Basic Research Article

CARTILAGE
1–9

DOI: 10.1177/19476035231155630
journals.sagepub.com/home/CAR
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Is the Synovium the First Responder to Posttraumatic Knee Joint Stress? The Molecular Pathogenesis of Traumatic Cartilage Degeneration

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Abstract

Objective. The aim of this study was to evaluate if a similar catabolic and inflammatory gene pattern exists between the synovium, hyaline cartilage, and blood of patients with the knee joint tissues and if one precedes the other. *Design*. A total of fifty-eight patients (34 females and 24 males) with a mean age of 44.7 years (range, 18-75) underwent elective knee arthroscopy due to previously diagnosed pathology. Full blood samples were collected preoperatively from synovium and cartilage samples intraoperatively. Real time PCR with spectrophotometric analysis was performed. Following genes taking part in ECM (extracellular matrix) remodeling were selected for analysis: MMP-1, MMP-2, MMP-8, MMP-9, MMP-13, MMP-14, ADAMTS-4 (Agg1) and ADAMTS-5 (Agg2) proteases, TIMP-1, and TIMP-2 – their inhibitors - and IL-1 and TNF-α cytokines. *Results*. Analysis revealed a strong and significant correlation between gene expression in synovial and systemic blood cells ($p < 0.05$ for all studied genes) with ADAMTS-4, ADAMTS-5, IL-1, TNF-α and TIMP-2 expression most positively correlated with an $R > 0.8$ for each. An analysis between chondrocytes and systemic blood gene expression shown no significant correlation for all genes. Bivariate correlation of International Cartilage Repair Society grading and genes expression revealed significant associations with synovial MMP-1, MMP-2, MMP-8, MMP-9, IL-1, TNF-α and TIMP-2. *Conclusion*. We suggest that the synovial tissue is the first responder for knee joint stress factors in correlation with the response of blood cells. The chondrocyte's genetic response must be further investigated to elucidate the genetic program of synovial joints, as an organ, during OA development and progression.

Keywords

articular cartilage, tissue, research methods, biomarkers, osteoarthritis, diagnosis, other, cytokines and growth, factors

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Introduction

Osteoarthritis (OA) is a chronic and slowly progressive disease that historically is described as a degenerative wear-and-tear disorder of the joint.[1,2] Pathologically, late stage of OA is characterized by synovial inflammation, articular cartilage destruction, and subchondral bone sclerosis.[1] Although OA has traditionally been viewed as a disease of the articular cartilage with accompanying subchondral bone involvement, it is now recognized as much more complex disease.

Modern imaging modalities and recent literature suggest that synovitis and resulting pro-inflammatory cascade of mediators play a crucial role in the pathogenesis of OA.[3]

Proinflammatory mediators present in synovial fluid stimulate the secretion of other cytokines, in addition to stimulating the synthesis of articular cartilage-damaging proteases like matrix metalloproteinases and aggrecanases. In addition to chondrocytes, recent literature has showed that local synoviocytes elicit pro-inflammatory mediators that progress the pathogenesis of OA.[3-5] Together with synoviocyte-derived factors, synovial inflammation is also attributed to cytokines attracting mononuclear cells—macrophages and lymphocytes. These factors include tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), chemokines, toll-like receptors (TLRs), and metalloproteinases (MMPs).[1,6] Also TNF-α was previously reported to upregulate production of MMPs, by chondrocytes, in rheumatoid arthritis.[7] Abovementioned cytokines stimulate production of MMPs, MMP-1 produced by synovial cells and MMP-13, MMP-2, MMP-3, and MMP-9 by chondrocytes.[8] In OA, principal matrix-degrading components are aggrecanases (ADAMTS-4 and 5) and collagenases (MMP 1, 8, and 13). These enzymes cooperatively function to degrade components of the extracellular matrix (ECM).[9-11] Recent literature suggests that matrix degradation in early OA stages may be due to MMP-13 and ADAMTS-5.[9] ADAMTS-5 breaks down aggrecan, the major proteoglycan of articular cartilage, that also protects collagen from degradation,[8,9,12] whereas MMP-13 has the highest activity toward collagen II and plays an important role in cartilage destruction.[13] When these proteolytic enzymes are released from activated chondrocytes, OA ensues. In a previous study on pro-inflammatory mediator's gene expression in knees with anterior cruciate ligament (ACL) rupture, we observed elevation in expression of IL-1 and TNF-α both in blood and in synovial cells, yet cartilage expression was not analyzed.[14] From the MMP family, ADAMTS-4 and ADAMTS-5 were found to be elevated in early OA stages.[15,16] However, a correlation between blood, synovial, and cartilage cells was not investigated. As the regulatory pathways between the joint tissue are yet to be established, we aimed to evaluate whether a similar catabolic and inflammatory gene pattern exists between the synovium, hyaline cartilage, and blood of patients with the cartilage lesions in the knee joint and whether any of them precedes. Our hypothesis entails that gene expression of blood and synovial MMPs may precede the expression of same genes in chondrocytes in early stages of OA.

Methods

Patients from the Department of Orthopedics and Pediatric Orthopedics at our home institution who underwent elective knee arthroscopy due to previously diagnosed pathology (i.e., ACL tear, meniscal tear, or cartilage lesion) during a 3-month period were enrolled into this study. Patients who were diagnosed with chronic diseases, including metabolic diseases; underwent hormonal and steroid therapy; underwent surgery of any kind; or were below 18 years were excluded from the study. All selected patients were informed of the study enrollment and were asked to sign the informed consent form that was approved by the Research Ethics Committee (Approval No. RNN/125/09/KE from May 12, 2009).

Following enrollment procedure to the study group, a peripheral venous blood sample was collected from each patient on admission to the hospital. The samples were collected into Monovette® EDTA KE test tubes and, after 20 minutes at room temperature, were frozen to –20°C.

For sample extraction, all patients were placed in the supine position on the surgical table, and the knee was prepared and draped in a sterile fashion after the anesthesia induction. Once the patient was positioned, the standard anterolateral portal was established by the senior author (A.G.) and diagnostic arthroscopy in the standard fashion was performed. After the first portal was obtained, the anteromedial portal was established to introduce the probe and other arthroscopic tools. Once all arthroscopic tools were in place, knee cartilage was evaluated and characterized according to International Cartilage Regeneration and Joint Preservation Society's (ICRS) Hyaline Cartilage Lesion Classification System (**Table 1**).[17] Samples were extracted from the intra-articular synovial membrane, surrounding the ACL, from its distal, tibial portion, or from the ACL lesion site if a tear was present, plus an articular cartilage punctate from an unloaded area in the intercondylar fossa of the femur. The cartilage sample was of a standardized size for the Autologous Chondrocyte Implantation (ACI) biopsy,[18] and cartilage sampling from a non-weightbearing areas of the knee has been done before.[19] The collected material was immediately placed in sterile and sealed 1.5 cm^3 tubes with RNAlater solution. Such prepared samples were cooled down to 4°C to be frozen to –20°C on the second day.

Total RNA was isolated, using TRI Reagent™ (Invitrogen®)[20] with in-solution DNase treatment using the

Table 1. Grades of Cartilage Lesions Evaluated and Characterized According to International Cartilage Regeneration and Joint Preservation Society's Hyaline Cartilage Lesion Classification System.

No	Age	Modified Grade
1	59	3D
2	56	3A
3	55	3D
4	59	3D
5	22	3A
6	56	2
7	18	1B
8	67	3B
9	39	3A
10	58	3C
11	68	3C
12	38	0
13	21	0
14	53	3C
15	62	3A
16	45	0
17	34	0
18	55	0
19	18	0
20	55	3B
21	18	3A
22	39	0
23	56	3B
24	43	3D
25	61	0
26	75	3C
27	49	3B
28	51	4A
29	30	0
30	18	0
31	34	3B
32	50	0
33	40	3B
34	61	3C
35	28	0
36	45	0
37	55	0
38	54	3B
39	27	3A
40	44	3B
41	24	0
42	33	0
43	26	0
44	30	0
45	40	0
46	63	3C
47	50	3D
48	67	3C
49	52	0
50	18	3A
51	55	3B
52	34	0
53	55	3B
54	61	3B
55	66	3D
56	28	0
57	25	3B
58	51	0

RNase-Free DNase set (Qiagen, Valencia, CA), followed by a clean-up using the spin columns from the PureLink Micro-to-Midi Total RNA purification system. The extracted RNA was analyzed by agarose gel electrophoresis and only cases with preserved 28S, 18S, and 5S ribosomal RNA bands indicating good RNA quality were used in the study. The isolated total RNA was immediately subjected to reverse transcription, supported by a SuperScript III RT kit with random hexamers (Invitrogen, Thermo Fisher Scientific corporation, Waltham, Massachusetts, U.S.). The qPCR assays had been preoptimized with regard to $MgCl_2$ and primer concentration, as well as annealing temperature. Amplification was performed for 10 minutes at 95°C to activate polymerase and 40 rounds for 15 seconds at 95°C and 1 minute at 60°C for amplification and signal analysis. The reactions were carried out 2 times, each in triplicate on an ABI Prism 7900 sequence detection system (Applied Biosystems, Foster City, CA) using Platinum SYBR Green qPCR SuperMix-UDG with ROX with 200 nM each forward and reverse primers and 2 μL cDNA in a final volume of 20 μL.

Controls without template cDNA were performed with each assay. The PCR products were identified based on their unique melting curve and confirmed by agarose gel electrophoresis. Validation of PCR efficiency was performed with a standard curve. Standard curves were prepared for each gene by serial dilution.

For the study, the following genes participating in ECM remodeling were selected: MMP-1, MMP-2, MMP-8, MMP-9, MMP-13, MMP-14, ADAMTS-4 (Agg1) and ADAMTS-5 (Agg2) proteases, tissue inhibitors of MMPs 1 and 2 (TIMP-1 and TIMP-2), and IL-1 and TNF-α cytokines. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an active and endogenous reference to correct for differences in the amount of total RNA added to reaction and to compensate for different levels of inhibition during reverse transcription of RNA, and during real-time PCR, the data were automatically calculated with the data analysis module. The results were analyzed according to the $2^{-\Delta\Delta^{Ct}}$ method.[21]

The Statistical software package of Statsoft Inc. (License No. SN AXAP911E504325AR-K) was used. The accepted level of statistical significance was $P < 0.05$. The normality of distribution was verified with the Shapiro-Wilk W test. In consideration of a substantial deviation from normality, the Mann-Whitney U test was applied. Correlations were evaluated by Pearson's correlation test.

Results

Patient Demographics

This study comprised 58 patients (34 females and 24 males) with a mean age of 44.7 (range = 18-75) years. The average

time from the injury to surgery was 9.67 (range = 1-36) months. Procedures were performed on 31 right and 27 left knees. Twenty-eight patients recalled knee trauma, averaging 17 months before surgery. On arthroscopic evaluation, 35 patients were diagnosed with cartilage lesion(s), whereas the other 23 cases were described as normal cartilage. In addition, 34 patients were identified with a medial meniscus lesion, 4 had lateral menisci lesions, and another 8 had ACL tears.

Tissue-Specific Genetic Comparisons

Analysis of all 12 factors, among the 58 subjects, revealed a strong and significant correlation between gene expression in synovial and systemic blood cells ($P < 0.05$ for all studied genes). More so, ADAMTS-4, ADAMTS-5, IL-1, TNF-α, and TIMP-2 expressions were most positively correlated with an $R > 0.8$ for each (**Table 2**). However, analysis between chondrocytes and systemic blood gene expression yielded no significant correlation for all genes. Similarly, when comparing chondrocytes and synovial cells, only MMP-1 revealed significant correlation between them. Overall, there was no single gene in which expression correlated between all tested tissues ($P > 0.05$).

Lesioned versus Nonlesioned Cartilage Gene Comparison

Bivariate correlation of ICRS grading and gene expression revealed significant associations with synovial MMP-1, MMP-2, MMP-8, MMP-9, IL-1, TNF-α, and TIMP-2. In addition, the extent of cartilage damage was correlated with blood cell MMP-2, ADAMTS-4, and IL-1 expression. However, only chondrocyte MMP-1 levels were correlated with ICRS grade (**Table 3**). Like the chondral defect data, most genes did not significantly correlate with meniscal lesions. Among the few exceptions were synovial ADAMTS-4, IL-1, and TIMP2; blood cell MMP-9, ADAMTS-4, ADAMTS-5, IL-1, and TIMP-2; and chondrocyte MMP-8 and ADAMTS-5 ($P < 0.05$). Unlike the chondral defect data, however, all mentioned expressions were identified in patients with medial meniscal lesions. Additional analyses were done for cases with ACL lesions and other cofounders; however, case numbers were too small to achieve proper statistics.

Discussion

Knee OA is a degenerative disease that is associated with various pathways promoting a cycle of pro-inflammatory and catabolic responses that degrade articular cartilage, subchondral bone, and synovium.[1,3] The precise etiology of OA is not fully understood, although hypotheses indicate that age-related changes in ECM and inflammatory molecules may directly activate chondrocytes.[10,22] During OA, the principal matrix-degrading components are aggrecanases and collagenases, members of the matrix MMP family.[9-11,23] ADAMTS-5 is involved in breakdown of aggrecan, which is vital cartilage proteoglycan that protects the collagen from degradation.[12] Therefore, the destruction of aggrecan begins at earlier stages of OA and precedes the destruction of collagen. As OA progresses, the activity of MMP-1 and MMP-13 increases, thereby intensifying collagen breakdown.[9,22,23] However, the specific molecular targets differ for each enzyme. MMP-1 is implicated in degrading collagen types I, II, and III, whereas MMP-3 interacts with collagen types II, III, IV, IX, and X; fibronectin; and various proteoglycans.[11] In addition, MMP-3 has been shown to upregulate the expression of other MMPs, escalating matrix destruction.[11] Of the MMPs, MMP-13 has the foremost ability to cleave collagen type II, the most prevalent collagen in articular cartilage, and therefore is commonly utilized as a biomarker for studying OA.[11] Based on that data, we chose to evaluate MMP-1, MMP-2, MMP-8, MMP-9, MMP-13, MMP-14, ADAMTS-4 (Agg1) and ADAMTS-5 (Agg2), TIMP-1, and TIMP-2 (MMP and ADAMT inhibitors) expression. Our study evidences the assertion that synovial proteases are the first indicator of intra-articular pathology. In this analysis, we found a significant correlation between gene expression in synovial and systemic blood cells, of which ADAMTS-4, ADAMTS-5, IL-1, TNF-α, and TIMP-2 expression were most positively correlated. Moreover, we found TNF-α and IL-1 expressions to be significantly higher in patients diagnosed with cartilage lesions. These changes are also notable within blood cells, suggesting an earlier systemic etiology for OA than previously proposed.

In response to the cellular stress and ECM damage within osteoarthritic joints, immune system activation ensues via damage-associated molecular patterns (DAMPs).[24] This stimulates pattern recognition receptors known as TLRs in response to tissue damage, which is an impetus for NF-κB signaling and subsequent pro-inflammatory cytokine production.[9,24,25] It should be noted that the role of TLR activation in synovitis and OA is not fully understood and requires additional investigation. The processes driving inflammation in OA are complex and there are numerous pro-inflammatory cytokines, many of which are measurable in the synovial fluid and joint tissues. As such, we sought to measure the gene expression of the most prominent pro-inflammatory molecules, IL-1β and TNF-α, within several tissues. We found that IL-1 gene expression was elevated in synovium and blood cells, and that this expression was significantly correlated with each other. IL-1β is documented to promote a catabolic state by suppressing collagen and aggrecan synthesis and promoting ADAMTS and MMP activity.[6,24,25] IL-1β causes

Table 2. Analysis Between Gene Expression of All 13 Factors in Synovial and Systemic Blood Cells ($P < 0.05$ for All Studied Genes).

	M	SD	R (X, Y)	r^2	*P*
MMP-1					
Synovium	0.218	0.055	**0.837**	0.700	<0.001
Blood	0.167	0.056			
MMP-2					
Synovium	0.202	0.052	**0.875**	0.766	<0.001
Blood	0.164	0.053			
MMP-8					
Synovium	0.019	0.007	0.295	0.087	0.024
Blood	0.012	0.005			
MMP-9					
Synovium	0.026	0.013	0.256	0.065	0.053
Blood	0.017	0.006			
MMP-9					
Synovium	0.024	0.007	**0.517**	0.267	<0.001
Blood	0.017	0.006			
MMP-13					
Synovium	0.082	0.022	**0.549**	0.301	<0.001
Blood	0.057	0.019			
MMP-14					
Synovium	0.023	0.007	0.077	0.006	0.565
Blood	0.018	0.006			
ADAMTS-4					
Synovium	0.086	0.021	**0.875**	0.766	<0.001
Blood	0.060	0.021			
ADAMTS-5					
Synovium	0.094	0.021	**0.834**	0.695	<0.001
Blood	0.066	0.020			
IL-1					
Synovium	0.127	0.030	**0.860**	0.739	<0.001
Blood	0.091	0.027			
TNF-α					
Synovium	0.098	0.021	**0.810**	0.656	<0.001
Blood	0.071	0.018			
TIMP-1					
Synovium	0.105	0.021	**0.528**	0.279	<0.001
Blood	0.084	0.021			
TIMP-2					
Synovium	0.085	0.019	**0.845**	0.715	<0.001
Blood	0.062	0.018			

MMP = metalloproteinase; IL-1 = interleukin-1; TIMP = tissue inhibitors of metalloproteinase.
$P < 0.001$ marked in bold.

chondrocytes to release more ADAMTS-4 and ADAMTS-5. Also, IL-1β stimulated the overexpression of ADAMTS-5 via the AP-1/microRNA-30a (miR-30a) axis.[26] In addition, chondrocytes exposed to IL-1β produce reactive oxygen species (ROS) that encourage apoptosis and articular cartilage destruction.[6,25] Numerous cell types are known to synthesize IL-1β, including chondrocytes, synoviocytes, and macrophages, which in advanced OA leads to a recurring cycle of inflammation and ECM destruction.[9,25,27] Surprisingly, our study indicates that IL-1 acts differently in chondrocytes than the other tested tissues.

In addition to IL-1β, IL-α is a prominent inflammatory mediator that is evident in arthritic synovial membranes.[24,25,28] TNF-α has the unique ability to bind 2 membrane receptor superfamily isotypes, namely, TNF-R1 and TNF-R2, both of which participate in a variety of signal

Table 3. Bivariate Correlation of ICRS Grading and Genes Expression.

Paired Variables	R Pearson	*P*
ICRS grade & Synovium—MMP-1	−0.266	0.044
ICRS grade & Synovium—MMP-2	−0.306	0.019
ICRS grade & Synovium—MMP-8	−0.390	0.003
ICRS grade & Synovium—MMP-9	−0.400	0.002
ICRS grade & Synovium—MMP-13	−0.014	0.918
ICRS grade & Synovium—MMP-14	−0.105	0.433
ICRS grade & Synovium—ADAMTS-4	−0.147	0.270
ICRS grade & Synovium—ADAMTS-5	−0.048	0.718
ICRS grade & Synovium—IL-1	−0.489	<0.001
ICRS grade & Synovium—TNF-α	−0.342	0.009
ICRS grade & Synovium—TIMP-1	0.015	0.909
ICRS grade & Synovium—TIMP-2	0.337	0.010
ICRS grade & Blood—MMP-1	−0.166	0.213
ICRS grade & Blood—MMP-2	−0.355	0.006
ICRS grade & Blood—MMP-8	−0.219	0.098
ICRS grade & Blood—MMP-9	−0.233	0.078
ICRS grade & Blood—MMP-13	−0.253	0.056
ICRS grade & Blood—MMP-14	−0.069	0.607
ICRS grade & Blood—ADAMTS-4	−0.300	0.022
ICRS grade & Blood—ADAMTS-5	−0.151	0.258
ICRS grade & Blood—IL-1	−0.413	0.001
ICRS grade & Blood—TNF-α	−0.223	0.093
ICRS grade & Blood—TIMP-1	−0.122	0.362
ICRS grade & Blood—TIMP-2	0.128	0.338
ICRS grade & chondrocyte—MMP-1	0.006	0.966
ICRS grade & chondrocyte—MMP-2	0.045	0.739
ICRS grade & chondrocyte—MMP-8	0.037	0.782
ICRS grade & chondrocyte—MMP-9	−0.066	0.619
ICRS grade & chondrocyte—MMP-13	0.058	0.666
ICRS grade & chondrocyte—MMP-14	−0.343	0.009
ICRS grade & chondrocyte—ADAMTS-4	0.033	0.807
ICRS grade & chondrocyte—ADAMTS-5	0.112	0.404
ICRS grade & chondrocyte—IL-1	−0.088	0.512
ICRS grade & chondrocyte—TNF-α	−0.152	0.254
ICRS grade & chondrocyte—TIMP-1	−0.193	0.147
ICRS grade & chondrocyte—TIMP-2	0.107	0.425

ICRS = International Cartilage Regeneration and Joint Preservation Society; MMP = metalloproteinase; IL-1 = interleukin-1; TIMP = tissue inhibitors of metalloproteinase.

pathways, including NF-κB, that stimulate an inflammatory response in the synovium.[25] Interestingly, synovial cell production of MMP-1, MMP-3, MMP-9, and MMP-13 was found to be NF-κB-dependent.[29] In addition to synovial inflammation, TNF-α inhibits chondrocyte proteoglycan and type II collagen synthesis while also inducing chondrocyte apoptosis, thereby negatively influencing articular cartilage integrity.[25,30] TNF-α together with IL-1 stimulate synovial fibroblasts to release MMP-1, 3, and 13, but also simulate the expression of intracellular MMP-8.[31] In OA, especially advanced stages, MMP-9 was found to be produced by synovial fibroblasts.[32] Overall, the effects of TNF-α are synergistic to IL-1β, as both initiate similar inflammatory pathways in OA.[24] In the present study, we found that TNF-α and IL-1 expressions were significantly higher in patients diagnosed with cartilage lesions. More so, there was a significant correlation with IL-1 and TNF-α expressions between synovium and blood cells.

Aggrecan breakdown represents an early stage of OA, preceding the collagen destruction, and thus aggrecanases have been identified as possible early OA biomarker.[33,34] ADAMTS are zinc metalloendopeptidases,[35] from which ADAMTS-4 and 5 are responsible for aggrecan breakdown. Of these two, ADAMTS-5 was identified to have a higher aggrecanolytic activity than ADAMTS-4 as shown in an *in vitro* model.[32] Interestingly, in an ADAMTS-5 knockout

mice (but not ADAMTS-4 knockout), the effects of surgically induced instability were reduced compared with healthy controls.[36] It is therefore that recently ADAMTS-5 has even been identified as possible serological marker of OA activity and recognized as a potential target for disease-modifying drugs.[15,16] On the contrary, ADAMTS-5 expression was found to be expressed constitutively in chondrocytes and synovial fibroblasts while ADAMTS-4 expression was triggered by inflammatory cytokines. Also, increased serum level of ADAMTS-4 was reported in patients with early OA and significantly higher than in early than intermediate and severe OA.[37] Our analysis has shown a significant correlation between gene expression in synovial and systemic blood cells, of which ADAMTS-4, ADAMTS-5, IL-1, TNF-α, and TIMP-2 expression were most positively correlated. And what is interesting is that the extent of cartilage injury was correlated with blood cell ADAMTS-4 but not ADAMTS-5. These findings are consistent with literature and may indicate that synovial and blood gene expression precede the expression of these genes in cartilage.[33,34]

Overall, the various chemokine families involved in the inflammatory response illustrate the complicated nature of OA pathogenesis. In conclusion, synovial lining hyperplasia, fibrosis, and infiltration of mononuclear cells are hallmarks of synovial inflammation in OA, which contribute to the progression of cartilage loss as well as symptoms of the disease, including joint pain, swelling, and stiffness.[3] The synovial membrane is reported to undergo lining hyperplasia, sublining fibrosis, and stromal vascularization. Several studies observed synovial infiltration with T-cell lymphocytes and macrophages as the most predominant response.[38-40] Moreover, higher cellular immunity was observed in OA patients, whose peripheral blood lymphocytes were stimulated by human cartilage protein.[41] Furthermore, T cells of OA patients present strong response to autologous chondrocytes.[42] These studies may suggest that T-cell lymphocytes infiltration, and secretion of the IL-1β and TNF-α can be a linkage between the synovial proliferation and systemic inflammatory reaction in the OA.[43] Moreover, the molecular crosstalk of pro-inflammatory mediators between cartilage and synovium could influence the impact of underlying OA development and pathogenesis in which cartilage damage in turn intensifies synovial inflammation and vice versa, creating a destructive cycle.[44] As we observed, the first response to the knee joint stress factors is elevated gene expression in the synovial membrane cells, followed by elevated expressions in the systemic blood. However, the chondrocyte reaction seems delayed when compared with the synovial response.

The principal finding of this study is that chondrocyte ECM remodeling and inflammatory cytokine gene expression do not correlate with blood and synovial expression in patients with OA. However, synovial and systemic blood gene expression are strongly correlated with ICRS grading. This may indicate that synovial membrane and systemic blood cells are the first responders in the knee joint tissues' regulatory processes.

Limitations of This Study

This study is not without the limitations. However, we managed to collect samples from as much as 58 patients, and we encountered unfortunate heterogeneity in diagnosed intra-articular lesions. As the subgroups appeared not to be large enough for separate analysis, we decided to exclude that data from statistical analysis. We are also aware that some other testing, like, for example, synovium histopathology, could possibly improve addressing the study hypotheses. Unfortunately, with our facility's limited budget, we decided not to convey additional analysis and focus on gene expression only. As we studied gene expression only, we cannot report the true levels of the intra-articular enzymes and their activities. But knowing that its secretion is determined by the genes we tested, we can assume that some correlations could be observed. However, as our aim was to compare catabolic and inflammatory gene expression between tissues, studying intra-articular fluid may not have provided insight on the enzyme origins. Additional studies investigating both gene and protein expression among various tissues and at various timepoints could provide greater understanding of the pathogenesis and progression of OA.

Another potential variable affecting results was chondral biopsy location. In the current study, we extracted chondrocytes from an unaffected, non-weightbearing surface of the femur, rather than the lesioned cartilage. Although this made tissue comparisons more consistent, Snelling and colleagues demonstrated that gene expression differs drastically between arthritic and normal cartilage within the same joint. They noted an upregulation in cell signaling, ECM remodeling, and inflammatory response genes, including TNF-receptor, ADAMTS-like2, CXCL14, and IL11.[45] However, we utilized this methodology for several key reasons: (1) to compare same cartilage each time for a more standardized approach; (2) our patients were rather young (average age of 43 years), so arthritic tissue was uncommon; and (3) OA was not the primary diagnosis and indication for surgery.

Conclusion

This study suggests that the synovial tissue is a first responder for knee joint stress factors, and its reaction correlates with the response of blood cells. The chondrocyte's genetic response should be further investigated to elucidate

the genetic program of synovial joints, as an organ, during OA development and progression.

Acknowledgment and Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

Research Ethics Committee (Approval No. RNN/125/09/KE from May 12, 2009).

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