppression on the inflammatory and catabolic pathways in articular tissue [11,16], little is known about the mechanism for suppression of PPARγ in OA and its expression levels in hip articular cartilage during disease progression. Assessing the expression of PPARγ and DNMTs during hip OA progression may help identify key players in the mechanism of hip OA.

Therefore, the aims of this study were: (1) to compare transcripts from cartilage at the impingement zone of hips with cam FAI and late stage hip OA disease and (2) investigate the expression of DNMT3B, DNMT1/DNMT3A, and PPARγ in human cartilage samples during the progression of hip OA (hip FAI and hip OA). We hypothesized that: (1) there would be a unique transcriptome profile of FAI (early stage) that is distinct from late FAI or OA (late stage) and (2) OA articular chondrocytes (ACs) have increased expression of DNMT1/DNMT3A and suppression of PPARγ and DNMT3B relative to those of early FAI ACs and normal ACs.

Material and Methods

Patients

This study was approved by the institutional review board (IRB No 2017703054). Full-thickness cartilage samples from the anterolateral head-neck junction (impingement zone) of 22 patients (22 hips) undergoing hip surgery were obtained. Of these patients, 9 underwent hip surgery to treat symptomatic cam FAI (early FAI; n = 9), and 13 underwent total hip replacement (THR) to treat advanced OA secondary to cam FAI (late FAI-OA; n = 13). The diagnosis of cam FAI or OA was determined by the treating surgeons (C.P-G. and J.C.C.). The Tönnis classification [17] was used to define the severity of hip OA: early FAI was diagnosed in patients with Tönnis 0-1 and late FAI in patients with Tönnis 2-3. A cam deformity was defined by an alpha angle greater than 55° on the preoperative anteroposterior (AP) pelvic, frog-leg lateral, and/or 45° on Dunn radiographs [18]. Interobserver and intraobserver reliability of the radiographic analysis of FAI was previously performed by our group [19]. All surgical procedures were performed by 2 experienced surgeons (C.P-G. and J.C.C.) between May 2018 and July 2021. Exclusion criteria included previous surgery, pincer morphology, infection, idiopathic osteonecrosis of the femoral head, psoriasis, and rheumatologic conditions. Patients were asked to stop taking nonsteroidal anti-inflammatory drugs (NSAIDs) a week before surgery. Figure 1 shows the experimental design.

Transcriptomes Analysis

Cartilage Sample Collection

During surgery, full-thickness cartilage samples were obtained using a liberator and an arthroscopic biter in arthroscopic procedures or a half-inch osteotome in patients undergoing open surgical

Table 1
Patient Characteristics.

Group	Early FAI	Late FAI OA	*P* Value
Number	9	13	
Age, y	43.5 ± 11.8	59.6 ± 9.1	.02
Sex, male/female	6/3	9/4	.85
BMI, kg/m^2	28.1 ± 1.2	31.5 ± 3.2	.19
α angle, deg	66.7 ± 7.9	75.1 ± 11.8	.22
Tönnis classification	1.1 ± 0.1	3.0 ± 0.0	.003
Tissue source, procedure	7 hip arthroscopies, 2 surgical dislocations	13 THR	

Values are expressed as mean ± standard deviation unless otherwise noted.
FAI, Femoroacetabular impingement; OA, osteoarthritis; BMI, body mass index; THR, total hip replacement.

dislocation or THR. All samples were obtained from the anterolateral aspect of the head-neck junction of the proximal femur. For the late FAI OA group, care was taken to harvest actual remaining cartilage tissue and not osteophytic tissue. The location of tissue harvesting was similar to what we did for the early FAI group. This was localized at the anterolateral aspect of the femoral head. All cartilage samples were stored in RNALater (AM7021, Ambion Life Technologies, Carlsbad, CA, USA) immediately after being harvested intraoperatively. Samples were kept on ice and transported to the laboratory within 2 hours of being harvested. If RNA was not extracted immediately, the tissues were stored at −80°C until RNA extraction.

Total RNA Preparation and Quality Assessment

RNA was extracted and purified as previously described [20]. RNA was purified using RNeasy MinElute Cleanup Kit (Qiagen Inc, Valencia, CA) and RNase-Free DNase Kit (Qiagen Inc, Valencia, CA). RNA was quantified with a NanoDrop ND-100 spectrophotometer (NanoDrop), while quality was assessed with an Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA) [20]. RNA samples with RNA Integrity Number >7 were selected for genome expression array experiments.

RNA Sequencing and Analysis

Samples were sequenced using an Illumina NovaSeq 6000 according to the manufacturer's protocol. Differential expression analysis was then performed to analyze for differences between conditions, and results were filtered for genes with Benjamini-Hochberg false-discovery rate (FDR) adjusted *P* values less than or equal to .05. Global perturbations in known Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were detected to test the log 2 fold-change. Perturbed KEGG pathways where the observed log 2 fold-changes of genes within the term were significantly perturbed in a single-direction versus background compared to other genes within a given term with *P* values less than or equal to .05 were rendered as annotated KEGG graphs [21].

Protein Interaction Network Analysis

Protein interactions among upregulated and downregulated genes in the gene expression analysis and pathway analysis in early FAI and late FAI-OA groups were analyzed using the search tool STRING [22].

RT-PCR Verification

Real-time RT-PCR was used to validate the expression levels of 3 genes identified as differentially expressed by the pathway analysis and had more than 5 interactions with other differentially expressed genes. These included AKT1, PPAR-γ, and HIF1α. Total RNA was extracted from cartilage tissue and reverse-transcribed into cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). RT-PCR amplification of the cDNA was performed in triplicates using SYBR Green reagent (Applied Biosystems). Relative gene expression was normalized against the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using the comparative cycle threshold method [23].

Cell Culture Analysis

Human Cartilage Explant Culture

Cartilage samples for transcriptome analysis were used to perform cell cultures. These included early FAI (n = 5) and late FAI-OA (n = 5). Control nondiseased (ND) healthy samples from fresh femoral head grafts were used (n = 5). Fresh femoral head grafts were used if they had macroscopically normal articular cartilage and the donor's age was less than 30 years old. The experiment was performed according to the method previously described [24]. All cartilage samples were stored in Hank's balanced salt solution (HBSS) immediately after being harvested and transported within 2 hours to the laboratory. Explanted cartilage tissue was washed several times with HBSS containing antibiotics and then incubated in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12) medium containing 10% Fetal Bovine Serum (FBS), penicillin/streptomycin (100 U/0.1 mg/mL) in a 6-well plate at 37°C and 5% CO2 for 48 hours (Fig. 1 shows the study design).

Histological Assessment

Following culture, cartilage explant specimens were fixed in 10% neutral buffered formalin for 24 to 48 hours, decalcified in Immunocal (Stat-Lab) for 3 days, dehydrated in graded ethanol, embedded in paraffin wax, and sectioned to thicknesses of 5 mm by standard protocol [1]. Safranin-O/fast green staining was performed to histologically evaluate the cartilage degeneration of 5 samples in each group. Cartilage degeneration was graded based on the Mankin score [25]. On this score, 0 is the best (hyaline) and 14 is the worst (OA) [25].

Gene Expression Analysis

Real-time polymerase chain reaction (RT-PCR) was performed to assess the expression levels of genes, including PPARγ, DNMT1, DNMT3A, DNMT3B, MMP13, COL2A1, according to the method described above. Relative gene expression was normalized to the mean value of the control ND group samples for each gene.

Immunofluorescence Analysis

After blocking with 2.5% donkey serum for 2 hours at room temperature, the paraffin sections were incubated with the primary antibodies: PPARγ (1:200, Cat# A0270, Abcam) and DNMT3B (1:200, Cat# A7239, Abcam) overnight at 4°C (n = 5 per group). After washing, the sections were incubated with the corresponding secondary antibody, Goat Anti-Rabbit IgG H&L (Alexa Fluor 488) (1:200, Cat# ab150077, Abcam), for 2 hours. The sections were probed with COL2 antibody (1:200, Cat# sc-518017, Santa Cruz Biotechnology) followed by TRITC-conjugated antirat secondary antibody (1:100, Cat# 712-295-153, Jackson ImmunoResearch). Nuclei were counter stained with DAPI solution (1:1,000, Vector Laboratories) for 5 minutes. The images were acquired with ZEISS LSM 880 Confocal Laser Scanning Microscope. The data were presented as the mean ratio of positive cells.

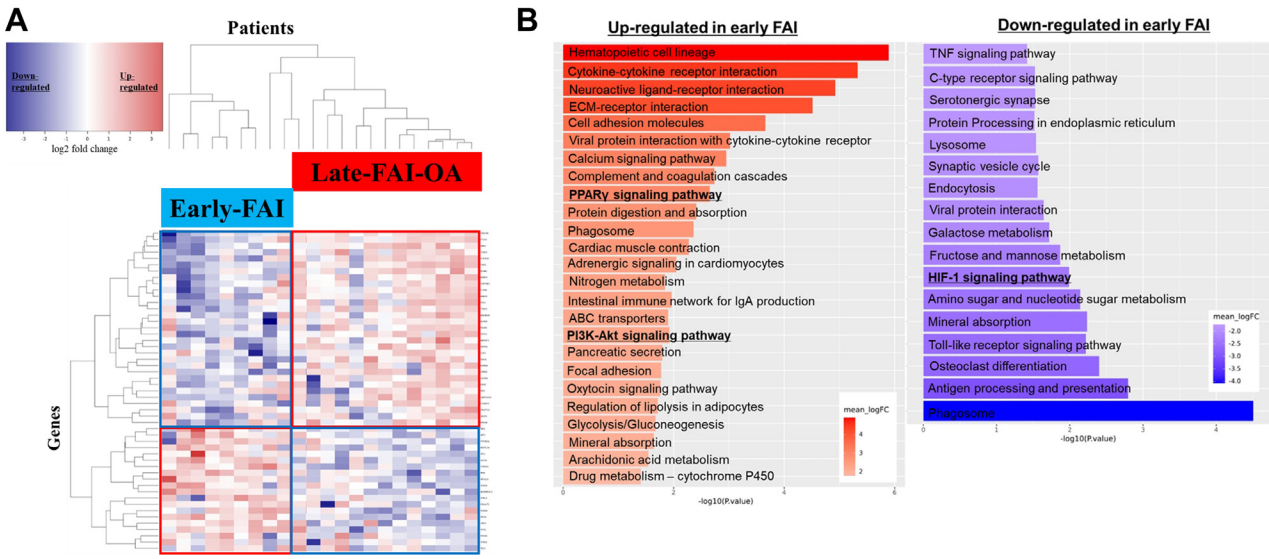

Fig. 2. A. Normalized gene expression levels of differentially expressed transcriptomes between early-FAI and late-FAI-OA were used to generate heatmaps. Patients were largely clustered into 2 distinct groups. Red indicated upregulated genes, and blue indicated downregulated genes. FAI, femoroacetabular impingement. OA, osteoarthritis. B. Bar plots showing upregulated (red) and downregulated (blue) pathways in early-FAI. FAI, femoroacetabular impingement.

Statistical Analysis

Data are presented as mean ± standard deviation (SD). The comparisons between groups were performed using Mann–Whitney U, ANOVA, or Kruskal Wallis test. Multiple comparisons were performed by post hoc test with Bonferroni correction if needed. The level of significance was set at *P* less than .05.

Results

Characteristics of Study Patients

Characteristics of study participants are presented in Table 1. The early FAI group included younger subjects with lower Tönnis

Table 2
Upregulated Genes in Early FAI Group.

Symbol	Gene Name	*P* Value (Adjusted)	Fold Change
TAC1	Tachykinin precursor 1	.0047	17.1
TPRG1	Tumor protein p63 regulated 1	.0446	16.7
MYOC	Myocilin	.0031	14.0
LMO3	LIM domain only 3	.0468	7.3
HIF3α	Hypoxia inducible factor 3 subunit alpha	.0468	3.6
PDZD8	PDZ domain containing 8	.0446	3.5
B3GALT1	beta-1,3-galactosyltransferase 1	.0282	2.9
SORL1	Sortilin-related receptor 1	.0284	2.8
AL109811.3	Novel protein	.0111	2.4
SCN4A	sodium voltage-gated channel alpha subunit 4	.0284	2.1
ARGLU1	Arginine and glutamate rich 1	.0479	1.9
SYNGR1	Synaptogyrin 1	.0483	1.9
KAT2B	Lysine acetyltransferase 2B	.0342	1.8
ISCU	Iron-sulfur cluster assembly enzyme	.0284	1.8
METTL7A	methyltransferase like 7A	.0084	1.8
PPP2R5α	Protein phosphatase 2 regulatory subunit B'alpha	.0370	1.8
NFIA	Nuclear factor I A	.008	1.8
IMP3	IMP U3 small nucleolar ribonucleoprotein 3	.0368	1.7
ADI2	Acireductone dioxygenase 1	.0468	1.7

Two genes (bold with underbar) have been previously reported as associated with osteoarthritis.
FAI, femoroacetabular impingement.

Table 3
Downregulated Genes in Early FAI Group.

Symbol	Gene Name	*P* Value (Adjusted)	Fold Change
TREM1	Triggering receptor expressed on myeloid cells 1	.0047	−14.6
GUCA1A	Guanylate cyclase activator 1A	.0446	−14.3
PTGES	Prostaglandin E synthase	.0031	−13.7
LINC01614	Long intergenic nonprotein coding RNA 1614	.0468	−7.78
HILPDA	Hypoxia inducible lipid droplet associated	.0468	−7.38
ANGPTL4	Angiopoietin like 4	.0446	−6.00
VEGFA	Vascular endothelial growth factor A	.0282	−4.41
SERPINE1	Serpin family E member 1	.0284	−4.38
TNFAIP6	TNF alpha-induced protein 6	.0111	−4.35
IER3	Immediate early response 3	.0284	−3.89
ENO2	Enolase 2	.0479	−3.67
BHLHE40	Basic helix-loop-helix family member e40	.0483	−3.35
PDK1	Pyruvate dehydrogenase kinase 1	.0342	−3.26
ACKR3	Atypical chemokine receptor 3	.0284	−3.19
PLAUR	Plasminogen activator, urokinase receptor	.0084	−3.16
ERO1A	Endoplasmic reticulum oxidoreductase 1 alpha	.0370	−3.12
THBS3	Thrombospondin 3	.0368	−3.11
SGK1	Serum/glucocorticoid regulated kinase 1	.0468	−2.83
ARRDC3	Arrestin domain containing 3	.0475	−2.46
C1GALT1	Core 1 synthase, glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase 1	.0302	−2.39
FNIP2	Folliculin interacting protein 2	.0031	−2.3
RCAN1	Regulator of calcineurin 1	.0483	−2.25
KDM3A	Lysine demethylase 3A	.0284	−2.13
NDRG1	N-myc downstream regulated 1	.0302	−2.12
IFITM3	Interferon-induced transmembrane protein 3	.0086	−2.12
PDGFA	Platelet-derived growth factor subunit A	.0022	−2.07
VMP1	Vacuole membrane protein 1	.0102	−1.96
CPNE8	Copine 8	.0284	−1.86
RAB29	RAB29, member RAS oncogene family	.0483	−1.81
WDR41	WD repeat domain 41	.0479	−1.58
SEL1L	SEL1L adaptor subunit of ERAD E3 ubiquitin ligase	.0309	−1.57

Two genes (bold with underbar) have been previously reported as associated with osteoarthritis.
FAI, femoroacetabular impingement.

Table 4
Differentially Expressed Genes With More Than 5 Interactions With Other Differentially Expressed Genes.

Upregulated Genes	Number of Connections
AKT1	13
PPARγ	7
PIK3CA	6
Downregulated genes	
HIF1α	17
VEGFA	15
PLUAR	7
SERPINE1	6

grades than the late FAI OA group. No significant differences in gender, BMI, LCEA, and α-angle were observed between groups.

Transcriptome Analysis

Differentially Expressed Genes and Functional Analysis

Our data showed 50 genes transcripts differentially expressed between early FAI and late FAI OA (fold change ≤ -1.5 or ≥ 1.5, P value <.01). Specifically, we observed 19 upregulated and 31 downregulated genes in early FAI compared to late FAI-OA. Principal component analysis and heat mapping revealed 2 distinct groups of samples, indicating a distinct set of transcripts at different disease stages (Fig. 2A). Upregulated and downregulated genes in the early FAI group are listed in Tables 2 and 3. Genes that have been previously reported to be associated with OA are boldly highlighted in Tables 2 and 3 [26–30].

Molecular Pathway Analysis

The entire list of differentially expressed genes was found to be significantly associated with 42 KEGG pathways, including 25 upregulated and 17 downregulated pathways in the early FAI group. Several pathways previously reported to be associated with OA are boldly highlighted in Figure 2B.

Protein Interaction Analysis

Network analysis was performed on identified upregulated or downregulated genes using STRING. In total, 3 up and 4 downregulated genes had more than 5 interactions with other genes (Table 4 and Fig. 3). Interestingly, the pathway analysis identified a possible role for PPARγ in OA. PPARγ was assigned as a hub with seven connections with other differentially expressed genes (AKT1, HIF3α, KDM3A, PTGES, ANGPTL4, SERPINE1, VEGFA).

RT-PCR Verification

RT-PCR was used to validate the differential expression levels of 3 genes (AKT1, PPAR-ɤ, and HIF1α) determined through pathway analysis. All 3 genes were confirmed as significantly differentially expressed by both techniques, with expression changes always in the predicted direction (Fig. 4).

Human Cartilage Explant Culture

Histology

Cartilage samples in the control ND group showed normal hyaline cartilage. Contrarily, early-FAI and late FAI-OA groups cartilage samples showed an osteoarthritic phenotype (Fig. 5).

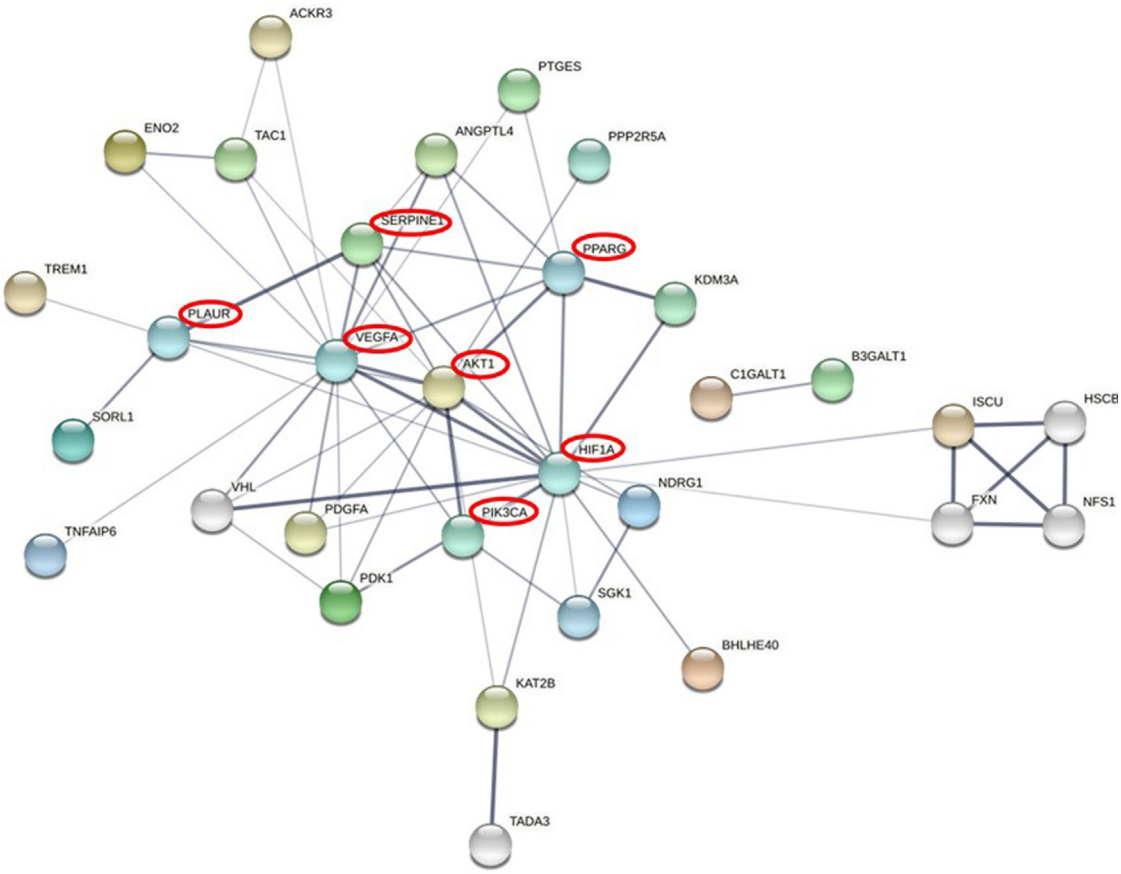

Fig. 3. Protein interaction network of genes. Network analysis was performed on all genes, either upregulated or downregulated in the gene expression analysis and pathway analysis in the early-FAI using STRING. FAI, femoroacetabular impingement.

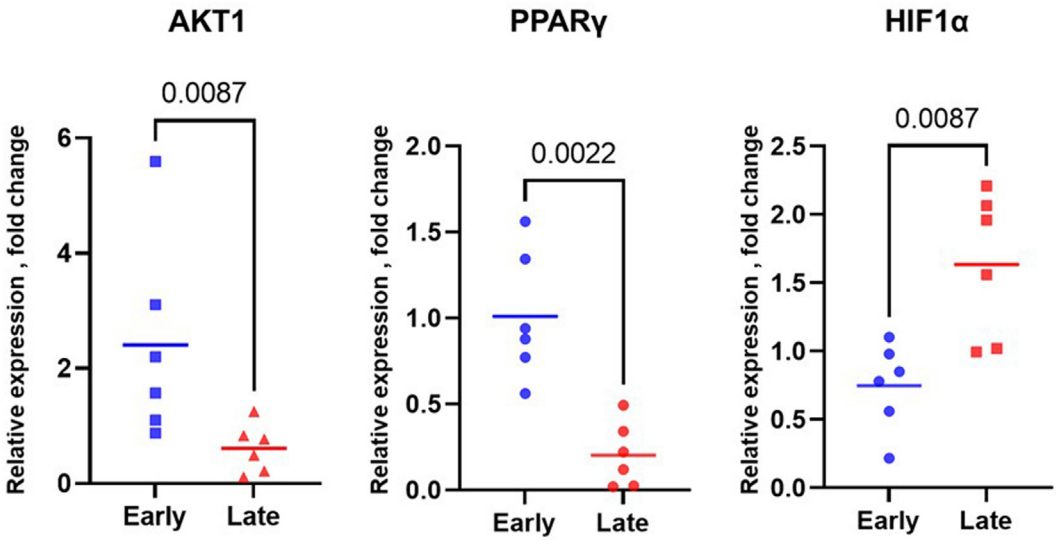

Fig. 4. Real-time-PCR validation of 3 genes (AKT1, PPARγ, and HIF1α) was identified as significantly differentially expressed between early-FAI and late-FAI-OA (n = 6/group). RT-PCR expression was normalized to GAPDH. FAI, femoroacetabular impingement. OA, osteoarthritis.

Gene Expression

RT-PCR revealed increased expression of DNMT1, DNMT3A, and MMP-13 and reduced expression of PPARγ and DNMT3B with disease progression (Fig. 6).

Immunofluorescence Analysis

Immunostaining of cartilage sections revealed that PPARγ and DNMT3B were abundantly expressed in chondrocytes of control ND cartilage (Fig. 7A) and progressively decreased with disease progression (Fig. 7).

Discussion

To our knowledge, this is the first study to comprehensively compare gene expression changes between early and late-stage human hip joint degeneration and confirm a specific molecular signature for each disease stage. Additionally, we have confirmed that there is marked suppression of PPARγ and DNTM3B and induction of DNMT1/3A with the progression of hip OA suggesting that epigenetic changes may play a critical role in the pathogenesis of this disease.

Transcriptome sequence analysis at the whole genome level is increasingly important for understanding altered gene expression in disease processes [31,32]. There have been a few transcriptome sequence studies [3,33] that identified novel catabolic transcripts in OA hip cartilage but only compared OA hips to nondiseased cartilage. Our study compared the transcript profile between FAI (early stage) and OA secondary to FAI (late stage). We demonstrated differential expression of 50 genes and 42 pathways, suggesting that early-stage disease is unique compared to nondiseased or advanced OA hips. Previously Xu et el reported 988 differentially expressed genes when comparing hip OA to nondiseased hips [3], confirming a more distinct molecular profile difference between OA and nondiseased hips than between early- and late-stage disease. We found

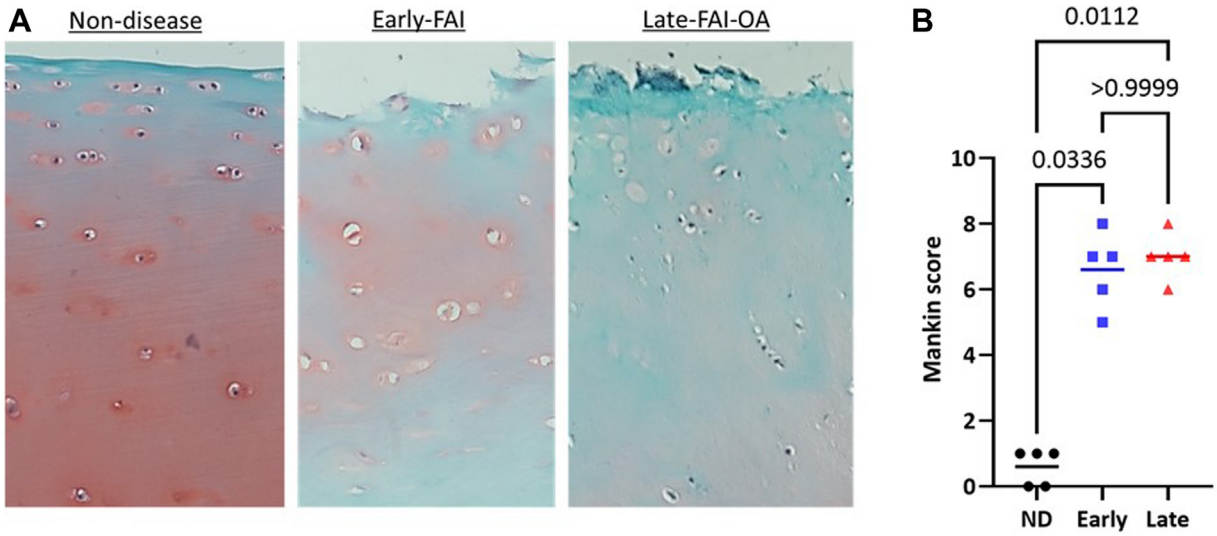

Fig. 5. A. Representative histologic sections of the cartilage stained with the safranin-O/fast green of the two groups. B. Bar graphs showing the comparison of cartilage degeneration in the Mankin score system. FAI, femoroacetabular impingement; OA, osteoarthritis; ND, nondisease; Early, early-FAI; Late, late-FAI-OA.

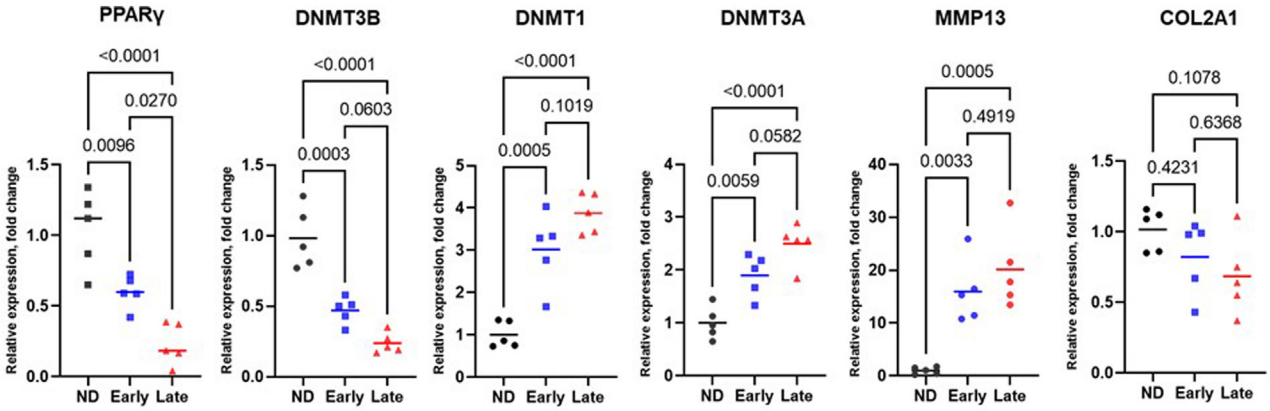

Fig. 6. Expression of PPARγ, DNMT3B, DNMT1, DNMT3A, MMP13, and COL2A1 in each group, as determined by RT-PCR (n = 5/group). ND, nondisease; Early, early-FAI; Late, late-FAI-OA. FAI, femoroacetabular impingement; OA, osteoarthritis.

Tachykinin precursor 1 (TAC1) and Hypoxia-inducible factor 3 subunit alpha (HIF3α) to be overexpressed during the early stage of the disease. In a similar analysis of human knee OA, TAC1 expression was significantly upregulated in early stage OA compared to the late stage [30]. TAC1 has been reported to be involved in the G protein-coupled receptor signal (GPCRs) transduction pathway. GPCRs are suggested to regulate pathological processes, including cartilage matrix degradation, synovitis, subchondral bone remodeling, and osteophyte formation. Further studies investigating TAC1 expression during disease progression may confirm its injurious role in early-stage OA. Similarly, we observed an overexpression of HIF3α during the early disease stage. Contrarily to TAC1, HIF3α has been previously reported to play a critical role in cartilage homeostasis and formation, energy matrix synthesis, and mechanotransduction [28]. It was shown that HIF3α levels indicate a hypertrophic stage of chondrogenic cells during early disease. We believe that the increased expression of this gene during the early stage of disease may reflect a metabolic adaptation of articular cartilage under stimulus from harmful impingement.

Our pathway analysis identified PPARγ upregulation during the early disease stage. Earlier, it was shown that PPARγ is widely expressed in chondrocytes and reported to be critical in cartilage homeostasis [34,35]. Moreover, natural and synthetic ligands of PPARγ were shown to inhibit the expression of several inflammatory and catabolic genes in cultured chondrocytes and exhibit anti-inflammatory and chondroprotective effects in an experimental OA animal model [12,13,36]. In addition, we found that PI3/Akt is upregulated in the early disease stage. Similarly, the PI3K/Akt pathway was reported to be critical for cartilage homeostasis [37]. According to our protein interaction network results, we confirmed these genes to be hub genes, with more than 5 interactions, indicating their essential role in gene regulation and biological

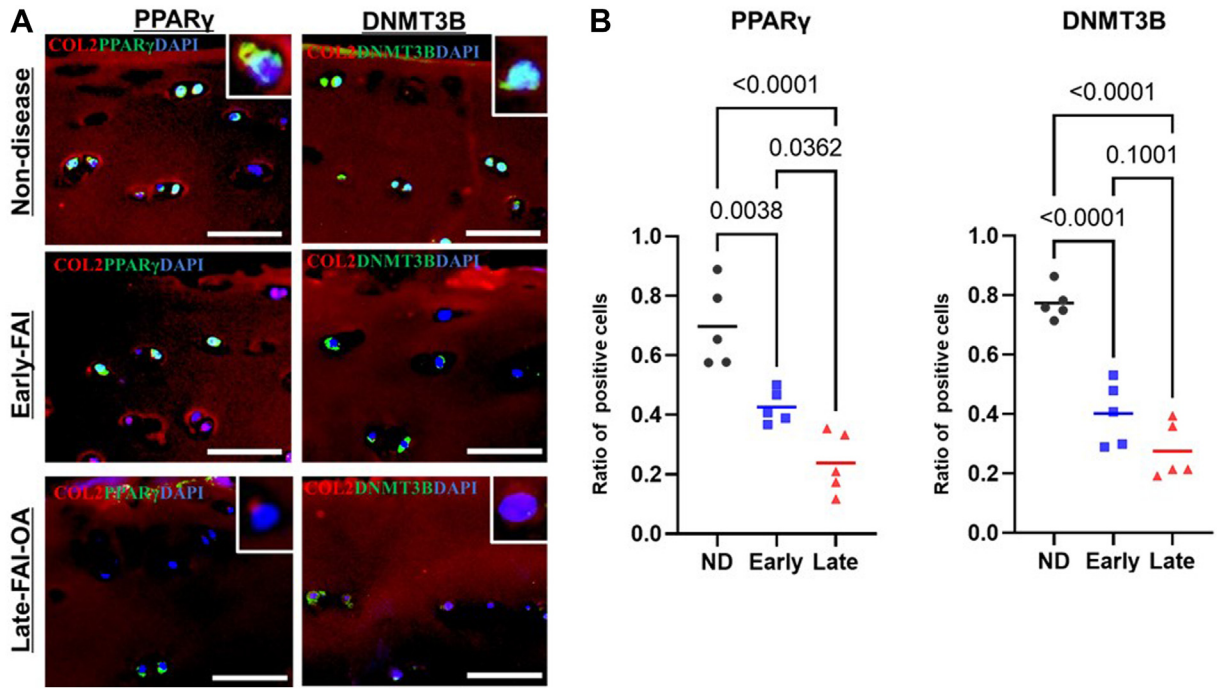

Fig. 7. A. Representative immunofluorescence staining of PPARγ (green) and DNMT3B (green) in cartilage sections in 3 groups. COL2 (red), type II collagen; DAPI (blue) stained nuclei. Scale bar: 50 μm. B. Quantitative analysis of the ratio of positive cells (PPARγ and DNMT3B) in the 3 groups (n = 5/group).

processes during early disease stages. Most importantly, the transcriptome data was validated using RT-PCR, confirming the gradual suppression of these genes in hip OA progression.

Since our data showed reduced PPARγ expression with disease progression, we confirmed this in ex-vivo culture and then investigated the mechanism of suppression. It has been suggested that PPARγ promoter (CpG island) hypermethylation could cause PPARγ suppression [16]. Since DNA methylation is normally catalyzed by DNMT1, DNMT3a, and DNMT3b, we thought to characterize DNMT1/3A and DNMT3B and PPARy expression between non-diseased hips and early and late FAI. IHC staining confirmed that PPARγ and DNMT3B expression is progressively reduced in disease progression. Contrarily, there is increased expression of DNMT1, DNMT3A, and MMP-13. This data suggests that aberrantly induced DNMT1/DNMT3a may significantly contribute to progressive suppression of PPARγ. Previously, Zhu et el [16] confirmed that PPARγ is suppressed in knee OA with significant contribution from aberrantly induced DNMT1/DNMT3. Unlike our study, the authors did not report the expression of this pathway during the early disease stage. We report for the first time PPARγ suppression during early-stage disease that may become more severe throughout disease progression. Contrarily, there is increased overexpression of DNMT1/DNMT3a with disease progression. This identifies PPARγ as a key target linking aberrant DNA methylation to OA and suggests that modulation of the PPARγ pathway should be investigated as a potential therapeutic approach for hip OA.

There were limitations to our study. First, our subjects were not matched for age. Although this would be ideal, previous studies reported no differences in expression of inflammatory and catabolic mediators in cartilage samples from the head-neck junction between younger (<30 years) and older (>30 years) patients [38]. Secondary, since OA is a whole joint disease, we believe that investigating in the future other key structures such as synovium will be critical to better understand the entire pathology during the OA progression. Moreover, a longitudinal comparison of the genetic expression of the same patient as the disease progresses, rather than a comparison of different patients at different stages as did in this study, would be ideal and desirable in the future. Additionally, we did not perform transcriptome analysis in nondiseased cartilage. However, we used historical data from other authors that reported on nondiseased hip cartilage for comparison [3]. Although our results suggest that PPARγ may contribute the progression of OA and DNA hypermethylation may cause PPARγ suppression, this has not been confirmed. Future studies will assess the DNA methylation status of the PPARγ promoter to determine if promoter hypermethylation occurs and ChIP assay to confirm that DNMT1/DNMT3A interacts with the PPARγ promoter.

In conclusion, this study provides novel data regarding gene expression in early and late-stage hip FAI. Most importantly, it suggests a distinct transcriptome profile of early-stage hip degeneration (FAI) compared to late stage hip OA secondary to FAI. Additionally, we identified a number of likely key molecular players contributing to the progression of hip OA. We believe that endogenous PPARγ preservation possesses therapeutic potential for the prevention or treatment of hip OA. Further studies in established animal models of hip OA will help assess the potential of modulating this pathway to preserve and protect the hip joint from degeneration.

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