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Human Genome-Wide Expression Analysis Reorients the Study of Inflammatory Mediators and Biomechanics in Osteoarthritis

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Abstract

A major objective of this article is to examine the research implications of recently available genome-wide expression profiles of cartilage from human osteoarthritis (OA) joints. We propose that when viewed in the light of extensive earlier work this novel data provides a unique opportunity to reorient the design of experimental systems toward clinical relevance. Specifically, in the area of cartilage explant biology this will require a fresh evaluation of existing paradigms, so as to optimize the choices of tissue source, cytokine/growth factor/nutrient addition, and biomechanical environment for discovery. Within this context, we firstly discuss the literature on the nature and role of potential catabolic mediators in OA pathology, including data from human OA cartilage, animal models of OA and ex vivo studies. Secondly, due to the number and breadth of studies on IL-1β in this area, a major focus of the article is a critical analysis of the design and interpretation of cartilage studies where IL-1β has been used as a model cytokine. Thirdly, the article provides a data-driven perspective (including genome-wide analysis of clinical samples, studies on mutant mice, and clinical trials), which concludes that IL-1β should be replaced by soluble mediators such as IL-17 or TGF-β1, which are much more likely to mimic the disease in OA model systems. We also discuss the evidence that changes in early OA can be attributed to the activity of such soluble mediators, whereas late-stage disease results more from a chronic biomechanical effect on the matrix and cells of the remaining cartilage and on other local mediator-secreting cells. Lastly, an updated protocol for in vitro studies with cartilage explants

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Contributions

All authors made substantial contributions to (1) the conception and design of this review paper, (2) drafting and revising of the article, and (3) final approval of the version for submission. JDS (jsandy44@gmail.com) takes responsibility for the integrity of the work as a whole, from inception to finished article.

Competing Interest Statement

The authors have no conflicts of interest, perceived or actual, to declare.

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and chondrocytes (including the use of specific gene expression arrays) is provided to motivate more disease-relevant studies on the interplay of cytokines/growth factors and biomechanics on cellular behavior.

Keywords

osteoarthritis; cytokines; growth factors; inflammation; biomechanics; bioinformatics

Introduction

Current research into OA is driven by at least three over-arching paradigms. Firstly, there is consensus that OA is a disease of the whole joint organ, which predicts a pathogenic role for all intra-synovial tissues including articular cartilage, synovial membrane, meniscal fibrocartilage, intra-articular ligaments, peri-articular tissues, and sub-chondral bone. Thus, the biological and biomechanical interplay between these tissues that ultimately leads to joint dysfunction remains a topic of high interest. Next, and closely related to the "whole joint" concept, is the notion that OA is not a single disease but a spectrum of pathologies that differ in the nature of the initiating event(s) and the subsequent natural history. Accordingly, OA subtypes can be characterized by the relative impact of factors such as genetic predisposition[1], joint trauma[2], aberrant biomechanics[3], and aging[4]. A third paradigm states that essentially all OA subtypes exhibit some degree of inflammation and that many of the pathogenic cellular changes are part of an innate pro-inflammatory response to stressors such as altered biomechanics, pH, radicals, and matrikines or DAMPs[5, 6]. Recognizing the importance of these paradigms, we discuss here how the ready availability of methods and instrumentation for analysis of the genome, epigenome, transcriptome, and proteome of OA has markedly increased the opportunities for seminal discovery of new pathogenic pathways.

Regardless of the emergence of these broad themes, much of the basic and translational research in OA continues to focus on articular cartilage and chondrocytes, as cell-mediated loss of this tissue from the bone end, is an event common to all subtypes of the disease, and the degree of cartilage damage is often viewed as the readout that can best measure the effectiveness of interventions. Further, biomechanically induced disturbance in the homeostatic control of articular cartilage tissue contributes substantially to OA pathology, and therefore the delineation of mechanistic links between mechanical forces and chondrocyte responses continues to occupy a central position in the area. In this review, we discuss how new "omics" data can be used to redesign experiments examining the interplay between biomechanical stimuli (compressive, shear, and tensile forces) and pro-inflammatory pathway responses that promote cartilage degeneration. As an example, we provide a critical evaluation of the published data that have focused on IL-1β as the key pro-inflammatory cytokine both in vitro and in vivo, and in both cell biological and biomechanical studies of OA.

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History of IL-1β Use in OA research

The use of IL-1β as a tool to study tissue matrix remodeling in OA was an almost inevitable outcome of the manner of its initial description. In 1977, the laboratory of Dame Honor Fell[7] described a protein that was synthesized by explanted porcine synovial tissue and induced the destruction of live cartilage such that (quote) "the cartilage became reduced to a mass of fibroblast-like chondrocytes without matrix." This factor was given the name "catabolin[8]" and subsequently shown by protein chemistry to be IL-1β[9]. Based largely on the impact of this seminal work, the effects of IL-1β have been studied in great detail by researchers interested in describing the cell responses in cartilage matrix turnover and synovial inflammation during OA pathogenesis. Indeed, data from investigations with IL-1β are a major component of the knowledge base that supports many of the present-day basic and pre-clinical studies on OA. Further, the consensus that OA is a chronic non-healing wound of the "joint organ," which has an innate inflammatory and fibrotic component[10-12], has further motivated studies on the role of cytokines in OA pathogenesis[6, 11]. As a result, investigators using IL-1β or examining its endogenous gene expression in OA research often introduce the studies with the premise that IL-1β is the "major cytokine" promoting joint tissue pathology in OA. However, the data in recent publications from disparate areas has persuasively argued against the notion of a central role for this cytokine.

IL-1β is present in essentially normal concentrations in joint fluids from early and late OA

A major role for IL-1β in mediating OA pathogenesis should be supported by an elevated level of the cytokine in the synovial fluid of OA patients. However, this is not the case, as multiple studies[13-15] have reported that the level in joint fluid aspirates collected early after injury or in advanced OA is essentially identical to that measured in normal fluids (~10 pg/mL). Moreover, the concentration of IL-1 receptor antagonist (IL-1Ra, ~10 ng/mL), which competes with IL-1β by binding to its receptor, is present in concentrations that far exceed the concentration of IL-1β itself. In one study of 42 OA patients[16], the average IL-1Ra/IL-1β (w/w) ratio in the synovial fluid was about 1800:1, making it highly unlikely that the IL-1β in the fluid ever binds to IL-1R1 on cells within the joint. It is notable that, in similar samples, IL-6 and IL-8 are found in 20-fold increased amounts (up to 20 ng/mL for IL-6)[17, 18].

Clinical trials targeted at blocking IL-1β signaling in OA patients have shown no marked efficacy

Treatment of OA patients with protein biologics to block IL-1 stimulation has given inconclusive results[19]. Monoclonal antibodies to IL-1R administered subcutaneously[20] were well tolerated in symptomatic patients; however, no robust clinical benefit was observed. In contrast, 150 mg of IL-1Ra given by intra-articular injection[21] showed an approximate 20% improvement in pain and global OA scores for 3 months. In a different study design[22], the same intra-articular dose of IL-1Ra was given at 2 weeks after ACL injury, and, in this case, a minor "clinically meaningful" benefit was seen at 2 weeks after the injection. However, there was no significant change in the level of synovial fluid IL-1β, and longer-term effects have not been reported.

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Studies with mutant mice do not support a direct role for IL-1β in murine OA

The elimination of genes that control IL-1β-stimulated pathways (such as IL-1β itself, caspase-1, or iNOS) actually results in the development of spontaneous OA[23]. In addition, blockade of NLRP3 (an inflammasome component) or IL-1RI[24] does not protect against loss of matrix components in murine cartilage explants, and ablation of MyD88[25] does not prevent development of experimental OA. Notably, several studies in a murine model of post-traumatic OA, induced by intra-articular fracture, have implicated IL-1β as a major inflammatory mediator of cartilage degeneration[26, 27]. However, to what extent the reported increases in serum and synovial fluid levels of IL-1β in the acute phase[26] of the model is due to an incipient OA or a response to bone fracture is unknown. Furthermore, it is difficult to interpret the reported protection against OA in this model, using the MRL/MpJ strain[27], which responds with a decrease in IL-1β response, since this mouse has a complex multigenic immune-modulated phenotype[28-30] and protection may be the result of modulation in other cytokine and growth factor responses.

Supra-physiologic concentrations of IL-1β are required to induce OA-like changes in cartilage explants and chondrocyte cultures

Although many in vitro studies (for example[31-34]) have demonstrated that the presence of IL-1β can induce degradation of the proteoglycan and collagen components of extracellular matrix (ECM), they were all performed using supra-physiological levels of IL-1β, ranging anywhere from 1 ng/mL to as much as 1000 ng/mL, compared to <10 pg/mL in body fluids. There is also ongoing research on the effect of IL-1β on micro-RNAs (miRs), including miR-145 and Smad pathway signaling[35], miR-140 and OA-like matrix changes[36, 37], as well as miR-146 and inflammation[38]; however, these studies also employ IL-1β at 5-10 ng/mL.

The presence of IL-1β-inducible factors in OA model systems does not implicate IL-1β itself

This widely-studied and complex topic is covered in a recent detailed review[39] on receptors and signaling pathways of IL-1 family members. Briefly, the major downstream effects in IL-1β stimulated cells are mediated by activation of AP-1 and NF-κB families of DNA-binding proteins and their interaction with TRE and κB sites in the promoter regions of target genes. In the regulation of transcription factor binding to κB sites, IL-1β is part of a group of over 150 different ligands that can signal through this pathway[40]. These include, for example, TLR ligands, LPS, AGE, cytokines such as IL-2, -12, -15, -17, and -18, LIF, TNFα/β, growth factors such as M-CSF, NGF and PDGF, stress molecules such as HSP60, hyaluronan oligosaccharides, anti-FAS, and Apo1. Binding at κB sites controls transcription of an equally large number of diverse target genes including growth factors (GM-CSF, IGFBP-2, M-CSF, PDGFB, VEGFc), metalloproteinase (ADAMTS5[41], MMP1, -9, and -13) and additional inflammatory mediators (IFN-γ, ICAM-1, tenascin C, Vcam1, uPA, COX2, iNOS, IL-1α/β, IL-1ra, IL-2, -6, -8, -11, -12, -15, TNFα/β). Relevant to this article, both IL-1R1 and IL-1R3 have a cytoplasmic subdomain (Toll/IL-1R) that has high structural and functional homology with the cytoplasmic domain of Toll-like receptors (TLRs), explaining why the cellular response to TLR ligands and IL-1 family ligands are often the same (for detailed reviews[42, 43]).

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Genome-wide expression data suggests that activation of matrix genes, but not IL-1β-responsive genes, is centrally important to cartilage pathology in human OA

The idea that IL-1β is not involved in OA has most recently been supported by ‘large data’ gene expression array studies. There are now at least eight papers[44-51] that have presented high-density coverage or genome-wide analysis of differentially expressed genes in cartilage from advanced human OA. For example, in one transcriptomic study[48], data was obtained for hip and knee cartilages taken at arthroplasty and compared to hip cartilage taken during surgery for femoral neck fracture. The data were examined for differential expression at the single gene level and then further analyzed for pathway associations. Firstly, in comparison with non-affected cartilage, only a relatively small number of genes were changed in the same direction (increased or decreased expression) for both hip and knee OA cartilage and therefore can be tentatively considered as disease-related, independent of the joint of origin. Increased mRNA transcript abundance in both types of OA cartilages were seen for collagens (COL2A1, COL5A1, COL5A2, COL8A2, COL11A1, COL13A1), PCOLCE (involved in collagen biosynthesis), glycosyltransferases involved in glycosaminoglycan synthesis (EXTL2, GALNT1, CHST6), cell-matrix ligands/receptors (NCAM1, ITGA11, SPARC), serine proteinases (HTRA1, SERBP1), and cell proliferation mediators (PDGFC, PDGFRL). Increased expression of cytokine genes was seen for IL-8, IL-12 and IL-17. Genes down-regulated in OA cartilage were stress response mediators (SOD2, MT1M, ELL2, HIG2, GADD45A, GYG1) and intracellular signaling molecules (HMGB2, AKT3, JAK2, EFNA1). In a separate study[50], gene expression was compared in cartilage from normal autopsy, early OA autopsy, and advanced OA arthroplasty. This study similarly revealed activation of ten collagen genes (excluding COL10A1) in cartilage from advanced stage OA. The authors discussed the possibility that the results may have been confounded by mRNA instability before tissue collection – a technical issue that remains a matter for further analysis. In fact, since all of the expression studies[44-49, 51] have been done on cartilage from joints with advanced OA, it is unknown whether similar or markedly different changes will be seen when cartilage from “early” OA (such as after ACL rupture) can be obtained for analysis.

Notably, the enhanced expression of multiple collagen genes in both early and late human OA cartilages is not seen in laser-dissected cartilage from murine surgically-induced OA[52], whether analyzed at early time points (1 and 2 weeks) or at the time of surface fibrillation (6 weeks). However, in the same model, enhanced expression of multiple collagen genes (Col3a1, Col4a2, Col5a1, Col12a1, Col14a1, Col16a1, Col22a1) and collagen processing enzymes (Pcolce and Lox1) was seen at 2 and 4 weeks in tissues pooled from the whole joint organ[53]. With regard to the topic of this review, most of the disease-related genes identified in human and murine OA are not typically altered by IL-1β, or they are altered in the opposite direction to the changes seen in diseased human cartilages. For example, while collagen and glycosaminoglycan synthesis genes are upregulated in OA, chondrogenesis is markedly inhibited by IL-1β in human MSCs[54]. This dichotomy has also been confirmed by proteomic analyses where samples were from human normal and OA cartilage explants either untreated[55] or treated with IL-1β (10 ng/mL)[56]. The major functional group identified as OA-related in untreated explants was ECM turnover proteins such as collagens; however,

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with IL-1β treatment, the major proteins identified as "OA-related" did not include the collagens.

Pathway analysis implicates IL-17, HMGB1 and COL5A1, but not IL-1β, in OA pathology

Another approach to identifying OA-related cellular responses is to describe the normal vs OA differential in terms of gene pathways. For example, using Ingenuity Canonical Pathway Analysis[48], 60 pathways emerged from such a search, but none were directly related to either "incoming" or "outgoing" IL-1 β signaling events. However, over half of these pathways appeared to be related to OA development and remarkably, ten were directly linked to IL-17 signaling. Other notable genes (with the number of pathways indicated here in brackets) were related to IL-15 [3], ILK [2], Wnt/β-catenin [2], and pluripotency [2], with a single pathway associated with the following: JAK, HMGB1, IL-6, IL-8, IL-12, OSM, p53, nitric oxide, O-glycan biosynthesis, AKT, p38/MAPK, NF-κB, caveolae, CCR5, hypoxia and insulin receptor.

Indeed, if the individual genes suggested from the differential between normal hip and both hip and knee OA are compared to the pathway analysis of normal hip vs OA hip, the combined data identifies IL-17, IL-15, IL-12, IL-8, HMGB1/2, COL5A1, HIG2, and GYG1 as some of the genes most highly "relevant" to OA pathology. Furthermore, if the analysis is confined to genes that have previously been implicated in OA, the authors[48] identified up-regulation of ADAMTS1, ADAMTS5, ADAMTS9, MMP1, MMP3, MMP23, SOD2, COL2A1, COL5A2, COL9A1, and COL11A1 and down-regulation of CCL20, ATF3, and GADD45a/b as OA-relevant. It should also be noted that some members of this particular list of "most-relevant" genes have been chosen primarily on the basis of the human OA transcriptome in end-stage hip disease[48]. Nonetheless, a central role for IL-17 in experimental arthritis has been a common theme in many studies[57] and is further supported by the fact that it inhibits (albeit at supra-physiologic concentrations) chondrogenic differentiation of human MSCs[58], synergizes with TL1A in the expression of Adamts5 by macrophage-like cells[59] and induces murine joint destruction after intra-articular injection[60].

Gene pathway analyses clearly implicate biomechanical effects in the development of OA

Biomechanical treatments, such as a single injurious compression of bovine cartilage explants (maximum peak contact stress of ~20-30 MPa), resulted in decreased expression of adhesion molecules, matrix proteases, and growth factors but an increase in metabolic-pathway enzymes, chemokine and cytokine receptors, signal transduction markers, and transport proteins[61, 62]. Gene pathway analysis of human OA cartilages has uncovered many examples of genes involved in the same pathways [49]. Another example is the modulation of the HMGB1 pathway seen in OA cartilage[48], which is also seen after mechanical injury to the superficial zone cartilage (through blunt impact or scratching in vitro[63]) that generates soluble HMGB1 resulting in activated migration of the resident progenitor population and induced expression of chondrogenic markers. Likewise, the many links to MAPKs in the protein interaction network for human OA hip cartilage[48] may be connected to the finding that MAPKs become transiently phosphorylated during a number of cyclic and static compression protocols imposed on cartilage explants[64, 65]. Of particular interest in the current context is the fact that late-stage human OA is invariably accompanied by an over-

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expression of multiple collagen genes in the cartilage, which is suggestive of a prolonged joint-wide activation of pro-fibrogenic growth factor signaling, including CTGF[66, 67] and TGF-β1[68]. Notably, treatment of normal cartilage with physiologic levels of unconfined dynamic compression for 30 min[69] also induced similar pro-fibrotic/anti-chondrogenic responses. Indeed, whether these pro-fibrotic factors are augmented by other mediators produced by mechanically challenged tissues in the OA joint, such as IL-17[48], should represent an area for further research.

Practical uses of genome-wide expression data: Application to in vitro cartilage and chondrocyte culture models for the discovery of new pathways that link biomechanical and biological stimuli during OA pathogenesis

Cartilage explant and 3D chondrocyte culture systems remain in wide use as experimental models for examining the interplay between biomechanical forces imposed on the specimen in vitro and corresponding cellular responses leading to OA pathogenesis pathways. We discuss here some practical uses of human OA-related expression data[44-51] and indicate how they might motivate what appear to be more clinically relevant studies in this area. The basic framework is presented in three sections that address the most critical issues in terms of study design, and should apply equally to experiments with biomechanical and/or biological perturbation.

(1) As a tissue source, full-depth (~1-2mm) human articular cartilage disks (3mm punch) from the tibial plateau or femoral condyle of normal knees (50-80 years) and the equivalent from full-depth, surface-intact articular cartilage from OA knees (50-80 years) should be used if possible. Human articular cartilage is preferred for consistency with the published OA-related expression analyses, but full-depth mature (>18 months) bovine or porcine articular cartilages can also be used, as they are readily available to most investigators.

(2) We suggest IL-17 should be included in any OA-inductive culture medium. The use of this cytokine (or pathway-linked cytokines such as IL-22[70]) is proposed on the bases of (1) the OA tissue gene expression and pathway analysis reviewed above, (2) its pro-arthritic activity in murine OA models[57, 60], (3) its capacity to inhibit chondrogenesis of human MSCs through suppression of PKA activity and inhibition of SOX9 phosphorylation[58], and (4) its capacity to induce ADAMTS1, -4 and, -5, synergistically with tumor necrosis factor-like protein 1A, in human macrophage cultures[59]. Furthermore, IL-17 signals via DNA binding of CEBPA components, which is important in vivo since CEBPA-positive M2 macrophages are abundant in chronic inflammation and fibrosis of the synovium[71]. In addition, increased expression of IL-17 is seen in synovial tissues in inflammatory phases of OA[72], and its binding to epithelial cells induces expression of COL5A1[73] via a TGF-β1/ALK5 signaling mechanism. Its relationship to TGF-β1-driven overexpression of collagen in scleroderma and fibrosis[74-77] is also relevant, since OA progression has been linked to both joint

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tissue fibrosis[12, 78, 79] and subchondral bone marrow fibrosis[80]. A pro-fibrotic effect of IL-17 is further underlined by the finding that mesenchymal-specific deletion of Cebpa attenuates murine pulmonary fibrosis[77]. As a separate issue, since the majority of OA cartilage expression profiling showed activation of matrix components (such as ACAN, COL2A1, COL3A1, COL4A1, COL12A1, PCOLCE), it seems reasonable that one or more growth factors found in elevated levels in OA synovial fluids (TGF-β1, CTGF, PDGF, EGF, FGF-2, VEGF, GDF5) should also be included in the OA-inductive medium. For example, TGF-β1 and CTGF could be added because they both have well-established pro-fibrotic effects highly relevant to OA pathogenesis. These include the action of TGF-β1 in induction and progression in murine OA models[11, 68], the correlation of elevated CTGF concentration in synovial fluids with radiographic severity in human OA[67] and the induction of synovial fibrosis in murine OA, with concurrent enhanced expression of MMP-13, ADAMTS-4, and ADAMTS-5[81]. Regarding the choice of IL-17, TGF-β1, and CTGF it should be noted that similar recommendations could be made for the addition of any one or more of the factors identified by genome-wide screening as “OA-related” (e.g. IL-15, IL-12, IL-8, PDGF, EGF, FGF-2, VEGF, GDF5).

(3) The primary readout should be gene expression analysis, which can be done on a small group of genes with known OA relevance, or on pathway-based arrays with predicted relevance. The sensitivity, specificity, accuracy, and reproducibility of these methods can generate important data from cartilage samples in the low milligram range. This is important because it makes it feasible to analyze for expression changes in different depth zones of articular cartilage after treatment in vitro. Indeed, paramount in all these studies is the recognition that there is a well-established[82, 83] depth-dependent difference in the response of cartilage cells to OA-inductive conditions in vivo. For example, expression comparisons of different depths of normal and surface-intact OA human articular cartilages[82] (obtained by micro-dissection into superficial, middle and deep zones) showed that the up-regulation (about 20-fold) of ACAN and COL2A1 in the deep zone of the OA samples was not seen in the superficial zone. This supports earlier studies[84] in which chondrocytes isolated from micro-dissected zones of mature bovine cartilage were shown to exhibit distinct metabolic profiles and responses to inflammatory mediators.

Summary

The availability of genome-wide expression profiles of cartilage from human OA joints can be used to reorient the design of clinically relevant, in vitro experimental systems in the laboratory. A fresh evaluation of existing paradigms can now be undertaken so as to optimize the choice of tissue source, cytokine, growth factor, or nutrient addition, biomechanical environment, and readout analysis for discovery. Since biomechanical modulation of genome wide alterations is relatively new to this area, it will be essential that experimental parameters (e.g tissue strain, boundary conditions, geometry) are reported and considered in the interpretation and reproduction of the generated data. Going forward, the

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use of commercially available pathway-linked gene expression arrays should help unravel the implications of the laboratory data for treatment of the human disease.

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REFERENCES

1. Reynard LN, Loughlin J. Insights from human genetic studies into the pathways involved in osteoarthritis. Nat Rev Rheumatol. 2013; 9:573–583. [PubMed: 23958796]
2. Ajuied A, Wong F, Smith C, Norris M, Earnshaw P, Back D, et al. Anterior cruciate ligament injury and radiologic progression of knee osteoarthritis: a systematic review and meta-analysis. Am J Sports Med. 2014; 42:2242–2252. [PubMed: 24214929]
3. Moyer RF, Ratneswaran A, Beier F, Birmingham TB. Osteoarthritis year in review 2014: mechanics--basic and clinical studies in osteoarthritis. Osteoarthritis Cartilage. 2014; 22:1989–2002. [PubMed: 25456294]
4. Hui W, Young DA, Rowan AD, Xu X, Cawston TE, Proctor CJ. Oxidative changes and signalling pathways are pivotal in initiating age-related changes in articular cartilage. Ann Rheum Dis. 2014
5. Liu-Bryan R, Terkeltaub R. Emerging regulators of the inflammatory process in osteoarthritis. Nat Rev Rheumatol. 2015; 11:35–44. [PubMed: 25266449]
6. Scanzello CR, Plaas A, Crow MK. Innate immune system activation in osteoarthritis: is osteoarthritis a chronic wound? Curr Opin Rheumatol. 2008; 20:565–572. [PubMed: 18698179]
7. Fell HB, Jubb RW. The effect of synovial tissue on the breakdown of articular cartilage in organ culture. Arthritis Rheum. 1977; 20:1359–1371. [PubMed: 911354]
8. Dingle JT. Catabolin--a cartilage catabolic factor from synovium. Clin Orthop Relat Res. 1981:219–231. [PubMed: 7014063]
9. Saklatvala J, Pilsworth LM, Sarsfield SJ, Gavrilovic J, Heath JK. Pig catabolin is a form of interleukin 1. Cartilage and bone resorb, fibroblasts make prostaglandin and collagenase, and thymocyte proliferation is augmented in response to one protein. Biochem J. 1984; 224:461–466. [PubMed: 6097218]
10. Li J, Anemaet W, Diaz MA, Buchanan S, Tortorella M, Malfait AM, et al. Knockout of ADAMTS5 does not eliminate cartilage aggrecanase activity but abrogates joint fibrosis and promotes cartilage aggrecan deposition in murine osteoarthritis models. J Orthop Res. 2011; 29:516–522. [PubMed: 21337391]
11. Plaas A, Velasco J, Gorski DJ, Li J, Cole A, Christopherson K, et al. The relationship between fibrogenic TGFbeta1 signaling in the joint and cartilage degradation in post-injury osteoarthritis. Osteoarthritis Cartilage. 2011; 19:1081–1090. [PubMed: 21624477]
12. Remst DF, Blom AB, Vitters EL, Bank RA, van den Berg WB, Blaney Davidson EN, et al. Gene expression analysis of murine and human osteoarthritis synovium reveals elevation of transforming growth factor beta-responsive genes in osteoarthritis-related fibrosis. Arthritis Rheumatol. 2014; 66:647–656. [PubMed: 24574225]
13. Kokebie R, Aggarwal R, Lidder S, Hakimiyan AA, Rueger DC, Block JA, et al. The role of synovial fluid markers of catabolism and anabolism in osteoarthritis, rheumatoid arthritis and asymptomatic organ donors. Arthritis Res Ther. 2011; 13:R50. [PubMed: 21435227]
14. Schlaak JF, Pfers I, Meyer Zum Buschenfelde KH, Marker-Hermann E. Different cytokine profiles in the synovial fluid of patients with osteoarthritis, rheumatoid arthritis and seronegative spondylarthropathies. Clin Exp Rheumatol. 1996; 14:155–162. [PubMed: 8737721]
15. Bertazzolo N, Punzi L, Stefani MP, Cesaro G, Pianon M, Finco B, et al. Interrelationships between interleukin (IL)-1, IL-6 and IL-8 in synovial fluid of various arthropathies. Agents Actions. 1994; 41:90–92. [PubMed: 8079829]

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16. Richette P, Francois M, Vicaut E, Fitting C, Bardin T, Corvol M, et al. A high interleukin 1 receptor antagonist/IL-1beta ratio occurs naturally in knee osteoarthritis. J Rheumatol. 2008; 35:1650–1654. [PubMed: 18597398]
17. Irie K, Uchiyama E, Iwaso H. Intraarticular inflammatory cytokines in acute anterior cruciate ligament injured knee. Knee. 2003; 10:93–96. [PubMed: 12649034]
18. Zysk SP, Fraunberger P, Veihelmann A, Dorger M, Kalteis T, Maier M, et al. Tunnel enlargement and changes in synovial fluid cytokine profile following anterior cruciate ligament reconstruction with patellar tendon and hamstring tendon autografts. Knee Surg Sports Traumatol Arthrosc. 2004; 12:98–103. [PubMed: 14504722]
19. Smelter E, Hochberg MC. New treatments for osteoarthritis. Curr Opin Rheumatol. 2013; 25:310–316. [PubMed: 23425965]
20. Cohen SB, Proudman S, Kivitz AJ, Burch FX, Donohue JP, Burstein D, et al. A randomized, double-blind study of AMG 108 (a fully human monoclonal antibody to IL-1R1) in patients with osteoarthritis of the knee. Arthritis Res Ther. 2011; 13:R125. [PubMed: 21801403]
21. Chevalier X, Giraudeau B, Conrozier T, Marliere J, Kiefer P, Goupille P. Safety study of intraarticular injection of interleukin 1 receptor antagonist in patients with painful knee osteoarthritis: a multicenter study. J Rheumatol. 2005; 32:1317–1323. [PubMed: 15996071]
22. Kraus VB, Birmingham J, Stabler TV, Feng S, Taylor DC, Moorman CT 3rd, et al. Effects of intraarticular IL1-Ra for acute anterior cruciate ligament knee injury: a randomized controlled pilot trial (NCT00332254). Osteoarthritis Cartilage. 2012; 20:271–278. [PubMed: 22273632]
23. Clements KM, Price JS, Chambers MG, Visco DM, Poole AR, Mason RM. Gene deletion of either interleukin-1beta, interleukin-1beta-converting enzyme, inducible nitric oxide synthase, or stromelysin 1 accelerates the development of knee osteoarthritis in mice after surgical transection of the medial collateral ligament and partial medial meniscectomy. Arthritis Rheum. 2003; 48:3452–3463. [PubMed: 14673996]
24. Bougault C, Gosset M, Houard X, Salvat C, Godmann L, Pap T, et al. Stress-induced cartilage degradation does not depend on the NLRP3 inflammasome in human osteoarthritis and mouse models. Arthritis Rheum. 2012; 64:3972–3981. [PubMed: 22933232]
25. Nasi S, Ea HK, Chobaz V, van Lent P, Liote F, So A, et al. Dispensable role of myeloid differentiation primary response gene 88 (MyD88) and MyD88-dependent toll-like receptors (TLRs) in a murine model of osteoarthritis. Joint Bone Spine. 2014; 81:320–324. [PubMed: 24703622]
26. Furman BD, Mangiapani DS, Zeitler E, Bailey KN, Horne PH, Huebner JL, et al. Targeting pro-inflammatory cytokines following joint injury: acute intra-articular inhibition of interleukin-1 following knee injury prevents post-traumatic arthritis. Arthritis Res Ther. 2014; 16:R134. [PubMed: 24964765]
27. Lewis JS Jr. Furman BD, Zeitler E, Huebner JL, Kraus VB, Guilak F, et al. Genetic and cellular evidence of decreased inflammation associated with reduced incidence of posttraumatic arthritis in MRL/MpJ mice. Arthritis Rheum. 2013; 65:660–670. [PubMed: 23203659]
28. Zins SR, Amare MF, Anam K, Elster EA, Davis TA. Wound trauma mediated inflammatory signaling attenuates a tissue regenerative response in MRL/MpJ mice. J Inflamm (Lond). 2010; 7:25. [PubMed: 20500883]
29. Okada Y, Nakamura T, Ichii O, Otsuka S, Kon Y. Pathogenetic role of an autoimmune susceptibility locus derived from MRL/MpJ strain chromosome 1 in chronic pancreas inflammation. Lupus. 2014; 23:1112–1123. [PubMed: 24847041]
30. Gornikiewicz B, Ronowicz A, Podolak J, Madanecki P, Stanislawska-Sachadyn A, Sachadyn P. Epigenetic basis of regeneration: analysis of genomic DNA methylation profiles in the MRL/MpJ mouse. DNA Res. 2013; 20:605–621. [PubMed: 23929942]
31. Koshy PJ, Lundy CJ, Rowan AD, Porter S, Edwards DR, Hogan A, et al. The modulation of matrix metalloproteinase and ADAM gene expression in human chondrocytes by interleukin-1 and oncostatin M: a time-course study using real-time quantitative reverse transcription-polymerase chain reaction. Arthritis Rheum. 2002; 46:961–967. [PubMed: 11953973]
32. Smith RJ, Rohloff NA, Sam LM, Justen JM, Deibel MR, Cornette JC. Recombinant human interleukin-1 alpha and recombinant human interleukin-1 beta stimulate cartilage matrix

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degradation and inhibit glycosaminoglycan synthesis. Inflammation. 1989; 13:367–382. [PubMed: 2668162]

33. Seed MP, Ismaiel S, Cheung CY, Thomson TA, Gardner CR, Atkins RM, et al. Inhibition of interleukin 1 beta induced rat and human cartilage degradation in vitro by the metalloproteinase inhibitor U27391. Ann Rheum Dis. 1993; 52:37–43. [PubMed: 8427512]
34. DiMicco MA, Patwari P, Siparsky PN, Kumar S, Pratta MA, Lark MW, et al. Mechanisms and kinetics of glycosaminoglycan release following in vitro cartilage injury. Arthritis Rheum. 2004; 50:840–848. [PubMed: 15022326]
35. Yang B, Kang X, Xing Y, Dou C, Kang F, Li J, et al. Effect of microRNA-145 on IL-1beta-induced cartilage degradation in human chondrocytes. FEBS Lett. 2014; 588:2344–2352. [PubMed: 24873879]
36. Liang ZJ, Zhuang H, Wang GX, Li Z, Zhang HT, Yu TQ, et al. MiRNA-140 is a negative feedback regulator of MMP-13 in IL-1beta-stimulated human articular chondrocyte C28/I2 cells. Inflamm Res. 2012; 61:503–509. [PubMed: 22273691]
37. Miyaki S, Nakasa T, Otsuki S, Grogan SP, Higashiyama R, Inoue A, et al. MicroRNA-140 is expressed in differentiated human articular chondrocytes and modulates interleukin-1 responses. Arthritis Rheum. 2009; 60:2723–2730. [PubMed: 19714579]
38. Gu SX, Li X, Hamilton JL, Chee A, Kc R, Chen D, et al. MicroRNA-146a reduces IL-1 dependent inflammatory responses in the intervertebral disc. Gene. 2015; 555:80–87. [PubMed: 25311550]
39. Garlanda C, Dinarello CA, Mantovani A. The interleukin-1 family: back to the future. Immunity. 2013; 39:1003–1018. [PubMed: 24332029]
40. Pahl HL. Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene. 1999; 18:6853–6866. [PubMed: 10602461]
41. Kobayashi H, Hirata M, Saito T, Itoh S, Chung UI, Kawaguchi H. Transcriptional induction of ADAMTS5 protein by nuclear factor-kappaB (NF-kappaB) family member RelA/p65 in chondrocytes during osteoarthritis development. J Biol Chem. 2013; 288:28620–28629. [PubMed: 23963448]
42. Dinarello CA. Overview of the interleukin-1 family of ligands and receptors. Semin Immunol. 2013; 25:389–393. [PubMed: 24275600]
43. Boraschi D, Tagliabue A. The interleukin-1 receptor family. Semin Immunol. 2013; 25:394–407. [PubMed: 24246227]
44. 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; 54:808–817. [PubMed: 16508957]
45. Aigner T, Fundel K, Saas J, Gebhard PM, Haag J, Weiss T, et al. Large-scale gene expression profiling reveals major pathogenetic pathways of cartilage degeneration in osteoarthritis. Arthritis Rheum. 2006; 54:3533–3544. [PubMed: 17075858]
46. Duan C, Guo X, Zhang XD, Yu HJ, Yan H, Gao Y, et al. Comparative analysis of gene expression profiles between primary knee osteoarthritis and an osteoarthritis endemic to Northwestern China, Kashin-Beck disease. Arthritis Rheum. 2010; 62:771–780. [PubMed: 20131229]
47. Wei T, Kulkarni NH, Zeng QQ, Helvering LM, Lin X, Lawrence F, et al. Analysis of early changes in the articular cartilage transcriptisome in the rat meniscal tear model of osteoarthritis: pathway comparisons with the rat anterior cruciate transection model and with human osteoarthritic cartilage. Osteoarthritis Cartilage. 2010; 18:992–1000. [PubMed: 20434574]
48. Xu Y, Barter MJ, Swan DC, Rankin KS, Rowan AD, Santibanez-Koref M, et al. Identification of the pathogenic pathways in osteoarthritic hip cartilage: commonality and discord between hip and knee OA. Osteoarthritis Cartilage. 2012; 20:1029–1038. [PubMed: 22659600]
49. 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. [PubMed: 25054223]
50. Tew SR, McDermott BT, Fentem RB, Peffers MJ, Clegg PD. Transcriptome-wide analysis of messenger RNA decay in normal and osteoarthritic human articular chondrocytes. Arthritis Rheumatol. 2014; 66:3052–3061. [PubMed: 25155964]

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51. 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; 18:581–592. [PubMed: 20060954]
52. Bateman JF, Rowley L, Belluoccio D, Chan B, Bell K, Fosang AJ, et al. Transcriptomics of wild-type mice and mice lacking ADAMTS-5 activity identifies genes involved in osteoarthritis initiation and cartilage destruction. Arthritis Rheum. 2013; 65:1547–1560. [PubMed: 23436205]
53. Loeser RF, Olex AL, McNulty MA, Carlson CS, Callahan M, Ferguson C, et al. Disease progression and phasic changes in gene expression in a mouse model of osteoarthritis. PLoS One. 2013; 8:e54633. [PubMed: 23382930]
54. Wehling N, Palmer GD, Pilapil C, Liu F, Wells JW, Muller PE, et al. Interleukin-1beta and tumor necrosis factor alpha inhibit chondrogenesis by human mesenchymal stem cells through NF-kappaB-dependent pathways. Arthritis Rheum. 2009; 60:801–812. [PubMed: 19248089]
55. Lourido L, Calamia V, Mateos J, Fernandez-Puente P, Fernandez-Tajes J, Blanco FJ, et al. Quantitative proteomic profiling of human articular cartilage degradation in osteoarthritis. J Proteome Res. 2014
56. Peffers MJ, Beynon RJ, Clegg PD. Absolute quantification of selected proteins in the human osteoarthritic secretome. Int J Mol Sci. 2013; 14:20658–20681. [PubMed: 24132152]
57. Koenders MI, Marijnissen RJ, Devesa I, Lubberts E, Joosten LA, Roth J, et al. Tumor necrosis factor-interleukin-17 interplay induces S100A8, interleukin-1beta, and matrix metalloproteinases, and drives irreversible cartilage destruction in murine arthritis: rationale for combination treatment during arthritis. Arthritis Rheum. 2011; 63:2329–2339. [PubMed: 21520013]
58. Kondo M, Yamaoka K, Sonomoto K, Fukuyo S, Oshita K, Okada Y, et al. IL-17 inhibits chondrogenic differentiation of human mesenchymal stem cells. PLoS One. 2013; 8:e79463. [PubMed: 24260226]
59. Ashlin TG, Kwan AP, Ramji DP. Regulation of ADAMTS-1, -4 and -5 expression in human macrophages: differential regulation by key cytokines implicated in atherosclerosis and novel synergism between TL1A and IL-17. Cytokine. 2013; 64:234–242. [PubMed: 23859810]
60. Chabaud M, Lubberts E, Joosten L, van Den Berg W, Miossec P. IL-17 derived from juxta-articular bone and synovium contributes to joint degradation in rheumatoid arthritis. Arthritis Res. 2001; 3:168–177. [PubMed: 11299057]
61. Lee JH, Fitzgerald JB, Dimicco MA, Grodzinsky AJ. Mechanical injury of cartilage explants causes specific time-dependent changes in chondrocyte gene expression. Arthritis Rheum. 2005; 52:2386–2395. [PubMed: 16052587]
62. Chan PS, Schlueter AE, Coussens PM, Rosa GJ, Haut RC, Orth MW. Gene expression profile of mechanically impacted bovine articular cartilage explants. J Orthop Res. 2005; 23:1146–1151. [PubMed: 16140194]
63. Seol D, McCabe DJ, Choe H, Zheng H, Yu Y, Jang K, et al. Chondrogenic progenitor cells respond to cartilage injury. Arthritis Rheum. 2012; 64:3626–3637. [PubMed: 22777600]
64. Vincent TL, Hermansson MA, Hansen UN, Amis AA, Saklatvala J. Basic fibroblast growth factor mediates transduction of mechanical signals when articular cartilage is loaded. Arthritis Rheum. 2004; 50:526–533. [PubMed: 14872495]
65. Fanning PJ, Emkey G, Smith RJ, Grodzinsky AJ, Szasz N, Trippel SB. Mechanical regulation of mitogen-activated protein kinase signaling in articular cartilage. J Biol Chem. 2003; 278:50940–50948. [PubMed: 12952976]
66. Bomer N, Cornelis FM, Ramos YF, den Hollander W, Storms L, van der Breggen R, et al. The effect of forced exercise on knee joints in Dio2–/– mice: type II iodothyronine deiodinase-deficient mice are less prone to develop OA-like cartilage damage upon excessive mechanical stress. Ann Rheum Dis. 2014
67. Honsawek S, Yuktanandana P, Tanavalee A, Chirathaworn C, Anomasiri W, Udomsinprasert W, et al. Plasma and synovial fluid connective tissue growth factor levels are correlated with disease severity in patients with knee osteoarthritis. Biomarkers. 2012; 17:303–308. [PubMed: 22416876]
68. Remst DF, Blaney Davidson EN, Vitters EL, Blom AB, Stoop R, Snabel JM, et al. Osteoarthritis-related fibrosis is associated with both elevated pyridinoline cross-link formation and lysyl hydroxylase 2b expression. Osteoarthritis Cartilage. 2013; 21:157–164. [PubMed: 23069856]

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69. Madej W, van Caam A, Blaney Davidson EN, van der Kraan PM, Buma P. Physiological and excessive mechanical compression of articular cartilage activates Smad2/3P signaling. Osteoarthritis Cartilage. 2014; 22:1018–1025. [PubMed: 24795273]

70. Carrion M, Juarranz Y, Martinez C, Gonzalez-Alvaro I, Pablos JL, Gutierrez-Canas I, et al. IL-22/IL-22R1 axis and S100A8/A9 alarmins in human osteoarthritic and rheumatoid arthritis synovial fibroblasts. Rheumatology (Oxford). 2013; 52:2177–2186. [PubMed: 24056519]

71. Yamamoto M, Shimizu Y, Takahashi H, Yajima H, Yokoyama Y, Ishigami K, et al. CCAAT/enhancer binding protein alpha (C/EBPalpha) M2 macrophages contribute to fibrosis in IgG4-related disease? Mod Rheumatol. 2014:1–3. [PubMed: 25119258]

72. van Baarsen L, Lebre M, van der Coelen D, Aarrass S, Tang M, Ramwadhdoebe TH, et al. Heterogeneous expression pattern of interleukin-17A (IL-17A), IL-17F and their receptors in synovium of rheumatoid arthritis, psoriatic arthritis and osteoarthritis: possible explanation for non-response to anti-IL-17 therapy? Arthritis Res Ther. 2014; 16:426. [PubMed: 25146432]

73. Vittal R, Fan L, Greenspan DS, Mickler EA, Gopalakrishnan B, Gu H, et al. IL-17 induces type V collagen overexpression and EMT via TGF-beta-dependent pathways in obliterative bronchiolitis. Am J Physiol Lung Cell Mol Physiol. 2013; 304:L401–414. [PubMed: 23262228]

74. Nakashima T, Jinnin M, Yamane K, Honda N, Kajihara I, Makino T, et al. Impaired IL-17 signaling pathway contributes to the increased collagen expression in scleroderma fibroblasts. J Immunol. 2012; 188:3573–3583. [PubMed: 22403442]

75. Fabro AT, da Silva PH, Zocolaro WS, de Almeida MS, Rangel MP, de Oliveira CC, et al. The Th17 pathway in the peripheral lung microenvironment interacts with expression of collagen V in the late state of experimental pulmonary fibrosis. Immunobiology. 2014

76. Vittal R, Mickler EA, Fisher AJ, Zhang C, Rothhaar K, Gu H, et al. Type V collagen induced tolerance suppresses collagen deposition, TGF-beta and associated transcripts in pulmonary fibrosis. PLoS One. 2013; 8:e76451. [PubMed: 24204629]

77. Hu B, Wu Z, Nakashima T, Phan SH. Mesenchymal-specific deletion of C/EBPbeta suppresses pulmonary fibrosis. Am J Pathol. 2012; 180:2257–2267. [PubMed: 22503555]

78. 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

79. Li J, Gorski DJ, Anemaet W, Velasco J, Takeuchi J, Sandy JD, et al. Hyaluronan injection in murine osteoarthritis prevents TGFbeta 1-induced synovial neovascularization and fibrosis and maintains articular cartilage integrity by a CD44-dependent mechanism. Arthritis Res Ther. 2012; 14:R151. [PubMed: 22721434]

80. Nakamae A, Engebretsen L, Bahr R, Krosshaug T, Ochi M. Natural history of bone bruises after acute knee injury: clinical outcome and histopathological findings. Knee Surg Sports Traumatol Arthrosc. 2006; 14:1252–1258. [PubMed: 16786336]

81. Blaney Davidson EN, Vitters EL, Mooren FM, Oliver N, Berg WB, van der Kraan PM. Connective tissue growth factor/CCN2 overexpression in mouse synovial lining results in transient fibrosis and cartilage damage. Arthritis Rheum. 2006; 54:1653–1661. [PubMed: 16646035]

82. 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; 58:154–163. [PubMed: 18163492]

83. Chou CH, Lee CH, Lu LS, Song IW, Chuang HP, Kuo SY, et al. Direct assessment of articular cartilage and underlying subchondral bone reveals a progressive gene expression change in human osteoarthritic knees. Osteoarthritis Cartilage. 2013; 21:450–461. [PubMed: 23220557]

84. Aydelotte MB, Kuettner KE. Differences between sub-populations of cultured bovine articular chondrocytes. I. Morphology and cartilage matrix production. Connect Tissue Res. 1988; 18:205–222. [PubMed: 3219850]