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Review

Human genome-wide expression analysis reorients the study of inflammatory mediators and biomechanics in osteoarthritis

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SUMMARY

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 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.

Introduction

Current research into osteoarthritis (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.

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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 sub-types of the disease, and the degree of cartilage damage is often viewed as the read-out 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.

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.

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,