

Review

Articular cartilage and changes in arthritis An introduction: Cell biology of osteoarthritis

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Abstract

The reaction patterns of chondrocytes in osteoarthritis can be summarized in five categories: (1) proliferation and cell death (apoptosis); changes in (2) synthetic activity and (3) degradation; (4) phenotypic modulation of the articular chondrocytes; and (5) formation of osteophytes. In osteoarthritis, the primary responses are reinitiation of synthesis of cartilage macromolecules, the initiation of synthesis of types IIA and III procollagens as markers of a more primitive phenotype, and synthesis of active proteolytic enzymes. Reversion to a fibroblast-like phenotype, known as 'dedifferentiation', does not appear to be an important component. Proliferation plays a role in forming characteristic chondrocyte clusters near the surface, while apoptosis probably occurs primarily in the calcified cartilage.

Keywords: cartilage, cell biology, chondrocyte phenotype, osteoarthritis

Introduction

Osteoarthritis (OA) involves the entire synovial joint, encompassing the cartilage, synovium, and underlying bone. The cells in each of these tissues have independent capacities to initiate and respond to injury in the joint, ultimately resulting in degeneration of cartilage. It is generally believed that degeneration of cartilage in OA is characterized by two phases: a biosynthetic phase, during which the cells resident in cartilage, the chondrocytes, attempt to repair the damaged extracellular matrix; and a degradative phase, in which the activity of enzymes produced by the chondrocytes digests the matrix, matrix synthesis is inhibited, and the consequent erosion of the cartilage is accelerated [1–4]. New techniques of molecular biology have provided invaluable insights into the function of cells during the onset and

perpetuation of OA. Analysis of mRNA levels in cartilage chondrocytes remaining even at joint replacement provided a surprise: the cells are not metabolically inert, but are actively synthesizing cartilage proteins. The proteins synthesized by OA chondrocytes are structural and functional macromolecules, and degradative enzymes. In addition, the areas of cellular activity and inactivity are now known to be regional. Unfortunately, at some point the biosynthetic anabolic activity is unable to keep pace with the degradative catabolic activity, and degeneration of the tissue results.

Influences of cytokines and growth factors

In normal adult cartilage, chondrocytes synthesize matrix components very slowly. During development, however, biosynthesis is stimulated by a variety of anabolic

BMP = bone morphogenetic protein; COL2A = type IIA procollagen; COL2B = type IIB procollagen; FGF = fibroblast growth factor; IGF = insulin-like growth factor; IL = interleukin; MMP = matrix metalloproteinase; NO = nitric oxide; NOS = nitric oxide synthase; OA = osteoarthritis; TGF = transforming growth factor; TIMP = tissue inhibitor of metalloproteinases; TNF = tumor necrosis factor.

cytokines and growth factors, such as transforming growth factor (TGF)- β , bone morphogenetic proteins (BMPs), and insulin-like growth factor I (IGF-I). In OA, many of these factors – and others, such as the inflammatory cytokines tumor necrosis factor (TNF)- α and interleukin 1 (IL-1) – are produced by the synovium and the chondrocytes. In normal cartilage, there is strict regulation of matrix turnover: a delicate balance between synthesis and degradation. In OA, however, this balance is disturbed, with both degradation and synthesis usually enhanced. The inflammatory cytokines IL-1, TNF- α , IL-17, and IL-18 act to increase synthesis of matrix metalloproteinases (MMPs), decrease MMP enzyme inhibitors, and decrease extracellular matrix synthesis. The anabolic cytokines IGF-I, TGF- β 1, 2, and 3, fibroblast growth factors (FGFs) 2, 4, and 8, and the BMPs act to stimulate extracellular matrix synthesis. It is believed that the production of the catabolic and anabolic cytokines activates the chondrocytes; however, no single cytokine can stimulate all the metabolic reactions observed in OA. Recent reviews explore in detail the role of cytokines and growth factors in the pathogenesis of OA [5,6].

Chondrocytes of articular cartilage produce and retain significant amounts of active and inactive BMPs, known to increase extracellular matrix synthesis and induce chondrogenesis and osteogenesis. For example, both normal and OA chondrocytes synthesize and retain BMP-7 (also called OP-1 [osteogenic protein 1]) [7]. BMP-7 is found in two forms: an active form generated by intracellular proteolytic cleavage, and an inactive precursor form (pro-BMP-7) [8]. Whereas the detection of mRNA encoding BMP-7 appeared to be the same in OA and normal adult tissues, the level of mature BMP-7 protein was downregulated in OA cartilage while the pro-BMP-7 remained high. In OA cartilage, mature BMP-7 was detected in the superficial layer, whereas the pro form was primarily in the deep layer. These results point to the possibility that one way in which proteinases could regulate anabolic activities is through the conversion of pro-BMPs to mature BMPs, converting inactive BMP to active BMP, which can then stimulate matrix synthesis.

Other molecular influences of cartilage degradation are beginning to emerge that have been found to be a result of initial molecular breakdown. It is now known that fragments of fibronectin can induce expression of metalloproteinases and matrix degradation in chondrocytes [9]. The molecular mechanism is probably the induction of enhanced gene expression of collagenase and stromelysin [10]. More recently, a fragment of link protein, part of the large proteoglycan aggregate in cartilage, was found to stimulate proteoglycan and collagen synthesis in cartilage explant culture [11]; consequently the fragments of protein degradation may stimulate the cells to attempt to repair the matrix, as proposed by Hering [12].

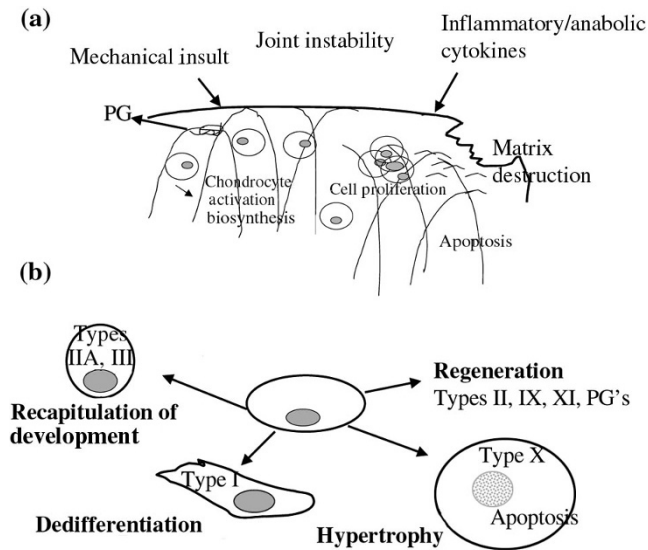
Cellular responses in OA cartilage

The cellular reaction pattern during the osteoarthritic disease process is at first glance rather heterogeneous. However, the reaction patterns can basically be summarized in five categories: (1) proliferation and cell death (apoptosis), (2) changes in synthetic activity, (3) changes in degradation, (4) phenotypic modulation of the articular chondrocytes, and (5) formation of osteophyte. A representation of these responses is shown in Fig. 1.

Cell proliferation and programmed cell death

Many studies [13–16] have shown that there is a very low proliferative activity in osteoarthritic chondrocytes, in contrast to normal articular chondrocytes, which have essentially no such activity. The activity seen in OA chondrocytes might be due to better access of chondrocytes to proliferative factors from the synovial fluid due to fissuring or loosening of the collagen network [13] or due to the damage to the collagen matrix itself [17]. In any case, proliferation of chondrocytes is most probably the biological activity that causes chondrocyte clustering, a characteristic feature of OA cartilage.

Several authors have suggested that cell death is a central feature in osteoarthritic cartilage degeneration, as it is in the terminal hypertrophic zone of the growth plate [18–21]. Recently, it was reported that apoptotic cell death is a dominant event in the degeneration of osteoarthritic cartilage, although the results are not in good agreement: for example, cell death in cartilage samples ranged from 5 to 11% and in patients with OA, from 22 to 51% of all cells [22–26]. We think it is very likely that these numbers are overestimates of the extent of apoptosis in cartilage, because if they are correct, other biosynthetic parameters of OA would be impossible; indeed even 'normal' cartilage would soon lose the capacity to undergo biosynthesis. In theory, a major degree of cell death would easily lead to a failure of turnover of the cartilage matrix, because chondrocytes are the only source of synthesis of matrix components in articular cartilage and there is no renewal of chondrocyte population. In our studies (T Aigner, unpublished findings), we have confirmed that apoptosis occurs in osteoarthritic cartilage, but at a very low rate with approximately 0.1% of the total cell population apoptotic at a given time point, indicating that the death of chondrocytes has only a limited impact on the pathology of osteoarthritis [13,15,27]. The only zone in which a large number of empty lacunae, indicative of cell death, has been found by us or others was the calcified cartilage layer [28,29]. The greatly reduced number of living chondrocytes in this cartilage zone does not seem to impair articular cartilage in normal conditions, but might be detrimental in more advanced stages of osteoarthritis, when this zone is considerably enlarged and represents a higher proportion of the residual cartilage. Because apoptotic cells are not removed effectively from cartilage, the products of cell

Figure 1

Chondrocyte response to injury. **(a)** Injury and response. Mechanical insult, joint instability and inflammatory (generally catabolic) or anabolic cytokines can cause matrix activation, cell proliferation, apoptosis and eventually matrix destruction. Proteoglycan fragments (PG) are lost from the matrix. **(b)** Phenotypic modulation. Chondrocyte activation can result in modulation of gene expression resulting in different patterns of protein synthesis characteristic of chondrocyte development, fibroblasts 'dedifferentiation', hypertrophy (as seen in the growth plate) or regeneration of mature cartilage.

death such as pyrophosphate and precipitated calcium may contribute to pathologic cartilage degradation.

The free radical nitric oxide (NO) has been implicated as a biological mediator in OA [30]. Articular chondrocytes produce the inducible enzyme nitric oxide synthase (NOS), and both NO and NOS are synthesized in OA. The role of NO in OA is not known, but it can inhibit proteoglycan synthesis *in vitro* and can inhibit chondrocytes' response to IGF-I [31]; in addition, some studies suggest that it may play a role in apoptosis of chondrocytes and synovial cells [32,33].

Metabolic activation and hypoanabolism

In osteoarthritic cartilage, a number of biochemical studies have demonstrated enhanced synthesis of extracellular matrix components [34–42]. Chondrocytes attempt to repair the damaged matrix by increasing their anabolic activity. Despite this increased activity, a net loss of proteoglycan content is one of the hallmarks of all stages of osteoarthritic cartilage degeneration [15]. This observation has led to the assumption that overall enzymatic degradation of matrix components might be the reason for the metabolic imbalance. However, most previous studies were based on an overall measurement of chondrocyte behavior or matrix composition within the whole

osteoarthritic cartilage. The techniques used did not allow detection of differences between cells of different cartilage zones. Our own analyses *in situ* showed that the loss of fixed charges (due to aggrecan glycosaminoglycan side chains) occurs in the upper zones of osteoarthritic cartilage, in which the cells downregulated their expression of matrix components, in particular of aggrecan: at the same time, the cells of the deeper zones are still activated [43]. In fact, the hyperactivity of matrix synthesis was restricted to the chondrocytes of the middle and deeper zones of osteoarthritic cartilage, where the extracellular matrix was histochemically still intact and no major loss of proteoglycan was detectable. This explains, at least in part, the loss of proteoglycan content in the upper zone, particularly if one assumes that the diffusion capacity of aggrecan monomers is limited and enhanced synthesis in one zone cannot compensate for the failure of synthesis in other zones. Notably, even in specimens with a very high Mankin's grade (>8), suggesting an advanced disease state, some chondrocytes showed strong anabolic activity and thus kept their capacity to be anabolically active.

Degradative enzymes

Articular cartilage chondrocytes are reported to synthesize many MMPs, namely, MMPs 1, 2, 3, 7, 8, 13, and 14 [44–46], as well as a variety of other serine and cysteine proteinases [47]. Most of these enzyme activities are increased in OA, whether by the mechanism of increased synthesis, increased activation of proenzymes by other MMPs or plasmin, or decreased inhibitor activity. In nearly all OA cells, MMP-3 (stromelysin), MMP-8 (collagenase-2), and MMP-13 (collagenase-3) were elevated. Many of these MMPs are stimulated by exposure of the cells to inflammatory cytokines [48]. To agonize the effects of MMPs, expression levels of inhibitors such as tissue inhibitor of metalloproteinases (TIMP)-1 are reduced in OA and rheumatoid arthritis [49,44,50], although the ratio of total MMPs to total inhibitors is not really known. In 92% of OA cases in one study [51], MMP-7 (matrilysin), an enzyme with a wide range of susceptible proteins, was localized in chondrocytes, mainly those in the superficial and transitional zones. Approximately 30% of the total chondrocytes were immunostained in the positive OA cartilage samples. The results of mRNA analysis were consistent with the localization of protein. The noncollagenase enzymes could act to disrupt the matrix, rendering it weaker and more susceptible to hydration.

The degradation of type II collagen has been studied extensively by the team of Dr Robin Poole, who have shown that MMP-13 is the enzyme responsible for most of the collagen degradation [52]. In addition, MMP-3 can cleave in the nonhelical telopeptide of type II and type IX collagens [53], leading to the disruption of a collagen crosslink. This cleavage could result in a disrupted fibril structure and, consequently, disrupted fibril function.

Indeed, Bonassar and associates have shown that treatment of cartilage plugs *in vitro* with stromelysin causes marked swelling of the tissue, whereas treatment with trypsin does not [54]. We have recently shown that the type II collagen telopeptide can also be cleaved by MMPs 7, 9, 13, and 14; this finding indicates the presence in OA of a host of enzyme candidates capable of disrupting the collagen network [55]. Disruption of this network will eventually lead to destabilization of the joint. Evidence for disrupted collagen structure in the pathophysiology of OA also comes from genetic studies showing that mutations in type II collagen lead to an unstable collagen network and eventually to premature OA [56,57].

Two new families of degradative enzymes have been detected in articular cartilage. Protein and mRNA for ADAM-10 (A Disintegrin-like And Metalloproteinase-like domain) was found in the most fibrillated areas of OA cartilage, especially in the cell clusters. Probably more importantly, two new enzymes, called aggrecanase 1 and 2, have been isolated that are ADAMs enzymes with an additional thrombospondin domain (ADAM-TS) capable of binding to chondroitin sulfate. The MMPs and aggrecanases cleave aggrecan at distinct sites in the core protein [58].

Cysteine peptidases, primarily cathepsins, have recently been found in OA cartilage and subchondral bone. Cathepsins L and K were localized subchondrally in association with cathepsin B, in osteophytes, in zones undergoing bone remodeling and at sites of inflammation, whereas cathepsin B was present and active in cartilage, particularly at sites where matrix neosynthesis takes place [59]. Inhibition of these cysteine enzymes had an effect on cartilage breakdown, indicating that they may play a role in the cascade of events leading to matrix degradation.

Phenotypic alterations of the chondrocytic phenotype

Potential phenotypic changes are characteristic of chondrocytes. Many studies have shown changes in phenotype during chondrocyte differentiation *in vivo* in the fetal growth-plate cartilage and of chondrocyte behavior *in vitro*. Several factors, such as retinoic acid, bromodeoxyuridine, and IL-1, induce so-called 'dedifferentiation', or modulation of the chondrocyte phenotype to a fibroblast-like phenotype. The chondrocytes stop expressing aggrecan and collagen type II, though they are still very active cells and express collagen types I, III, and V [60–63]. This example clearly demonstrates the implications of phenotypic alterations of chondrocytes: despite potentially high synthetic activity, dedifferentiated chondrocytes do not express cartilage-specific anabolic genes such as aggrecan or type II collagen. Therefore, in addition to deactivation, phenotypic alteration represents another potential reason for anabolic failure of chondrocytes in osteoarthritic cartilage.

Classically, chondrocyte phenotypes are categorized largely by subtyping of collagen gene expression [64,65]. Thus, chondroprogenitor cells are characterized by the expression of the alternative splice variant of type II collagen, type IIA procollagen (COL2A) [66]. Mature chondrocytes express the typical cartilage collagen types II (COL2B), IX, and XI as well as aggrecan and link protein [67–69]. Hypertrophic chondrocytes are marked by the expression of type X collagen. These cells are found in the lowest zone of the cartilage of the fetal growth plate [70,71] and in the calcified zone of adult cartilage thought to be a remnant of the lower hypertrophic zone of the fetal growth-plate cartilage [72]. Chick chondrocytes can undergo post-hypertrophic differentiation to osteoblast-like cells, expressing type I collagen [73–75].

In our laboratories, we performed *in situ* expression analyses in normal and osteoarthritic cartilage specimens, using the markers for chondrocyte differentiation, collagen type II and aggrecan (activated functional chondrocytes), collagen types I and III (dedifferentiated chondrocytes), collagen type IIA (chondroprogenitor cells), and collagen type X (hypertrophic chondrocytes). Activated chondrocytes were found mostly in the middle zones of osteoarthritic cartilage. These cells also expressed type IIA procollagen and deposited it primarily in the cell-associated cartilage. This indicates that on the molecular level, a significant proportion of adult articular chondrocytes starts to re-express a chondroprogenitor phenotype in osteoarthritic cartilage degeneration, which is comparable to the chondroprogenitor phenotype observed in fetal skeletal development [66,76]. Cells expressing type III collagen were mainly found in the upper middle zone. Interestingly, a reversion to a fetal phenotype and the reinitiation of fetal skeletal developmental processes also occurs in the deepest zones of osteoarthritic cartilage: here, the cells start to express type X collagen [77], which is a specific marker for hypertrophy of growth-plate chondrocytes [78,70]; apoptosis occurs; and the cartilage matrix calcifies: all these events are processes taking place in the lowest zone of fetal growth-plate cartilage.

The uppermost chondrocytes of OA cartilage often do not demonstrate expression of any of the collagen types investigated. This pattern is not replicated by the established modulations of the chondrocyte phenotype known *in vivo* and *in vitro*. None of the discussed marker genes were expressed by the chondrocytes in the upper zone of osteoarthritic cartilage [77,79] and no really specific markers have been established yet for these cells, although one good candidate could be the cartilage surface protein gp-30 [80]. This stresses the need to establish a broader gene expression profile by modern screening technologies.

Secondary cartilage formation (osteophytes)

One of the most remarkable and consistent features of joints affected by OA, whether naturally occurring or experimentally induced, is the development of prominent osteochondral nodules known as osteophytes (also called osteochondrophytes or chondro-osteophytes). Indeed, the presence of osteophytes in a joint, more than any other pathological feature, distinguishes OA from other arthritides [81]. It seems likely that both mechanical and humoral factors are involved in stimulating the formation of osteophytes. Osteophytes are an example of new cartilage and bone development in OA joints and arise from tissue associated with the chondro-synovial junction or from progenitor cells residing in the perichondrium [82–84] – indicating that there is a population of pluripotential cells that is responsive to the mechanical and humoral sequelae of joint injury [84]. Though the exact functional significance of osteophyte growth remains unclear, osteophytes might help to stabilize joints affected by OA [85]. It is conceivable that the pathogenesis of osteophytes is related to the induction of bone spurs called exostoses, which also probably arise from the perichondrium or periosteum.

Analyzing osteophytes of different developmental stages from human patients, we could show a sequential process of differentiation. The first indications of chondrogenic differentiation were within fibrous, mesenchymal tissue marked by the onset of type IIA collagen. The next stage was characterized by the appearance of transitory, fibrocartilaginous cells expressing types II and III collagen. Chondrocytes synthesizing collagen type II (and very probably also the other collagens typical of cartilage) then appeared, followed by hypertrophic chondrocytes characterized by the onset of expression of type X collagen [84]. Although extremely variable and heterogeneous in the amount of collagen and local distribution, various cell and tissue types in osteophytes correlate with those seen in a normally developing fetal epiphysis.

In some of the larger osteophytes, areas of hyaline cartilage extended to the surface of the osteophyte. These cartilaginous tissues resemble genuine articular cartilage in chondrocyte morphology and in an extracellular matrix showing a predominance of type II collagen, absence of type I collagen, and an even staining with toluidine blue. It is questionable whether the biomechanical stability and the collagen architecture of these cartilaginous tissues correspond to those of original articular cartilage and its arcade structure. Interestingly, the anabolic factors TGF- β and TGF- β 2 were found in osteophytes from human femoral heads [86,84]. In any case, the ability of joint tissue to regenerate cartilaginous structures is a fascinating phenomenon, stimulating numerous experimental approaches to cartilage healing in degenerating joints.

Conclusions

The cellular response in OA is complex, and the more information becomes available, the more complex it seems. Of integral importance is the question of why the cartilage retains function for many years, and then begins to erode rapidly. A great deal of information in OA has come from studies at joint replacement and in animal models; however, such studies focus on the beginning and end of the process. More studies are needed that fill the gaps in between by studying high-risk populations, mild ongoing OA in humans, and following animal models to end-stage OA. Preliminary studies in this area are encouraging, showing that the information obtained from both animal models and end-stage human OA is valid. Our challenge in the future will be to sort out the primary and secondary stimuli and cellular responses and determine at what level the disease process can be attenuated.

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