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Distinct degenerative phenotype of articular cartilage from knees with meniscus tear compared to knees with osteoarthritis

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SUMMARY

Objective: To compare the transcriptome of articular cartilage from knees with meniscus tears to knees with end-stage osteoarthritis (OA).
Design: Articular cartilage was collected from the non-weight bearing medial intercondylar notch of knees undergoing arthroscopic partial meniscectomy (APM; $N = 10$, 49.7 ± 10.8 years, 50% females) for isolated medial meniscus tears and knees undergoing total knee arthroplasty (TKA; $N = 10$, 66.0 ± 7.6 years, 70% females) due to end-stage OA. Ribonucleic acid (RNA) preparation was subjected to SurePrint G3 human 8 × 60K RNA microarrays to probe differentially expressed transcripts followed by computational exploration of underlying biological processes. Real-time polymerase chain reaction amplification was performed on selected transcripts to validate microarray data.
Results: We observed that 81 transcripts were significantly differentially expressed (45 elevated, 36 repressed) between APM and TKA samples (≥ 2 fold) at a false discovery rate of ≤ 0.05. Among these, *CFD, CSN1S1, TSPAN11, CSF1R* and *CD14* were elevated in the TKA group, while *CHI3L2, HILPDA, COL3A1, COL27A1* and *FGF2* were highly expressed in APM group. A few long intergenic non-coding RNAs (lincRNAs), small nuclear RNAs (snoRNAs) and antisense RNAs were also differentially expressed between the two groups. Transcripts up-regulated in TKA cartilage were enriched for protein localization and activation, chemical stimulus, immune response, and toll-like receptor signaling pathway. Transcripts up-regulated in APM cartilage were enriched for mesenchymal cell apoptosis, epithelial morphogenesis, canonical glycolysis, extracellular matrix organization, cartilage development, and glucose catabolic process.
Conclusions: This study suggests that APM and TKA cartilage express distinct sets of OA transcripts. The gene profile in cartilage from TKA knees represents an end-stage OA whereas in APM knees it is clearly earlier in the degenerative process.

Introduction

Osteoarthritis (OA) is the most common form of arthritis and is characterized primarily by the degeneration and loss of articular cartilage. OA is considered a heterogeneous disease with a variety of pathogenic factors, all of which result in similar patterns of cartilage degeneration. Meniscal tears are likely to be an important early event in the initiation and propagation of degenerative changes in the knee[1] and are known to predispose about 50% of individuals to develop knee OA within 10–20 years after injury[2,3]. However, the molecular basis for the initiation and progression of OA following meniscus injury still remains largely unknown. While the relationship between meniscus lesions and OA is complex, it is thought that meniscus tears in healthy knee may lead to OA and OA can also lead to meniscus tears that may exacerbate the OA process[3]. Yet, little is known about the biological basis of this relationship. Recently, we have reported that a number of transcripts (including *CSN1S1, COL10A1, WIF1, SPARCL1, POSTN* and *VEGFA*) with potential relevance to the pathogenesis of OA are differentially

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expressed in OA and non-OA meniscus tissues providing some molecular clues to the relationship between the meniscus and OA[4].

Gene expression profiles of human cartilage procured from patients undergoing total knee arthroplasty (TKA) and injured meniscus procured from patients during arthroscopic partial meniscectomy (APM) identified transcripts differentiating between hyaline and fibrocartilage tissues[5]. However, we are not aware of any existing study that has directly compared gene expression in the cartilage from APM knees and TKA knees.

A few studies compared degenerated cartilage with the "normal" either taken from within the same diseased (e.g., arthritis, anteromedial gonarthrosis) joints[6–9], normal joints[10,11] or from trauma patients[12] providing important insights into the changes in the gene expression pattern. However, "normal" cartilage in most of these studies was largely excised from areas of grossly intact cartilage in an end-stage degenerated joint, which was exposed to the inflammatory and catabolic mediators found in OA synovium and synovial fluid. In addition, normal-looking and degenerated cartilage is not always taken from the same location in the joint, a potentially important confounder for any comparison. We have previously reported that cartilage from some patients with a meniscus tear, but no OA, exhibits a pre-OA phenotype[13] compared to data coming from OA and non-OA cartilage[10] as well as from genome-wide association studies[14].

Here, we aim to investigate the molecular differences between cartilage from knees undergoing APM and TKA. We hypothesize that cartilage from knees undergoing APM has a degenerative phenotype earlier in the disease process than end-stage OA based on transcript signatures and exhibits a repair response while cartilage from OA patients demonstrates a distinct and more advanced degenerative phenotype. Comparing the molecular phenotypes of cartilage from knees undergoing APM and TKA is an important step to elucidate how the molecular biology of cartilage changes after meniscus injury along the pathway to OA. We believe that transcriptome differences between these types of cartilage may shed light on the very early response of the knee joint to meniscus injury.

Methods

Patients

The Institutional Review Board at the study institution approved the protocol. Eligible patients undergoing APM or TKA provided written and signed informed consent prior to surgery. The operating surgeon (Dr. Brophy or Dr. Wright) collected the cartilage samples during the procedure. Male and female patients of any age and body mass index (BMI) were included (Table I). All patients had preoperative X rays which were reviewed and assessed using the Kellgren–Lawrence (K-L) scale for OA.

Patients undergoing APM had a tear in the medial meniscus posterior horn and were clinically indicated for surgical intervention based on history and physical examination. We noted whether each patient had a specific trauma or an acute onset of symptoms without a specific injury. All APM patients had preoperative bilateral Rosenberg weight-bearing views, bilateral Merchant views and a lateral view of the involved knee as well as magnetic resonance imaging (MRI) of the knee. The X rays and MRIs were reviewed as part of routine clinical practice by a musculoskeletal radiologist and an orthopedic surgeon. None of the patients had any degenerative changes on X ray or any evidence for chondral damage or other injury on MRI prior to their surgery. Patients who were considered for inclusion in the study based on pre-operative imaging were excluded if there was any chondral damage in the tibiofemoral compartments or more than focal grade 2 changes in the patellofemoral joint at the time of APM. The pattern of each meniscus tear was also recorded at the time of surgery. TKA patients met the American College of Rheumatology criterion for knee OA, had moderate to severe pain and functional limitations, and had failed non-operative measures.

Cartilage sampling

The techniques for collecting articular cartilage were the same as described in a previous study[13]. During APM, a ring curette was used to collect a small fragment of cartilage from a non-weight bearing portion of the medial intercondylar notch. During TKA, a size-matched fragment of cartilage was collected from a similar area of the medial intercondylar notch. The samples were limited to articular cartilage without collecting any subchondral bone. Immediately after harvest, the cartilage was placed in a tube of RNAlater solution (Thermo Fisher Scientific) for quick transport to the laboratory.

Ribonucleic acid (RNA) preparation

RNA was isolated using a previously described technique[4]. Briefly, isolation was performed using both TRIzol:Chloroform method and Minispin columns (Qiagen). The cartilage samples were immersed in liquid nitrogen and homogenized using Mikro dismembrator (B. Braun, Biotech International). One milliliter of TRIzol-Reagent (Invitrogen) was added before transferring the solution to a microfuge tube for incubation at room temperature for 5 min. After the addition of 200 μL chloroform, the solution was mixed vigorously and incubated at room temperature for five additional minutes before being transferred to a phase-lock gel tube. Once the gel collected at the bottom, the tube was centrifuged for 15 min at 12,000 rpm and 4°C. The upper aqueous phase containing RNA was decanted into a clean microfuge tube. Same volume of 70% RNase-free ethyl alcohol was mixed before applying to RNeasy spin columns (Qiagen) and centrifuged for 15 s at 8000 rpm at room temperature. The flow-through was decanted and prior to washing the column four times with the supplied wash buffers (each buffer twice). The resulting RNA was eluted in 30 μL of RNase-free water. Nanodrop ND 1000 (Thermo Fisher Scientific) was used to determining the RNA concentrations and the quality of the total RNA samples was assessed by Agilent 2100 Bioanalyzer (Agilent Technologies) using the RNA integrity numbering system.

Microarray hybridization

Microarray hybridization procedure was essentially the same as we reported previously[4]. Briefly, A Sigma WTA2 kit (Sigma Aldrich) was used to amplify 20 ng of RNA and the resulting complementary deoxyribonucleic acid (cDNA) was labelled with the Kreatech ULS RNA labeling kit (Kreatech Diagnostics). Three milligrams of cDNA was mixed with Kreatech labeling buffer and Kreatech cyanine 5 (Cy5)/DY-ULS, incubated for 15 min in the dark at 85°C, and then quenched on ice for 3 min. Columns (Qiagen) were used to purify labelled cDNA. The amplified RNA was suspended in Agilent Gene Expression buffer, blocking agent, and Kreablock (Agilent) for hybridization. The hybridization solution was applied to SurePrint G3 Human 8 × 60K microarrays (Agilent) and incubated with streptavidin Cy5 for 20 h at 65°C. Washing procedures were performed according to the Agilent Gene Expression protocol. An Agilent C class microarray scanner was used to laser scan the hybridized arrays to detect Cy5 fluorescence. Fluorescent Cy5 signals were recorded in focal units for each spotted probe bound to the array surface. Each spot on the array consists of oligos made up of a known 60 base-pair sequence for a gene of interest and the intensity in focal units corresponded to the number of Cy5-labelled

Table I
Characteristics of study patients

Sample ID	*Group*	*Age (years)*	*Sex*	*BMI (kg/m^2)*	*Side*	*Surgery*	*K-L score*	*Onset of symptoms*	*Tear pattern*	*Smoking*	*Diabetes*	*Chondrosis*
P4-001	APM	37	Female	21.92	Left	PMM	0	Nonspecific	Complex	No	No	No
P4-002	APM	45	Male	28.84	Right	PMM	0	Nonspecific	Complex	No	No	No
P4-003	APM	62	Female	27.76	Left	PMM	0	Nonspecific	Complex	No	No	No
P4-004$	APM	58	Female	20.52	Left	PMM/Ch	0	Injury	Complex	No	No	Yes
P4-005$	APM	31	Female	24.27	Left	PMM	0	Nonspecific	Complex	No	No	No
P4-007	APM	53	Male	26.62	Left	PMM/Ch	0	Acute	Complex	No	No	Yes
P4-008$	APM	50	Male	28.48	Right	PMM/Ch	0	Nonspecific	Complex	No	No	No
P4-009$	APM	65	Female	27.07	Left	PMM	0	Injury	Complex	No	No	No
P4-010*	APM	53	Male	31.99	Right	PMM	0	Injury	Oblique	No	Yes	No
P4-011$	APM	43	Male	34.43	Right	PMM	0	Acute	Radial	No	No	No
P4-012*	APM	40	Male	24.54	Left	PMM	0	Acute	Oblique	No	No	No
P4-013$	APM	53	Male	26.32	Left	PMM	0	Injury	Complex	No	No	No
P4-102$	TKA	57	Male	31.80	Left	TKA	3	–	–	No	No	–
P4-103$	TKA	64	Female	28.72	Right	TKA	3	–	–	Yes	No	–
P4-104*	TKA	53	Female	30.04	Left	TKA	3	–	–	No	No	–
P4-105$	TKA	62	Female	46.51	Right	TKA	4	–	–	No	Yes	–
P4-106	TKA	80	Male	30.42	Right	TKA	4	–	–	No	No	–
P4-107	TKA	67	Female	38.62	Right	TKA	4	–	–	No	No	–
P4-108*	TKA	70	Female	38.47	Left	TKA	4	–	–	No	No	–
P4-109$	TKA	64	Female	44.62	Right	TKA	4	–	–	No	No	–
P4-111	TKA	61	Male	31.46	Left	TKA	3	–	–	No	No	–
P4-112	TKA	62	Female	46.05	Left	TKA	4	–	–	No	No	–
P4-113$	TKA	79	Female	31.28	Left	TKA	4	–	–	No	No	–
P4-114$	TKA	64	Female	37.29	Left	TKA	4	–	–	No	No	–

APM = arthroscopic partial meniscectomy; TKA = total knee arthroplasty; BMI = body mass index; PMM = partial medial meniscectomy; Ch = chondroplasty; K-L = Kellgren–Lawrence; * = excluded from microarray analysis; $ = used for real-time polymerase chain reaction.

cDNA fragments hybridized to the spot of oligos bound to the surface of the array. The focal units were interpreted as relative measures of expression as the intensity represents the relative abundance of a given gene in the sample. Feature Extraction software (Agilent) was used to perform gridding and image analysis. RNA samples were randomized on the microarrays to avoid expression and/or detection bias.

Data mining and statistical analysis

The function *read.maimages* was used to import the Agilent Feature Extraction data into the R/Bioconductor package Limma. The intensity measures were transformed to the $\log_2$ scale, background subtracted, filtered for reliable signals above background (10% greater than the 95th percentile of negative control probes), and then quantile normalized to account for array to array variance. The probes were then filtered again to only include probe ID's that matched known and current Ensembl gene ID's, limiting analysis to probes. An additive generalized linear model (Limma GLM) including coefficients accounting for patient status, sex, BMI, age, and two latent unknown effects as derived by the R package surrogate variable analysis (SVA) was then created.

A Spearman correlation matrix and multidimensional scaling plots were used to assess the sample performance. Gene/transcript performance was assessed with plots of residual standard deviation of every gene to their average log count with a robustly fitted trend line of the residuals. Gene level analysis was performed assuming that $\log_2$ focal unit intensity was an independent observation for that gene and that the data was nearly Gaussian in distribution. Therefore, a Limma GLM fitted to the two conditions of interest along with blocking factors for other known or confounding sources of variation yielded genes whose low P values correspond to the difference in variance within vs across conditions and low uncertainty under the null hypothesis that there was no difference in relative expression between the two groups due to TKA or APM alone. We calculated post hoc statistical power on specimens using the R/Bioconductor package *sizepower* and found that we had a final statistical power of 94%. In the case of whole transcriptome analysis, statistical power of a study by itself does not serve as an accurate means of determining the uncertainty of the results. Instead, we controlled for false positives by limiting our focus to genes whose Benjamini Hochberg multiple testing correction false discovery rate (FDR) adjusted $P \leq 0.05$ and whose reported fold changes were in excess of an absolute value of 1.5. Data were shown in fold change for each comparison with 95% confidence interval (CI, lower limit to upper limit).

All microarrays were performed by the Genome Technology Access Center at the study institution. The raw array data are accessible through the accession number GSE117999 at http://www.ncbi.nlm.nih.gov/projects/geo). Based on our prior experience with Agilent microarray studies where RNA mass and quality are not limited, the correlation of technical replicates for high quality samples was typically >95% and mirrors the reported technical reproducibility described in Agilent's technical literature and of that reported by the MicroArray Quality Control (MAQC) consortium[15].

Gene ontology (GO) analysis

The R/Bioconductor packages Generally Applicable Gene Set Enrichment (GAGE) and Pathview were used to elucidate global transcriptomic changes in known GO terms from the biological interpretation of the large set of features found in the Limma results. GAGE measures perturbations in GO terms based on changes in observed $\log_2$ fold changes for the genes within that term vs the background $\log_2$ fold changes observed across features not contained in the respective term as reported by Limma. For GO terms with a statistical significance of $P \leq 0.05$, heatmaps were automatically generated for each respective term to show how genes co-vary/co-express across the term in relation to a given biological process or molecular function.

Real-time polymerase chain reaction (PCR)

Real-time PCR was used to validate the expression pattern of 12 transcripts (Table II), including six randomly selected patients in the APM group and six randomly selected patients in the TKA group. Briefly, 160 ng of RNA was subjected to DNase I (Life

Table II
Primers used for real-time PCR validation

Gene symbol	Accession #	Gene name	Forward primer (5' - 3')	Location		Reverse primer (5' - 3')	Location		Amplicon size (base-pair)
				from	to		from	to	
CFD	NM_001928	Complement factor D	CTCCAAGCGCCTGTACGAC	301	319	CAGTGTGGCCTTCTCCGAC	409	391	109
CSN1S1	NM_001025104.1	Casein alpha S1	CTCACCTGTCTTGTGGCTGT	65	84	GGCTCACTGCTCTCTGATGG	156	137	92
TSPAN11	NM_001080509.2	Tetraspanin 11	TACTTGTCATGGTGACCGGC	319	338	ATGACGAGCAACAGGCAGAA	412	393	94
CD14	NM_000591.3	CD14 molecule	AGAACCTTGTGAGCTGGACG	445	464	TGCAGACACACACTGGAAGG	541	522	97
HOXC8	NM_022658.3	Homeobox C8	CCTCCGCCAACACTAACAGT	526	545	GCTGTAAGTTTGCCGTCCAC	650	631	125
TMEM176A	NM_018487.2	Transmembrane protein 176A	GCTCGAGTGACTGGAACACT	542	561	CATGGCCTGAAGGGTTCTGA	660	641	119
CHI3L2	NM_004000.2	Chitinase 3 like 2	GCTGGACCCATCACAGAGTC	945	964	GGAACCTGCTGATCCTGGAG	1045	1026	101
HILPDA	NM_013332.3	Hypoxia inducible lipid droplet associated	TGCAGAGGAGTAGGGTCCTT	237	256	AGGCGATGGGCTCTCTAGTA	370	351	134
COL3A1	NM_000090.3	Collagen type III alpha 1 chain	TCGAGGCAGTGATGGTCAAC	1092	1111	TTTGAACCAGGAGACCCTGC	1202	1183	111
COL27A1	NM_032888.3	Collagen type XXVII alpha 1 chain	TGGACAGACGTGTCTCAAGC	5587	5606	AGTGGATGGTGATGTGCTGG	5705	5686	119
FGF2	NM_002006.5	Fibroblast growth factor 2	GTGCTAACCGTTACCTGGCT	700	719	TCAGTGCCACATACCAACTG	849	830	150
COL5A1	NM_000093.4	Collagen type V alpha 1 chain	GACACCGCAGTACCTGACAC	1116	1135	GGGCTCCTTCCCTAGGTCTT	1253	1234	138
PPIA	NM_021130.4	Peptidylprolyl isomerase A	TCTGCACTGCCAAGACTGAG	430	449	TGGTCTTGCCATTCCTGGAC	546	527	117

Technologies) to remove genomic DNA contamination. Random hexamers and the SuperScript II First Strand Synthesis System (Invitrogen) were used to synthesize first strand cDNA. PCR was performed on a 7500 Fast Real-Time PCR System (Applied Biosystems). *PPIA (*Peptidylprolyl isomerase A) acted as the housekeeping gene for normalization of fluorescence threshold (Ct) values of target genes using SYBR™ Green PCR Master Mix (Applied Biosystems). This housekeeping gene demonstrated stable expression with negligible variation across samples and has been used as a housekeeping gene in cartilage for both OA related and non-OA related studies and exhibited no differential expression in impacted and control cartilage specimens[16]. Amplification steps were essentially the same as reported previously[4]. Ct values were normalized to *PPIA* for each sample (ΔCt) and then $\log_2$ transformed. GraphPad Prism (GraphPad Software) was used to detect the significant deference via the Mann-Whitney *U* test.

Results

Study patients

Our initial query with principal component analysis (PCA) indicated that two patients from APM group (P4-010, P4-012) and two patients from TKA group (P4-104, P4-108) were extreme outliers as they were far from the cluster of majority and were therefore excluded (Table I). These samples also had low Spearman correlations or confounding deviations in hierarchical clustering analysis. The final study cohort included 10 patients without radiographic OA (K-L Score = 0) undergoing APM and 10 patients with radiographic OA (K-L score = 3–4) undergoing TKA. None of the APM patients had any chondrosis in the medial or lateral compartment at the time of surgery. Only 3/10 APM patients had any chondrosis in the knee at the time of surgery, always limited to Grade 2 changes involving the patellofemoral compartment. Age [mean ± standard deviation years] was significantly ($P = 0.001$) different between APM (49.7 ± 10.8) and TKA (66.0 ± 7.6) groups. BMI [mean (kg/m^2) ± standard deviation] was significantly ($P = 0.001$) higher in TKA (36.4 ± 7.0) than APM (26.6 ± 3.9) group. Similarly, K-L score (mean ± standard deviation) was significantly ($P < 0.0001$) higher in TKA (3.7 ± 0.5) than APM (0.00 ± 0.00) group. The distribution by sex (70% female TKA cohort, 50% female APM cohort) was not significant between two groups ($P = 0.650$). The clinical history and tear pattern varied for the APM cohort, with the majority of patients lacking a specific injury or acute onset of symptoms and complex tears were the most common.

Quantitative transcriptomic differences

PCA revealed that patients were clustered into two distinct clusters: one cluster comprised of samples from APM patients and the other cluster had samples from TKA patients based on PC1 (Fig. 1A). Patients were clustered by condition based on gene expression signatures on hierarchical clustering heatmaps (Fig. 1B). Expression fold change and averaged expression level of differentially expressed transcripts are shown as MA plot (Fig. 1C). Furthermore, we displayed the differentially expressed transcripts concurrently through volcano plots (Fig. 1D) to indicate the trend of transcription expression in magnitude (fold change) and significance (*P* value). The transcripts shown in the upper right (up-regulated) and left (down-regulated) corners are statistically most significant (lowest *P* value) and are greatest in magnitude (fold change). Both the MA and Volcano plots serve to illustrate the relationship of the statistical uncertainty and the observed changes in expression where data points of individual genes on the extremes (*y* axis for the MA plot and *x* axis for the Volcano plot) represent genes with the greatest statistical certainty and highest practical/biological relevance for gene expression changes between the two cohorts. The GAGE GO analysis where we measured the changes in $\log_2$ fold change expression within well-characterized gene sets vs the background of all other genes tested serves as our interpretation of biological relevance.

Among all human RNAs spotted on the microarray chip (40,146), Limma analysis generated a list of 1301 (3.24%) transcripts differentially-expressed between APM and TKA at FDR ≤ 0.05 regardless of fold change. While these differentially expressed genes were largely protein-coding, eight lincRNAs (long intergenic non-coding RNAs, a class of long non-coding RNAs that do not overlap with the bodies of known protein-coding genes), 10 snoRNAs (small nuclear RNAs, a class of small non protein-coding RNA molecules that primarily guide site-specific chemical modifications of other RNAs) and three antisense RNAs (single stranded RNAs that are complementary to a protein-coding mRNA (messenger ribonucleic acid) with which it hybridizes, and thereby blocks its translation into protein) were also detected as differentially expressed.

Differentially expressed transcripts (mRNAs)

In total, 582 protein coding transcripts showed at least ≥ 1.5 fold magnitude of difference between APM and TKA cartilage at FDR ≤ 0.05 (Supplemental Table I). At 2 fold, 81 transcripts (45 elevated in TKA, 36 elevated in APM) were differentially expressed (Table III). Notably, *CFD* (5.99 fold [95% CI, 2.15–16.67], FDR = 0.040), *CSN1S1*

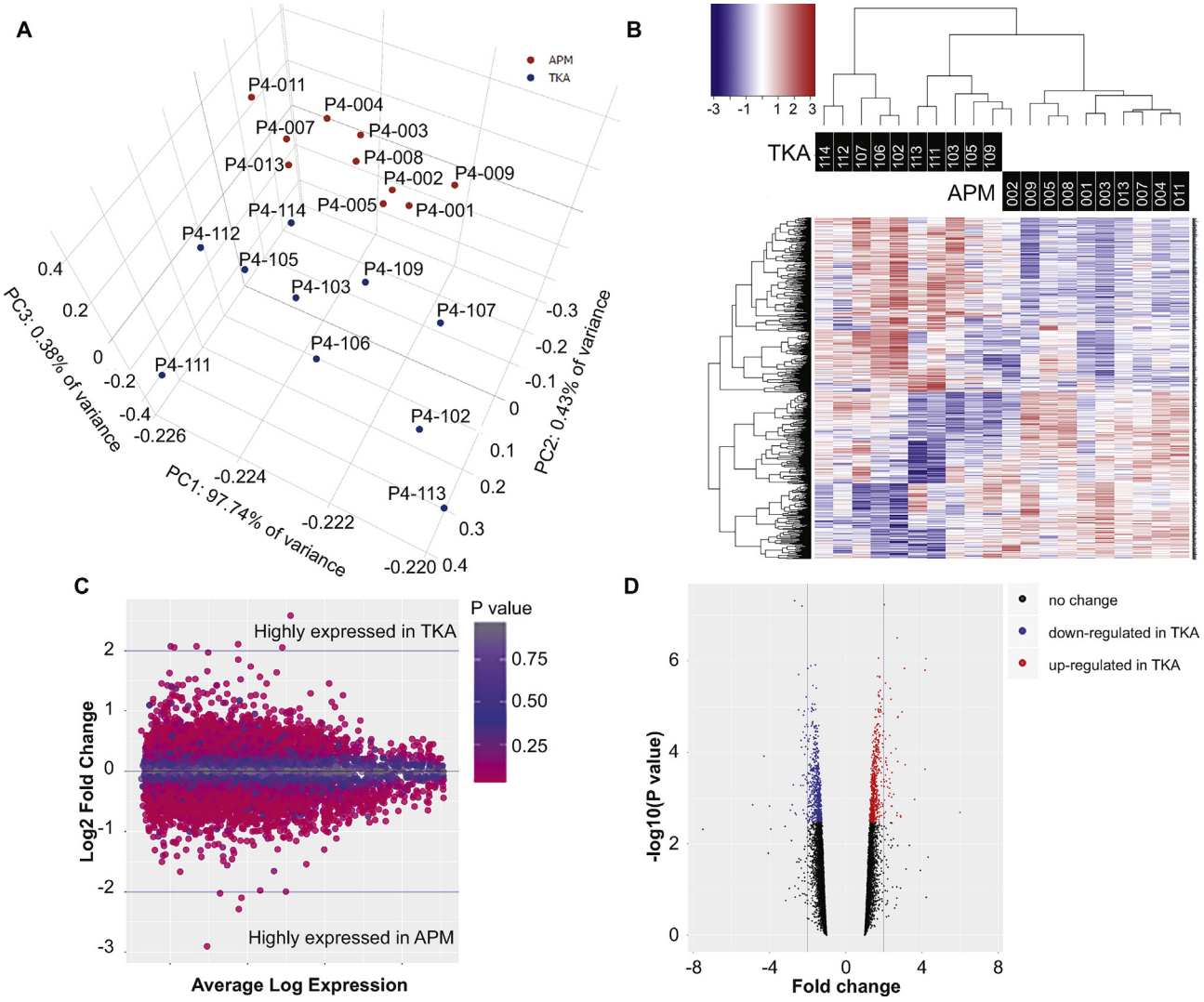

Fig. 1. A). Principal components analysis of 10 APM and 10 TKA samples showed clear distinction between two groups. Each dot represents one patient. B). Normalized gene expression level (z score) of differentially expressed transcripts between APM and TKA patients were used to generate heatmaps. Color bar above heatmaps indicates patients' metadata in which patients were mainly clustered. Based on differentially expressed transcripts, TKA and APM samples were distinctly separated. C). Expression fold change and averaged expression level of differentially expressed transcripts between APM and TKA patients are shown in the form of an MA plot. D). The differentially expressed transcripts at ≥ 2 fold-change are depicted in volcano plots to pictorially represent trend of expression by *P* value (*y* axes) and fold change (*x* axes). APM = arthroscopic partial meniscectomy; TKA = total knee arthroplasty.

(4.21 fold [95% CI, 2.81–6.33], FDR = 0.003), *TSPAN11* (4.16 fold [95% CI, 2.74–6.34], FDR = 0.003), *CSF1R* (3.62 fold [95% CI, 1.83–7.16], FDR = 0.029) and *CD14* (2.96 fold [95% CI, 2.02–4.34], FDR = 0.005) were highly elevated in TKA group while *CHI3L2* (−4.87 fold [95% CI, −3.53 to −1.04], FDR = 0.033), *HILPDA* (−3.99 fold [95% CI, −3.10 to −0.89], FDR = 0.034), *COL3A1* (−2.91 fold [95% CI, −2.42 to −0.66], FDR = 0.038), *COL27A1* (−2.81 fold [95% CI, −2.31 to −0.67], FDR = 0.033) and *FGF2* (−2.60 fold [95% CI, −2.19 to −0.57], FDR = 0.042) were repressed in TKA.

Biological interpretation

GO analysis revealed that a large number of differentially expressed transcripts belonged to numerous enriched biological processes. Transcripts up-regulated in TKA cartilage were enriched for protein localization to endoplasmic reticulum, protein activation cascade, chemical stimulus, immune response, toll-like receptor signaling pathway and nuclear transcribed mRNA catabolic process (Fig. 2A). Transcripts up-regulated in APM cartilage were enriched for mesenchymal cell apoptosis, epithelial morphogenesis, canonical glycolysis, connective tissue development, cartilage development, extracellular structure organization and glucose catabolic process (Fig. 2B).

Differentially expressed lincRNAs, snoRNAs and antisense RNAs

Eight lincRNAs were differentially expressed between APM and TKA (two were elevated and six were repressed in TKA group). There were 10 snoRNAs that showed a differential expression between APM and TKA (all elevated in TKA group). Finally, three antisense RNAs (all elevated in TKA group) were also detected as differentially expressed between the two groups (Table IV).

Real-time PCR

Real-time PCR confirmed that all the transcripts tested (total 12, 6 elevated and 6 repressed in TKA) showed a concordance between microarrays and real-time PCR. Notably, the expression pattern was

Table III
Gene transcripts differentially expressed between APM and TKA cartilage*

Gene symbol	*Gene name*	*P value*	*FDR*	*Fold change*	*95% CI*	
					Lower limit	*Upper limit*
Gene transcripts elevated in TKA cartilage						
CFD	complement factor D	0.002	0.040	5.99	2.15	16.67
CSN1S1	casein alpha s1	<0.001	0.003	4.21	2.81	6.33
TSPAN11	tetraspanin 11	<0.001	0.003	4.16	2.74	6.34
CSF1R	colony stimulating factor 1 receptor	0.001	0.029	3.62	1.83	7.16
C1QB	complement C1q B chain	<0.001	0.014	3.11	1.88	5.15
INCENP	inner centromere protein	<0.001	0.003	3.09	2.21	4.32
CD14	CD14 molecule	<0.001	0.005	2.96	2.02	4.34
GFPT2	glutamine-fructose-6-phosphate transaminase 2	0.003	0.044	2.88	1.54	5.40
TPPP	tubulin polymerization promoting protein	0.002	0.043	2.87	1.55	5.35
PPP6R1	protein phosphatase 6 regulatory subunit 1	<0.001	0.006	2.74	1.90	3.95
HOXC8	homeobox C8	<0.001	0.013	2.74	1.76	4.25
C1orf61	chromosome 1 open reading frame 61	<0.001	0.006	2.74	1.90	3.95
LEO1	LEO1 homolog, Paf1/RNA polymerase II complex component	<0.001	0.001	2.70	2.06	3.52
NCF1	neutrophil cytosolic factor 1	0.002	0.040	2.69	1.52	4.76
CYP1B1	cytochrome P450 family 1 subfamily B member 1	0.002	0.043	2.68	1.50	4.79
C11orf96	chromosome 11 open reading frame 96	0.001	0.025	2.52	1.56	4.08
TMEM176A	transmembrane protein 176A	<0.001	0.016	2.43	1.61	3.67
UHRF2	ubiquitin like with PHD and ring finger domains 2	0.001	0.034	2.43	1.48	3.97
DEFB1	defensin beta 1	0.001	0.027	2.40	1.51	3.83
CRIP1	cysteine rich protein 1	0.001	0.034	2.39	1.47	3.90
Gene transcripts elevated in APM cartilage						
CHI3L2	chitinase 3 like 2	0.001	0.033	−4.87	−3.53	−1.04
HILPDA	hypoxia inducible lipid droplet associated	0.001	0.034	−3.99	−3.10	−0.89
COL3A1	collagen type III alpha 1 chain	0.002	0.038	−2.91	−2.42	−0.66
COL27A1	collagen type XXVII alpha 1 chain	0.001	0.033	−2.81	−2.31	−0.67
CCDC80	coiled-coil domain containing 80	0.001	0.020	−2.74	−2.17	−0.74
CLEC18B	C-type lectin domain family 18 member B	<0.001	0.000	−2.68	−1.76	−1.08
C10orf10	chromosome 10 open reading frame 10	0.002	0.041	−2.65	−2.22	−0.59
FGF2	fibroblast growth factor 2	0.002	0.042	−2.60	−2.19	−0.57
RHOBTB3	Rho related BTB domain containing 3	<0.001	0.005	−2.47	−1.77	−0.85
ZRANB2	zinc finger RANBP2-type containing 2	<0.001	0.003	−2.46	−1.70	−0.90
INSIG1	insulin induced gene 1	0.001	0.021	−2.36	−1.85	−0.62
PFKFB3	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	<0.001	0.009	−2.34	−1.72	−0.73
UCKL1	uridine-cytidine kinase 1 like 1	<0.001	0.010	−2.31	−1.70	−0.71
DUT	deoxyuridine triphosphatase	<0.001	<0.001	−2.28	−1.48	−0.90
ITM2C	integral membrane protein 2C	<0.001	0.017	−2.25	−1.72	−0.62
LYNX1	Ly6/neurotoxin 1	0.001	0.021	−2.23	−1.74	−0.58
COL5A1	collagen type V alpha 1 chain	0.002	0.038	−2.23	−1.82	−0.49
CD72	CD72 molecule	<0.001	0.008	−2.23	−1.60	−0.71
SLC25A37	solute carrier family 25 member 37	0.002	0.041	−2.18	−1.78	−0.47
VSIG10	V-set and immunoglobulin domain containing 10	<0.001	0.017	−2.18	−1.65	−0.59

* = only top 20 elevated/repressed gene transcripts are shown; APM = arthroscopic partial meniscectomy; TKA = total knee arthroplasty; FDR = false discovery rate; CI = confidence interval.

same between the two assays with many genes showing statistical significance between APM and TKA even in a subset ($N = 6$ each group) of samples (Fig. 3).

Discussion

This investigation into the gene expression of cartilage from knees undergoing APM without radiographic OA demonstrates a distinct expression profile compared to cartilage from knees undergoing TKA with end-stage radiographic OA. Several transcripts were differentially expressed between the two groups and GO analyses further revealed their significance in a number of biological processes and pathways. These differentially expressed genes and pathways are promising targets for further investigation into their mechanistic role in OA disease process as they reflect differences in gene expression at distinct time points during the disease process.

There are a number of interesting findings in terms of the individual transcripts differentially expressed between the two groups. While we know that cartilage from APM patients looks grossly normal and the knees have no radiographic features of OA, our recent analysis of this normal appearing cartilage suggests that it exhibits molecular signatures reflective of OA[13]. Thus it appears that both APM and TKA cartilage, despite having a distinct gene expression profile, are both related to OA phenotype, albeit at different time points in disease progression. The expression pattern of these transcripts was confirmed by real-time PCR and was found to be concordant with the microarray data.

Transcripts that were highly expressed in TKA knees are clearly associated with OA. CFD (adipsin) is responsible for activating the alternative pathway of the complement system. Little work has examined the role of adipsin in OA. Adipsin was higher in the synovial fluid and serum among individuals with OA undergoing TKA compared to those without OA[17]. CFD was one of the top differentially expressed gene in meniscus from TKA patients compared to APM patients[4]. Serum levels of CFD in OA patients were correlated with increased cartilage volume in the global knee and medial femur at baseline and 2 years[18]. CSN1S1 is known to mediate pro-inflammatory properties through the activation of granulocyte-macrophage colony-stimulating factor (GM-CSF) via p38 mitogen-activated protein kinase (MAPK) pathway[19]. It shows higher expression in capsule from OA joints[20], OA cartilage[10] and OA synovium[21]. Moreover, another study confirmed that CSN1S1 exhibited significantly higher expression in OA cartilage and

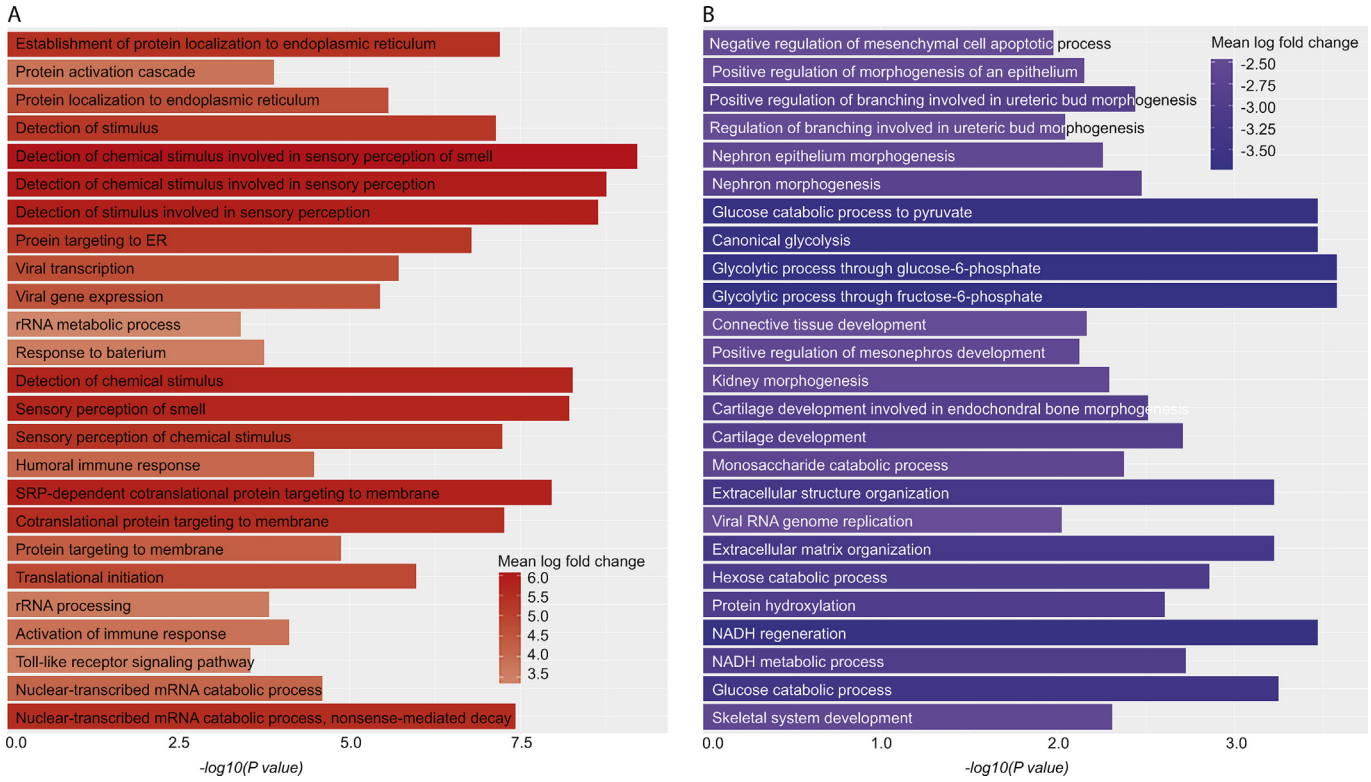

Fig. 2. Barplots showing different biological processes. Gene ontology biological process are shown for transcripts elevated in TKA group (A) and for transcripts elevated in APM group (B). Top 25 biological processes are shown in each category. ER = endoplasmic reticulum, rRNA = ribosomal ribonucleic acid, SRP = signal recognition particle, mRNA = messenger ribonucleic acid, NADH = nicotinamide adenine dinucleotide.

Table IV
Antisense, lincRNAs and snoRNAs differentially expressed between APM and TKA cartilage

Gene symbol	*Gene biotype*	*Gene name*	*P value*	*FDR*	*Fold change*	*95% CI*		*Description*
						Lower limit	*Upper limit*	
RAD21-AS1	antisense	RAD21 antisense RNA 1	0.002	0.035	1.66	1.24	2.21	Up in TKA
C1QTNF9B-AS1	antisense	C1QTNF9B antisense RNA 1	0.003	0.044	1.51	1.18	1.94	Up in TKA
WT1-AS	antisense	WT1 antisense RNA	<0.001	0.019	1.51	1.24	1.84	Up in TKA
PWRN1	lincRNA	Prader–Willi region non-protein coding RNA 1	0.001	0.023	1.69	1.29	2.21	Up in TKA
FAM157A	lincRNA	Family With Sequence Similarity 157 Member A	<0.001	0.019	1.52	1.24	1.87	Up in TKA
TTTY12	lincRNA	testis-specific transcript, Y-linked 12 (non-protein coding)	<0.001	0.016	−1.50	−1.80	−1.24	Up in APM
C9orf163	lincRNA	chromosome 9 open reading frame 163	0.001	0.031	−1.53	−1.93	−1.21	Up in APM
C22orf34	lincRNA	chromosome 22 open reading frame 34	<0.001	0.004	−1.54	−1.77	−1.34	Up in APM
MGC45922	lincRNA	uncharacterized LOC28	0.001	0.021	−1.88	−2.58	−1.37	Up in APM
C10orf91	lincRNA	chromosome 10 open reading frame 91	<0.001	0.016	−1.98	−2.72	−1.44	Up in APM
MEG3	lincRNA	maternally expressed 3 (non-protein coding)	<0.001	0.012	−4.28	−7.87	−2.32	Up in APM
SNORA23	snoRNA	small nucleolar RNA, H/ACA box 23	<0.001	0.015	4.16	2.19	7.90	Up in TKA
SNORD114-14	snoRNA	small nucleolar RNA, C/D box 114-14	0.001	0.030	1.73	1.28	2.34	Up in TKA
SNORD116-28	snoRNA	small nucleolar RNA, C/D box 116-28	<0.001	0.013	1.60	1.30	1.98	Up in TKA
SNORD3B-1	snoRNA	small nucleolar RNA, C/D box 3B-1	0.001	0.023	1.58	1.25	2.00	Up in TKA
SNORA54	snoRNA	small nucleolar RNA, H/ACA box 54	0.002	0.034	1.57	1.22	2.02	Up in TKA
SNORD116-29	snoRNA	small nucleolar RNA, C/D box 116-29	<0.001	0.008	1.57	1.32	1.86	Up in TKA
SNORA36A	snoRNA	small nucleolar RNA, H/ACA box 36A	<0.001	0.018	1.55	1.25	1.91	Up in TKA
SNORD116-10	snoRNA	small nucleolar RNA, C/D box 116-10	<0.001	0.018	1.52	1.24	1.86	Up in TKA
SNORD116-19	snoRNA	small nucleolar RNA, C/D box 116-19	0.001	0.025	1.52	1.22	1.89	Up in TKA
SNORD116-26	snoRNA	small nucleolar RNA, C/D box 116-26	<0.001	0.013	1.51	1.26	1.81	Up in TKA

FDR = false discovery rate; CI = confidence interval; APM = arthroscopic partial meniscectomy; TKA = total knee arthroplasty.

synovium than in normal tissues[22]. TSPAN11 (CD151 like) is a member of the tetraspanins family, also called transmembrane 4 superfamily but nothing is known about its role in OA.

CSF1R blockage reduces inflammation in human and rodent models of rheumatoid arthritis (RA)[23]. CSF through binding to the tyrosine kinase receptor CSF1R promotes cell survival and proliferation[24]. In RA, CSF is produced by synovial endothelial cell[25] and *in vitro* by IL-1β and TNFα[26]. In mice, blockage of CSF1R stopped cartilage damage, systemic bone loss and bone erosion[27]. Inhibition of CSF1 and CSF1R is a promising target and therapeutic alternative for arthritis and related conditions[28]. CD14 is considered a reflection of inflammatory activation status of macrophages as it is abundant on monocyte and macrophages[29] and serves as a receptor for the bacterial lipopolysaccharide (LPS) and LPS binding protein

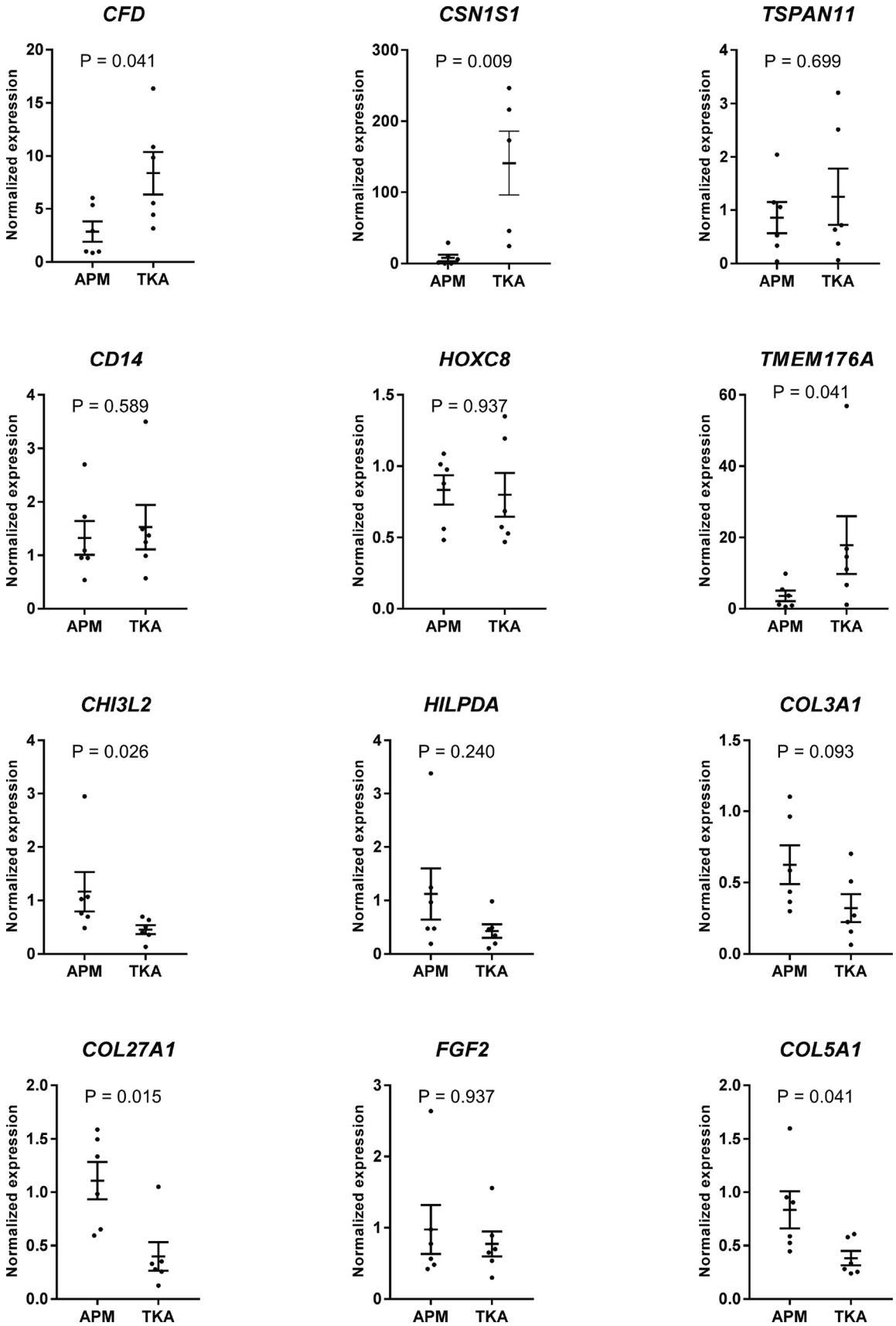

Fig. 3. Validation of transcripts by real-time PCR. The expression of 12 transcripts differentially expressed between APM and TKA was validated by real-time PCR. The expression profile of the entire set of transcripts obtained from real-time PCR amplification was concordant with the microarray data. The data is presented as mean with standard error of the mean. (top two rows = genes elevated in TKA cartilage, bottom two rows = genes elevated in APM cartilage). Significance was determined by Mann-Whitney *U* test. APM = arthroscopic partial meniscectomy; TKA = total knee arthroplasty.

complex[30]. Available literature suggests that pain in OA may be, due in part, to the release of CD14 from activated macrophages in inflamed joint tissues and from infiltrated macrophages in deep root ganglia. Synovial fluid CD14 has been shown to be significantly associated with osteophyte severity and joint space narrowing[31]. Our findings appear to be in line with the aforementioned studies.

Transcripts that were highly expressed in the APM group may arise from three plausible scenarios: (1) some genes are clearly associated with cartilage degenerations, (2) other genes have been implicated in mechanotransduction or response to injury, and (3) some have a role in joint homeostasis. CHI3L2 (YKL-39) is a novel growth and differentiation factor involved in cartilage homeostasis. It has been reported that CHI3L2 enhances colony forming activity, cell proliferation, and type II collagen expression in ATDC5 cells[32]. CHI3L2 activates phosphorylation of extracellular signal regulated kinases ERK1/ERK2 in HEK293 and U87 malignant glioma (MG) cell lines[33]. YKL-39 mRNA has been shown to be significantly up-regulated in the cartilage of patients with OA[34]. Moreover, the level of YKL-39 mRNA expression was positively correlated with collagen type II up-regulation in both early and late stages of the disease[35]. It appears that YKL-39 is involved in a variety of physiological processes (e.g., tissue remodeling, chondrocyte repair, inflammation) and serves as specific biomarkers for OA progression[36]. Higher expression of CHI3L2 in APM cartilage likely represents a repair response and a surrogate for disease progression at an early stage e.g., after a meniscus tear. Gene expression of HILPDA decreases with age as well in cartilage from OA patients and gets stimulated in synovial fibroblasts by IL-17[37], an inflammatory cytokine synovial levels of which are negatively correlated with OA severity[38]. This may, in part, explain why HILPDA is highly expressed in APM cartilage.

A number of transcriptome studies have identified COL3A1 as a differentially expressed gene between normal and arthritic cartilage. Consistent with our study, lower expression of COL3A1 has also been shown in degenerated cartilage compared to macroscopically intact cartilage from the same knee[39]. One study, analyzing microarray data from gene expression omnibus, did find highly expressed COL3A1[40], although it was under powered and under analyzed. COL27A1 is a fibrillary collagen gene, which is highly expressed in developing skeletal cartilage[41] and is known to be regulated by SOX9, as are COL2A1, COL11A2 and COL9A1[42]. Literature on the role and expression pattern of COL27A1 in OA is limited, but our findings suggest it responds to injury, as its expression was higher in APM cartilage compared to TKA cartilage, and may represent a marker for early OA. A genome-wide association study has found that a single nucleotide polymorphism in COL27A1 shows association (at nominal significance) with radiographic OA[43].

FGF2 was also significantly up-regulated in APM cartilage compared to TKA cartilage. It has been implicated in OA[44], impedes anabolism and promotes catabolism potentially via up-regulation of MMP13[45]. The role of FGF2 in OA and cartilage homeostasis, however, remains controversial as it is a novel endogenous chondroprotective agent in cartilage that suppresses ADAMTS5 and delays cartilage degradation in murine OA[46]. It also plays a functional role in chondrocyte mechanotransduction[47].

There were several transcripts found to be up-regulated in TKA cartilage compared to APM which were associated with immune response. These findings were interesting because involvement of immune response systems has also been implicated in OA. An integrative meta-analysis of differentially expressed genes in OA cartilage has demonstrated that immune response was a highly-enriched GO term[48]. Transcriptome analysis of equine cartilage[49] and human meniscus[50] has shown that biological processes related to immune response were elevated with age, which is consistent with our current findings and previous findings from APM cartilage[13] as well as TKA meniscus[4]. These findings suggest that TKA cartilage has a higher tendency to express genes related to immune response than APM cartilage.

GO terms referring to extracellular matrix organization, including several collagens (e.g., COL1A2, COL2A1, COL3A1), was enriched for highly changed genes in preserved (non-OA) cartilage[8]. The repression of processes related to extracellular matrix organization in the TKA cartilage relative to APM cartilage is a significant finding with a number of biological and clinical implications. Several studies have shown that loss of extracellular matrix occurs in the degenerative phenotype of cartilage as a cause and consequence of OA[51]. As we did not compare cartilage between healthy knees and knees with OA, we cannot conclude that this identifies a degenerative phenotype in the cartilage. It does, however, show that the cartilage from TKA patients had higher degeneration than cartilage from APM patients. The functional processes related to connective tissue development were also repressed in TKA cartilage. Suppression of connective tissue development, epithelial morphogenesis and cartilage development in TKA cartilage would minimize any potential for regeneration in this group.

While these results show statistically significant differences in gene expression, clinical relevance of these findings is not immediately apparent since the downstream effects of these differences in gene expression are not known. Nevertheless, this study is an initial step towards advancing our understanding of biological events after meniscal injury. In the light of above discussion, several transcripts and biological processes differentially represented in APM and TKA are characteristic of the OA disease process[8,10,13]. These findings stress the importance of studying the knee joint as an organ, with inclusion of other tissues (bone, synovium, meniscus, ligaments) and healthy controls. Since the gene expression in the cartilage was compared between injured and diseased (OA) joints, further research is needed to compare these findings to normal cartilage in intact knees. In this study, the prevalence of females was 20% higher in TKA patients than APM patients. Considering that females have a higher risk for OA than males[52], we added patient sex in our model to account for this discrepancy in the percentage of female patients. While some studies[53] have reported sex-related differences in cartilage, we did not have a large enough sample size to determine the sex-related differences in transcript expression. Another limitation was the inclusion of three patients with focal grade 2 chondrosis in the patellofemoral compartment, reflecting the fact that it is extremely rare to find knees completely free of any chondral wear or damage at the time of APM. On the other hand, it is impossible to determine exactly why these patients do not have cartilage damage at the time of APM. This selected subsample of subjects may be highly resilient to cartilage wear and potentially less susceptible to the development of knee OA compared to more commonly encountered patients with concomitant meniscus tears and chondrosis.

Despite these limitations, our study has several strengths. First, the comparison between injured and diseased cartilage provides a unique assessment of the condition of each tissue after knee injury vs OA. As discussed, changes consistent with the disease phenotype are seen in both groups. Second, based on initial analysis of these data, and considering published work[13], all the confounders were included in the model. Finally, our validation assay i.e. real-time PCR substantiated our findings on the differences seen by microarray as we observed high concordance of expression pattern between the two assays.

In conclusion, our study clearly demonstrated that numerous transcripts were differentially expressed between cartilage from knees undergoing APM and TKA. Despite significant differences, both express genes and pathways related to OA. Therefore, the gene

profile in APM cartilage likely represents an earlier stage in degeneration while TKA cartilage is end-stage. Future mechanistic studies, as well as comparison with normal (uninjured) cartilage, could shed light on how injury alters the joint homeostasis, ultimately leading to OA and irreversible joint destruction. Improving our knowledge of early events after meniscal injury and surgery may advance our understanding of how and why this injury impacts the knee joint as a whole, and what could be done to delay or prevent subsequent joint damage.

Author contributions

All authors were involved in drafting and revision of the manuscript and all authors approved the final version to be published. Drs. Rai and Brophy had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of interest

All authors declare that there exists no conflict of interest with regard to this study.

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Supplementary data

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References

1. Lewandrowski KU, Muller J, Schollmeier G. Concomitant meniscal and articular cartilage lesions in the femorotibial joint. Am J Sports Med 1997;25:486–94.
2. Lohmander LS, Englund PM, Dahl LL, Roos EM. The long-term consequence of anterior cruciate ligament and meniscus injuries: osteoarthritis. Am J Sports Med 2007;35:1756–69.
3. Roos H, Adalberth T, Dahlberg L, Lohmander LS. Osteoarthritis of the knee after injury to the anterior cruciate ligament or meniscus: the influence of time and age. Osteoarthritis Cartilage 1995;3:261–7.
4. Brophy RH, Zhang B, Cai L, Wright RW, Sandell LJ, Rai MF. Transcriptome comparison of meniscus from patients with and without osteoarthritis. Osteoarthritis Cartilage 2018;26:422–32.
5. Ochi K, Daigo Y, Katagiri T, Saito-Hisaminato A, Tsunoda T, Toyama Y, *et al.* Expression profiles of two types of human knee-joint cartilage. J Hum Genet 2003;48:177–82.
6. Dunn SL, Soul J, Anand S, Schwartz JM, Boot-Handford RP, Hardingham TE. Gene expression changes in damaged osteoarthritic cartilage identify a signature of non-chondrogenic and mechanical responses. Osteoarthritis Cartilage 2016;24:1431–40.
7. Snelling S, Rout R, Davidson R, Clark I, Carr A, Hulley PA, *et al.* A gene expression study of normal and damaged cartilage in anteromedial gonarthrosis, a phenotype of osteoarthritis. Osteoarthritis Cartilage 2014;22:334–43.
8. Ramos YF, den Hollander W, Bovee JV, Bomer N, van der Breggen R, Lakenberg N, *et al.* Genes involved in the osteoarthritis process identified through genome wide expression analysis in articular cartilage; the RAAK study. PLoS One 2014;9:e103056.
9. Sato T, Konomi K, Yamasaki S, Aratani S, Tsuchimochi K, Yokouchi M, *et al.* Comparative analysis of gene expression profiles in intact and damaged regions of human osteoarthritic cartilage. Arthritis Rheum 2006;54:808–17.
10. Karlsson C, Dehne T, Lindahl A, Brittberg M, Pruss A, Sittinger M, *et al.* Genome-wide expression profiling reveals new candidate genes associated with osteoarthritis. Osteoarthritis Cartilage 2010;18:581–92.
11. Fu M, Huang G, Zhang Z, Liu J, Zhang Z, Huang Z, *et al.* Expression profile of long noncoding RNAs in cartilage from knee osteoarthritis patients. Osteoarthritis Cartilage 2015;23:423–32.
12. Liu Q, Zhang X, Dai L, Hu X, Zhu J, Li L, *et al.* Long noncoding RNA related to cartilage injury promotes chondrocyte extracellular matrix degradation in osteoarthritis. Arthritis Rheumatol 2014;66:969–78.
13. Rai MF, Sandell LJ, Zhang B, Wright RW, Brophy RH. RNA microarray analysis of macroscopically normal articular cartilage from knees undergoing partial medial meniscectomy: potential Prediction of the risk for developing osteoarthritis. PLoS One 2016;11:e0155373.
14. Reynard LN, Loughlin J. Insights from human genetic studies into the pathways involved in osteoarthritis. Nat Rev Rheumatol 2013;9:573–83.
15. Consortium M, Shi L, Reid LH, Jones WD, Shippy R, Warrington JA, *et al.* The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements. Nat Biotechnol 2006;24:1151–61.
16. Ashwell MS, O'Nan AT, Gonda MG, Mente PL. Gene expression profiling of chondrocytes from a porcine impact injury model. Osteoarthritis Cartilage 2008;16:936–46.
17. Fernandez-Puente P, Mateos J, Fernandez-Costa C, Oreiro N, Fernandez-Lopez C, Ruiz-Romero C, *et al.* Identification of a panel of novel serum osteoarthritis biomarkers. J Proteome Res 2011;10:5095–101.
18. Martel-Pelletier J, Raynauld JP, Dorais M, Abram F, Pelletier JP. The levels of the adipokines adipsin and leptin are associated with knee osteoarthritis progression as assessed by MRI and incidence of total knee replacement in symptomatic osteoarthritis patients: a post hoc analysis. Rheumatology 2016;55:680–8.
19. Vordenbaumen S, Braukmann A, Petermann K, Scharf A, Bleck E, von Mikecz A, *et al.* Casein alpha s1 is expressed by human monocytes and upregulates the production of GM-CSF via p38 MAPK. J Immunol 2011;186:592–601.
20. Campbell TM, Trudel G, Wong KK, Laneuville O. Genome wide gene expression analysis of the posterior capsule in patients with osteoarthritis and knee flexion contracture. J Rheumatol 2014;41:2232–9.
21. Ungethuem U, Haeupl T, Witt H, Koczan D, Krenn V, Huber H, *et al.* Molecular signatures and new candidates to target the pathogenesis of rheumatoid arthritis. Physiol Genomics 2010;42A:267–82.

22. Park R, Ji JD. Unique gene expression profile in osteoarthritis synovium compared with cartilage: analysis of publicly accessible microarray datasets. Rheumatol Int 2016;36:819–27.
23. Garcia S, Hartkamp LM, Malvar-Fernandez B, van Es IE, Lin H, Wong J, *et al.* Colony-stimulating factor (CSF) 1 receptor blockade reduces inflammation in human and murine models of rheumatoid arthritis. Arthritis Res Ther 2016;18:75.
24. Hume DA, MacDonald KP. Therapeutic applications of macrophage colony-stimulating factor-1 (CSF-1) and antagonists of CSF-1 receptor (CSF-1R) signaling. Blood 2012;119:1810–20.
25. Nakano K, Okada Y, Saito K, Tanikawa R, Sawamukai N, Sasaguri Y, *et al.* Rheumatoid synovial endothelial cells produce macrophage colony-stimulating factor leading to osteoclastogenesis in rheumatoid arthritis. Rheumatology (Oxford) 2007;46:597–603.
26. Campbell IK, Ianches G, Hamilton JA. Production of macrophage colony-stimulating factor (M-CSF) by human articular cartilage and chondrocytes. Modulation by interleukin-1 and tumor necrosis factor alpha. Biochim Biophys Acta 1993;1182:57–63.
27. Toh ML, Bonnefoy JY, Accart N, Cochin S, Pohle S, Haegel H, *et al.* Bone- and cartilage-protective effects of a monoclonal antibody against colony-stimulating factor 1 receptor in experimental arthritis. Arthritis Rheumatol 2014;66:2989–3000.
28. El-Gamal MI, Al-Ameen SK, Al-Koumi DM, Hamad MG, Jalal NA, Oh CH. Recent advances of colony-stimulating factor-1 receptor (CSF-1R) kinase and its Inhibitors. J Med Chem 2018;61:5450–66.
29. Landmann R, Muller B, Zimmerli W. CD14, new aspects of ligand and signal diversity. Microbes Infect 2000;2:295–304.
30. Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science 1990;249:1431–3.
31. Daghestani HN, Pieper CF, Kraus VB. Soluble macrophage biomarkers indicate inflammatory phenotypes in patients with knee osteoarthritis. Arthritis Rheumatol 2015;67:956–65.
32. Miyatake K, Tsuji K, Yamaga M, Yamada J, Matsukura Y, Abula K, *et al.* Human YKL39 (chitinase 3-like protein 2), an osteoarthritis-associated gene, enhances proliferation and type II collagen expression in ATDC5 cells. Biochem Biophys Res Commun 2013;431:52–7.
33. Areshkov PA, Kavsan VM. Chitinase 3-like protein 2 (CHI3L2, YKL-39) activates phosphorylation of extracellular signal-regulated kinases ERK1/ERK2 in human embryonic kidney (HEK293) and human glioblastoma (U87 MG) cells. Tsitol Genet 2010;44:3–9.
34. Steck E, Breit S, Breusch SJ, Axt M, Richter W. Enhanced expression of the human chitinase 3-like 2 gene (YKL-39) but not chitinase 3-like 1 gene (YKL-40) in osteoarthritic cartilage. Biochem Biophys Res Commun 2002;299:109–15.
35. Knorr T, Obermayr F, Bartnik E, Zien A, Aigner T. YKL-39 (chitinase 3-like protein 2), but not YKL-40 (chitinase 3-like protein 1), is up regulated in osteoarthritic chondrocytes. Ann Rheum Dis 2003;62:995–8.
36. Ranok A, Wongsantichon J, Robinson RC, Suginta W. Structural and thermodynamic insights into chitooligosaccharide binding to human cartilage chitinase 3-like protein 2 (CHI3L2 or YKL-39). J Biol Chem 2015;290:2617–29.
37. Hattori T, Ogura N, Akutsu M, Kawashima M, Watanabe S, Ito K, *et al.* Gene expression profiling of IL-17A-Treated synovial fibroblasts from the human Temporomandibular joint. Mediators Inflamm 2015;2015:436067.
38. Liu Y, Peng H, Meng Z, Wei M. Correlation of IL-17 level in Synovia and severity of knee osteoarthritis. Med Sci Monit 2015;21:1732–6.
39. Fukui N, Ikeda Y, Ohnuki T, Tanaka N, Hikita A, Mitomi H, *et al.* Regional differences in chondrocyte metabolism in osteoarthritis: a detailed analysis by laser capture microdissection. Arthritis Rheum 2008;58:154–63.
40. Sun J, Yan B, Yin W, Zhang X. Identification of genes associated with osteoarthritis by microarray analysis. Mol Med Rep 2015;12:5211–6.
41. Pace JM, Corrado M, Missero C, Byers PH. Identification, characterization and expression analysis of a new fibrillar collagen gene, COL27A1. Matrix Biol 2003;22:3–14.
42. Jenkins E, Moss JB, Pace JM, Bridgewater LC. The new collagen gene COL27A1 contains SOX9-responsive enhancer elements. Matrix Biol 2005;24:177–84.
43. Yau MS, Yerges-Armstrong LM, Liu Y, Lewis CE, Duggan DJ, Renner JB, *et al.* Genome-wide association study of radiographic knee osteoarthritis in North American Caucasians. Arthritis Rheumatol 2017;69:343–51.
44. Meo Burt P, Xiao L, Dealy C, Fisher MC, Hurley MM. FGF2 high molecular weight Isoforms Contribute to Osteoarthropathy in male mice. Endocrinology 2016;157:4602–14.
45. Yan D, Chen D, Cool SM, van Wijnen AJ, Mikecz K, Murphy G, *et al.* Fibroblast growth factor receptor 1 is principally responsible for fibroblast growth factor 2-induced catabolic activities in human articular chondrocytes. Arthritis Res Ther 2011;13:R130.
46. Chia SL, Sawaji Y, Burleigh A, McLean C, Inglis J, Saklatvala J, *et al.* Fibroblast growth factor 2 is an intrinsic chondroprotective agent that suppresses ADAMTS-5 and delays cartilage degradation in murine osteoarthritis. Arthritis Rheum 2009;60:2019–27.
47. Vincent TL, McLean CJ, Full LE, Peston D, Saklatvala J. FGF-2 is bound to perlecan in the pericellular matrix of articular cartilage, where it acts as a chondrocyte mechanotransducer. Osteoarthritis Cartilage 2007;15:752–63.
48. Wang X, Ning Y, Guo X. Integrative meta-analysis of differentially expressed genes in osteoarthritis using microarray technology. Mol Med Rep 2015;12:3439–45.
49. Peffers M, Liu X, Clegg P. Transcriptomic signatures in cartilage ageing. Arthritis Res Ther 2013;15:R98.
50. Rai MF, Patra D, Sandell LJ, Brophy RH. Transcriptome analysis of injured human meniscus reveals a distinct phenotype of meniscus degeneration with aging. Arthritis Rheum 2013;65:2090–101.
51. Squires GR, Okouneff S, Ionescu M, Poole AR. The pathobiology of focal lesion development in aging human articular cartilage and molecular matrix changes characteristic of osteoarthritis. Arthritis Rheum 2003;48:1261–70.
52. Srikanth VK, Fryer JL, Zhai G, Winzenberg TM, Hosmer D, Jones G. A meta-analysis of sex differences prevalence, incidence and severity of osteoarthritis. Osteoarthritis Cartilage 2005;13:769–81.
53. Pan Q, O'Connor MI, Coutts RD, Hyzy SL, Olivares-Navarrete R, Schwartz Z, *et al.* Characterization of osteoarthritic human knees indicates potential sex differences. Biol Sex Differ 2016;7:27.