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CD44 and integrin matrix receptors participate in cartilage homeostasis

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Abstract. Articular chondrocytes express the matrix receptors CD44 and integrins. Both of these receptors exhibit interactions with adjacent extracellular matrix macromolecules. In addition, both integrins and CD44 have the capacity for signal transduction as well as modulated interactions with the actin cytoskeleton. As such, both receptor families provide the chondrocytes a means to detect changes in matrix composition or to function as mechanotransducers. Disruption of CD44 or integrin-mediated cell-matrix interactions, either experimentally induced or when present in osteoarthritis, have profound effects on cartilage metabolism. Thus, CD44 and integrin receptors play a critical role in maintaining cartilage homeostasis.

Key words. CD44; hyaluronan; proteoglycan; integrin; collagen; fibronectin; chondrocyte.

In many tissues, carefully regulated cell-matrix interactions are responsible for maintaining tissue homeostasis [1–4]. Most cell-matrix interactions are mediated via transmembrane receptors. Articular chondrocytes have been shown to express both integrin [5–7]) as well as nonintegrin (e.g., annexin V and CD44) extracellular matrix (ECM) receptors [8, 9] (fig. 1). Members of each receptor class, through interactions with their principal ligands, provide chondrocytes the means to 'sense' changes in the ECM environment – changes that may elicit a reparative response, matrix remodeling or, alternatively, cellular quiescence. This review discusses two matrix receptor classes: CD44s and integrins.

CD44 as an ECM receptor in cartilage

CD44 is a single-pass transmembrane receptor expressed by many cell types including chondrocytes [9, 10]. CD44 was initially characterized as a lymphocyte-homing receptor. However, subsequent studies demonstrated that this receptor was identical to the cell surface 'hyaluronan-binding protein' sought by many groups [4, 11, 12]. In chondrocytes, CD44 represents the primary receptor responsible for hyaluronan (HA) binding [4, 9]. However, in cartilage, HA is seldom present as a pure molecule. Often more than 50 aggrecan proteoglycan (PG) monomers together with an associated link protein become bound to a single filament of HA [13]. Thus, the binding of HA to CD44 in cartilage facilitates the retention of large, highly hydrated, HA/PG/link protein aggregates at the surface of chondrocytes (fig. 1). This retention can be visualized as a gel-like pericellular matrix surrounding chondrocytes in vitro [9, 14, 15]. This matrix can be removed via treatment with the HA-specific enzyme, *Streptomyces* hyaluronidase, or incubation with excess HA oligosaccharides. After washing the cells, a gel-like coat can be reestablished by the addition of new HA/PG aggregates (with or without link protein). The re-establishment of a gel-like pericellular matrix can be inhibited by the inclusion of anti-CD44 antibodies [9], suggesting that the HA/PG aggregates must rebind to the CD44 receptor. Treatment with CD44 antisense oligonucleotides [16] or transfection and overexpression of mutant CD44 isoforms [17] also inhibit the retention of a gel-like coat around chondrocytes. All of these experiments demonstrate that CD44 is an important mediator in chondrocyte cell-matrix interactions that involve PG/HA/link protein aggregates.

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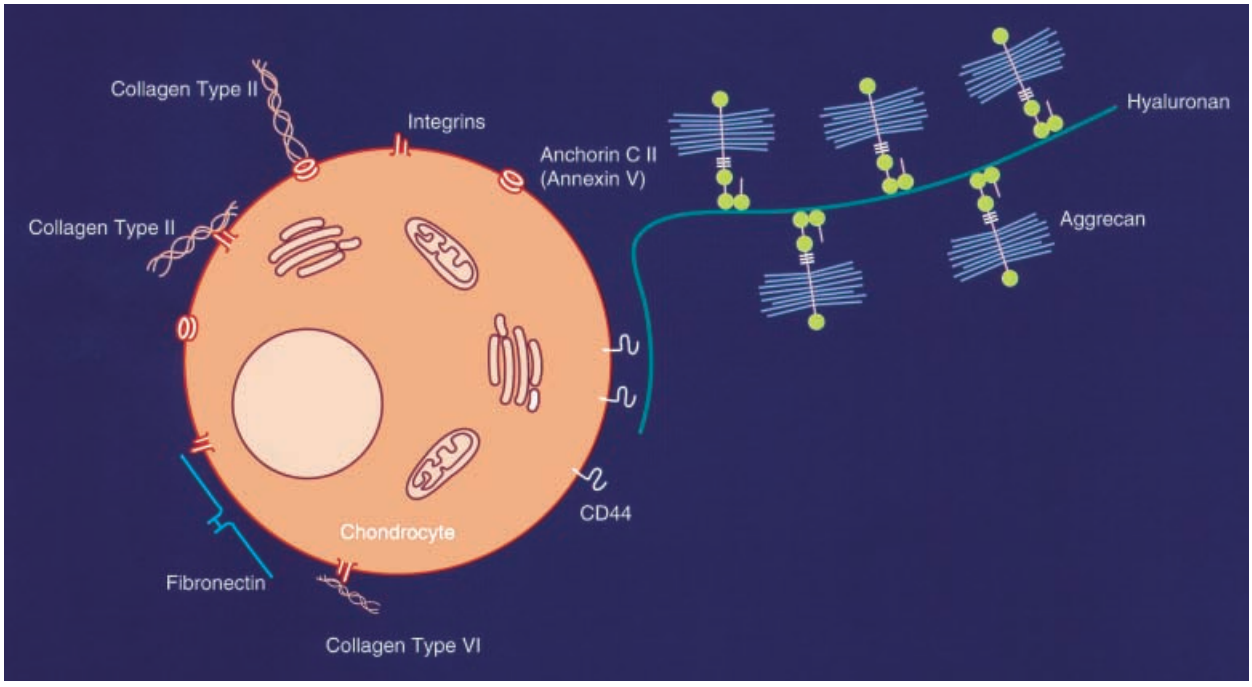

Figure 1. Model of chondrocyte receptors mediating cell-matrix interactions.

One question that arises is whether there are connections between the CD44-anchored HA/PG-rich matrix and other ECM components present on chondrocytes – components such as collagen or fibronectin, presumably bound to other matrix receptors (fig. 1). In an attempt to determine whether these HA/PG-rich pericellular matrices are present within cartilage tissue, chondrocytes were isolated from chick embryonic tibia using purified collagenase in the presence of 20% fetal bovine serum [18]. The chondrocytes were readily liberated from the tissue with the gel-like HA-dependent matrix intact. Presumably the collagenase degraded the collagen-rich matrix with little effect on the HA/PG-rich matrix that remained intact and associated with the chondrocytes through the cell isolation protocol. In reverse, hyaluronidase is often used to expose collagen epitopes [19], a treatment that has little effect on the collagenous network. Thus, the collagen-rich, the fibronectin-rich, and the HA/PG-rich ECMs, although possibly intangled, are likely anchored to the chondrocyte plasma membrane via separate and distinct receptor systems.

Cell signaling mediated by chondrocyte CD44

As described above, CD44 is a single-pass membrane glycoprotein. It contains a hydrophobic domain (encoded by exon 18) followed by a 70-amino acid intracellular domain (exon 20) [20]. The intracellular domain of CD44 contains motifs with the potential for transducing signals and controlling the spatial organization of the receptor in the plasma membrane. However, as yet, no downstream intracellular event has been directly or conclusively linked to CD44 signaling. The intracellular domain contains no tyrosine residues but does possess several serine residues that undergo differential phosphorylation, two in particular, Ser336 and Ser337. In some cell systems, phosphorylation of CD44 appears to increase CD44 binding of the actin-binding protein, ankyrin and increase the capacity of the receptor to bind HA [21]. Conversely, phosphorylation-defective mutants of CD44 expressed in T lymphoma cells display reduced HA binding [22]. However, in a different study using transfected AKR1 cells, phosphorylation-incompetent mutants of CD44 did not exhibit altered HA binding or receptor distribution into detergent-soluble and cytoskeletal-associated (detergent-insoluble) pools [23]. Macrophages appear to exhibit two pools of CD44 with respect to phosphorylation. In resting peritoneal macrophages, the two pools are balanced between the phosphorylated and nonphosphorylated forms. In elicited macrophages, there is a shift, such that more than 90% of the CD44 becomes phosphorylated. In chondrocytes, removal of HA and the HA/PG-rich matrix via *Streptomyces* hyaluronidase treatment results in a reduction in phosphorylated CD44, a reduction that is reversed by subsequent addition and the rebinding of exogenous HA [16]. These results suggest that chondrocyte CD44 does have the capacity to respond to changes in the HA/PG-rich ECM and alter the phosphorylation state of the receptor. However, as yet, the true

consequence(s) of this change in phosphorylation is not known.

In addition to serine phosphorylation sites, the intracellular domain of CD44 exhibits protein motifs that permit the binding and/or utilization of two different families of actin-binding proteins. Both are responsible for linking the cytoplasmic domain of CD44 to the cortical actin cytoskeleton. One family is called 'ERM' to designate its component proteins (ezrin, radixin, and moesin). The other is called the 'ankyrin' family. As discussed above, serine phosphorylation enhanced CD44 association with ankyrin in T lymphocytes [21]. The variant-10 CD44 isoform expressed on endothelial cells also utilizes an interaction with ankyrin for its linkage to the cytoskeleton. However, the 140-kDa CD44 expressed in endothelial cells interacts with the cytoskeleton via ERM proteins [24]. Chondrocytes have been shown to express moesin (a component of the ERM), and ankyrin simultaneously. However, anti-CD44 monoclonal antibody-mediated immunoprecipitation studies of lysed and solubilized chondrocytes revealed only the coimmunoprecipitation of ankryin with CD44 [16]. Whether both of these linkage systems operate together or selectively (possibly for different functions) is not known at present but remains an intriguing possibility. What does association of CD44 with ankyrin or ERM have to do with changes in the HA/PG-rich matrix? In the absence of an HA/PG-rich matrix (i.e., following *Streptomyces* hyaluronidase treatment), a higher percentage of CD44 is extractable by mild detergent conditions, conditions that do not solubilize CD44 associated with the actin cytoskeleton [16]. The percentage of cytoskeleton-associated CD44 increases significantly when CD44 is solubilized from cells exhibiting a more intact HA/PG-rich pericellular matrix or, when HA is added back to chondrocytes following *Streptomyces* hyaluronidase treatment [16]. Thus, changes in the ECM are 'sensed' by the chondrocytes by receptor occupancy enhancing receptor linkage to the underlying cytoskeleton, a linkage mediated via various actin-binding proteins. Changes in receptor-cytoskeletal interactions also have the potential to affect chondrocyte metabolism.

Chondrocyte CD44 participates in HA internalization and catabolism

In addition to the role of chondrocyte CD44 in anchoring the HA/PG-rich pericellular matrix and possibly 'sensing' changes in that matrix, CD44 is also responsible for the internalization of extracellular HA, leading to its complete catabolism intracellularly. Bovine articular chondrocytes were shown to internalize, sequester, and degrade fluorescein- or ^{3}H-labeled HA within an intracellular, chloroquine-sensitive compartment [25]. Pretreatment of the chondrocytes with either HA hexasaccharides or anti-CD44 antibodies blocked the endocytosis of HA. In addition, the chondrocytes displayed no capacity to bind or internalize control fluorescein-conjugated dextran (2×10^3 kDa) ruling out fluid-phase pinocytosis as a possible mechanism for HA internalization. In subsequent studies, bovine or human articular chondrocytes treated with interleukin (IL)-1 to induce a catabolic-like phenotype resulted in a six- to eightfold increase in CD44 mRNA, a six- to eightfold increase in CD44 protein and a threefold increase in uptake and accumulation of intracellular HA [10, 26]. Does this capacity of CD44 play a role in affecting chondrocyte metabolism? The answer is unknown. One suggestion would be that CD44 not only has the capacity to 'sense' changes in the adjacent ECM and transduce those signals but is also an effector, actively participating in the catabolism of ECM components, namely HA.

Disruption of cell-matrix interactions mediated via CD44

As discussed above, artificially removing pericellular HA from chondrocytes in vitro affects CD44 phosphorylation as well as the association with the actin cytoskeleton. Removal of HA also enhances the receptor recycling rate (i.e., receptor turnover from the cell surface) [27]. However, what are the consequences of inhibiting CD44-mediated cell-matrix interactions within intact cartilage? Two approaches have been used to address this question. Both were led by practical concerns as to how to manipulate the CD44-HA interactions within intact cartilage or full- thickness cartilage slices grown in tissue culture. The first was the use of CD44 antisense oligonucleotides. Small oligonucleotides, such as a rhodamine-conjugated 16-nucleotide phosphorothioate probe, were found to readily penetrate bovine articular cartilage slices and accumulate within the chondrocytes (data not shown). This penetration was enhanced by the use of DNA:liposome complexes (e.g., lipofectamine, Life Technologies [28]). The goal of using the CD44 antisense oligonucleotides was to selectively inhibit CD44 protein expression and thus limit the chondrocyte capacity for CD44-mediated cell-matrix interactions (fig. 2A, C). The outcome of this treatment was the induction of a state of 'chondrocytic chondrolysis' [29] throughout the full thickness of the cartilage slices [30]. Frozen sections from the treated cartilage displayed a near total loss of safranin-O staining as well as prominent staining for the aggrecanase neoepitope, NITEGE. Control, untreated tissue or slices treated with the control, 'sense' oligonucleotide displayed rich safranin-O staining and little NITEGE epitope expression. Thus, limiting the expression of CD44 and the subsequent loss of HA/PG-rich cell-matrix interactions had a dramatic effect on the overall metabolism of the cartilage.

A second approach to inhibit CD44-mediated cell-matrix interactions was the addition of small HA oligosaccharides, a mixture predominately composed of HA hexa- and octasaccharides. These small oligosaccharides also have the capacity to readily penetrate into intact cartilage tissue slices. These small HA oligosaccharides can compete for the binding of HA to CD44 but are not large enough to affect the displacement of aggrecan proteoglycan monomers from HA [9]. In addition, most, if not all of the aggrecan monomers are stabilized via the tandem binding of link proteins, making the monomers nondisplaceable even in the presence of larger HA oligosaccharides. The goal of this experiment was not to affect CD44 expression but to competitively inhibit the interaction of CD44 with the HA/PG-rich matrix (fig. 2C). The outcome of HA oligosaccharide treatment was the same as the effect of CD44 antisense oligonucleotides (fig. 2B), that is, induction of a state of 'chondrocytic chondrolysis' throughout the full thickness of the cartilage slices [31]. As with the antisense oligonucleotide treatment, the cartilage slices displayed a dramatic loss of safranin-O staining, a loss of immunohistological staining for aggrecan (5-D-4) and a prominent increase in the neoepitope for aggrecanase, NITEGE. Both matrix metalloproteinase (MMP)-2 and MMP-9, detected by gelatin zymography, were also found to be upregulated following treatment with HA oligosaccharides. In this study, HA oligosaccharides were tested on bovine as well as human articular chondrocytes with similar results. While chondrocytic chondrolysis was apparently induced by this treatment, the presence of the HA oligonucleotides also affected chondrocyte biosynthesis. However, unlike the effects of IL-1 on cartilage (i.e., a downregulation of chondrocyte biosynthesis [10, 32]), HA oligosaccharide treatment resulted in enhanced mRNA expression for aggrecan and HA synthase (HAS-2) [31]. Incorporation of $^{35}S\text{-}SO_4$ into PG was also elevated. Interestingly, the HA oligosaccharides had no effect on CD44 mRNA expression.

In summary, perturbation of CD44-mediated cell-matrix interactions through the use of antisense oligonucleotides or HA oligosaccharides resulted in a chondrocytic chondrolysis cascade. While the effects on biosynthesis were only examined in the study using HA oligosaccharides, interference with CD44 cell-matrix interactions results in more of a 'matrix-remodeling' response. Matrix remodeling would conceivably include chondrolysis coupled with enhanced matrix biosynthesis. Although the exact mechanism for these events is currently unknown, CD44-mediated signal transduction is one possibility. The signal transduction may involve traditional intracellular mediators, such as protein kinases or attachment factors. Alternatively, signals may be transduced via altered receptor organization within the membrane. Loss of extracellular contacts serving to organize CD44s within the membrane may result in a disorganization of the linked intracellular

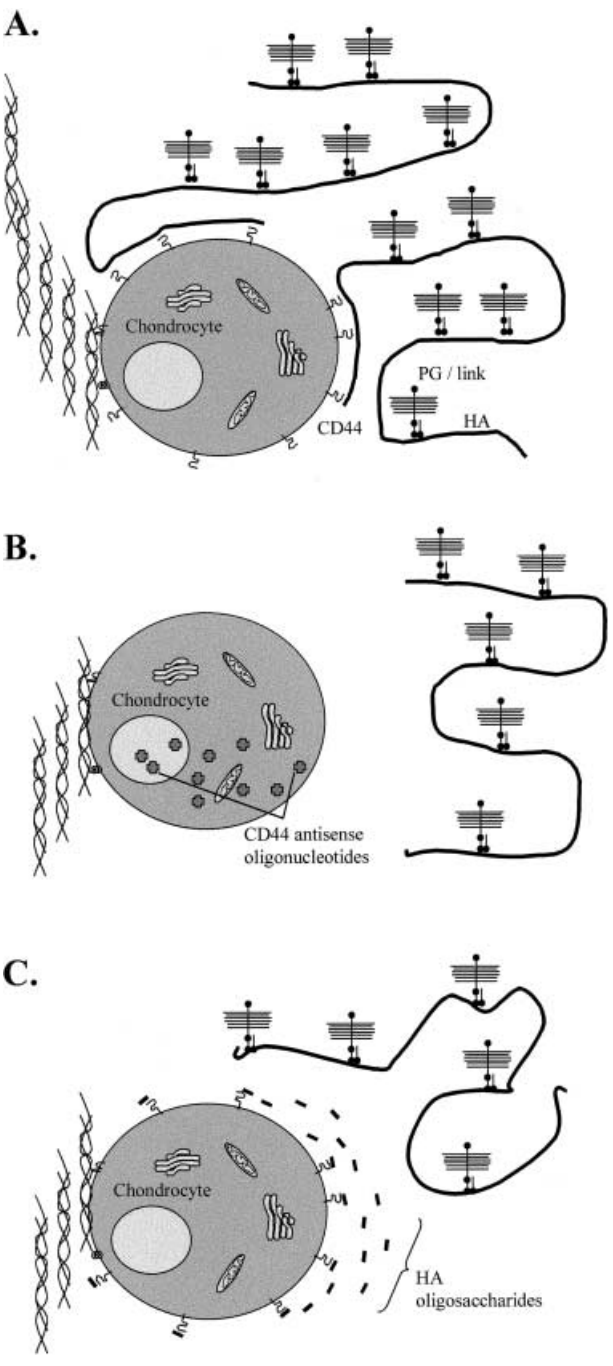

Figure 2. Model of two approaches used to disrupt cell-matrix interactions mediated via CD44, without disrupting cell-matrix interactions mediated via other receptors (e.g., collagen-integrin or collagen-annexin interactions). (*A*). The normal state of a chondrocyte cell-associated matrix. (*B*) CD44 interactions with the HA/PG-rich matrix are disrupted through the introduction of CD44 antisense phosphorothioate oligonucleotides. (*C*) CD44 interactions with the HA/PG-rich matrix are disrupted through the introduction of small HA oligosaccharides.

cytoskeleton. However, regardless of the mechanism, improper cell-matrix interactions mediated via CD44 clearly have the capacity to significantly alter cartilage metabolism. Thus, in reverse, maintaining proper interactions between HA/PG and CD44 is likely critical to the maintenance of cartilage homeostasis.

Chondrocyte integrin expression

The integrins are a large family of cell adhesion receptors that are expressed on most cell types including chondrocytes. Integrins are heterodimeric (one α and one β subunit) transmembrane glycoproteins that contain a large globular extracellular domain capable of binding specific ECM proteins (cell-matrix adhesion) as well as other cell surface receptors (cell-cell adhesion). The integrin cytoplasmic domains interact with cytoskeletal and signaling proteins providing a means by which integrins can mediate changes in cell shape and gene expression in response to matrix changes [33, 34]. At least 20 different integrin heterodimers have been described resulting from the combination of 9 types of β subunits (designated β–β9) with 14 types of α subunits. Two of the major subfamilies of integrins which mediate binding of cells to ECM proteins contain the β1or αV subunits. Chondrocytes have been found to express members of both integrin subfamilies and these integrins mediate adhesion to several different ECM proteins found in cartilage (fig. 3).

Adult articular chondrocytes express $\alpha1\beta1$, $\alpha3\beta1$, $\alpha5\beta1$, and $\alpha10\beta1$ integrins as well as $\alpha V\beta3$ and $\alpha V\beta5$ both in vitro and in situ [5–7, 35]. Relative to adult chondrocytes, fetal chondrocytes [36–38], chondrosarcoma cells [39], and immortalized chondrocytes [40] appear to have higher levels of $\alpha2\beta1$. Fetal chondrocytes also express $\alpha6\beta1$ [36–38]. These various studies of chondrocyte integrin expression have used immunohistochemical methods and/or measurement of integrins after isolation of cells from cartilage. Some caution must be taken when interpreting the results of these studies. The type of fixation and processing used as well as the potential masking of cell surface epitopes by matrix proteins can affect immunohistochemical results. Integrin levels measured on chondrocytes after isolation from cartilage can be affected by the proteolytic conditions needed to remove the cells from their matrix. In addition, changes in chondrocyte integrin expression can occur during cell culture where the levels of certain integrins such as $\alpha1\beta1$ increase [41]. Compared to histologically normal cartilage, increased immunostaining for chondrocyte integrins has been observed in osteoarthritic cartilage, with a particular increase noted in $\alpha1\beta1$ in monkey cartilage with osteoarthritis (OA)-like changes [6] and the appearance of $\alpha2\beta1$ noted in OA cartilage in human femoral heads [42]. These observations suggest that chondrocyte expression of certain integrins may be stimulated by removal or damage of the ECM as seen during cell isolation and OA, respectively. In addition, chondrocyte integrin expression may be affected by the differentiated state of the cell, as seen in the studies of chondrogenesis, chondrosarcoma cells, and immortalized cells.

Treatment of cultured chondrocytes with serum [41, 43] and synovial fluid [43] as well as transforming growth factor (TGF)-β [41], insulin-like growth factor (IGF)-I [41] and IL-1 [44] has been shown to modulate chondrocyte integrin expression. TGF-β increased cell surface expression of the $\alpha5\beta1$ integrin but not $\alpha1\beta1$ while IGF-I was shown to increase expression of both [41]. Changes in cell surface levels of integrins appeared to correlate with changes in adhesion to specific ECM proteins measured in adhesion assays. TGF-β treatment resulted in a 50% decrease in the adhesion of chondrocytes to type VI collagen, while adhesion to type II collagen and fibronectin was stimulated. IGF-1 stimulated adhesion to all three proteins, consistent with the increased levels of $\alpha1$ and $\alpha3/\alpha5$ on the surface of IGF-1-treated cells. These results are consistent with adhesion inhibition studies which indicate that $\alpha1\beta1$ is a primary chondrocyte receptor for type VI collagen [41]. IL-1 was shown to stimulate expression of the αV and $\beta1$ integrin subunits [44]. The effect of IL-1 on expression of other integrin subunits has not been reported. The regulation of chondrocyte integrin expression by growth factors and cytokines as well as the increased expression noted when matrix is removed and the cells are placed in culture suggests an important role for integrins in mediating cell-matrix interactions in cartilage.

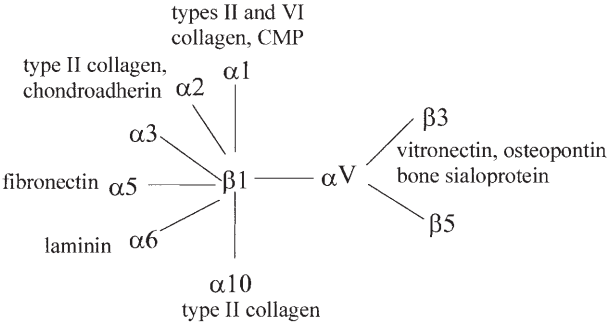

Figure 3. Chondrocyte integrins and their ligands. The members of the $\beta1$ and αV integrin subfamilies reported to be expressed by chondrocytes are shown along with the extracellular matrix proteins which have been demonstrated to bind to the specific integrin subunits. (CMP, cartilage matrix protein).

Chondrocyte integrin-mediated ECM adhesion

Integrins would be expected to directly interact with proteins found in the pericellular matrix. The chondrocyte and pericellular matrix together form a structural unit sometimes called the ‘chondron’ [45]. In addition to HA

and PG discussed above, chondrons have been shown to contain collagen types II, VI, and IX, and fibronectin [45, 46]. Other proteins potentially capable of mediating adhesive interactions in cartilage include tenascin [47], laminin [48], thrombospondin [49], osteopontin [50], cartilage matrix protein [51], and chondroadherin [52]. Several of these proteins, including fibronectin [53], type VI collagen [54], osteopontin [50], tenascin [55], and cartilage matrix protein [56], have been found in greater amounts in OA cartilage, possibly representing increased synthesis of proteins involved in matrix repair. Clearly, HA binding to the CD44 receptor plays an important role in the formation and perhaps repair of the pericellular matrix. The role of integrins in maintaining or repairing the pericellular matrix is less clear.

Cell adhesion assays have been used to determine the specificity of integrin-ECM protein interactions. Chondrocytes were found to use integrins to attach to fibronectin, matrix Gla protein (MGP), types II and VI collagen, vitronectin, osteopontin, and bone sialoprotein II [57]. Other studies have also shown chondrocyte integrin-mediated adhesion to cartilage matrix protein [51], laminin [36], chondroadherin [52], and a collagen-associated protein containing the RGD integrin recognition sequence (RGD-CAP or βig-h3 [58]). By using monoclonal antibodies which block cell adhesion mediated by specific integrin subunits, > 90% of chondrocyte adhesion to fibronectin and types II and VI collagen could be inhibited by an anti-β1 integrin antibody, demonstrating the importance of β1 integrins in binding these proteins [57]. Antibodies to the β3 integrin subunit inhibited > 90% of the adhesion of chondrocytes to osteopontin and about 50% of the adhesion to bone sialoprotein. Using α subunit antibodies, more than 80% of the adhesion of chondrocytes to fibronectin was blocked with an α5 antibody [6] and about 75% of the adhesion to type VI collagen was blocked with an antibody to α1 [40]. Only about 38% of the adhesion to type II collagen was blocked with the α1 antibody [6]. These results indicate that $\alpha1\beta1$ is a primary chondrocyte receptor for type VI collagen, while $\alpha5\beta1$ is a primary chondrocyte fibronectin receptor. Although the $\alpha1\beta1$ integrin partially mediates chondrocyte adhesion to type II collagen, other β1 integrins capable of binding type II collagen must be present. The recent discovery of $\alpha10\beta1$ by affinity chromatography using type II collagen suggests that this integrin may be a key receptor for type II collagen [35]. Blocking antibodies to α10 have not been available to test this directly. Immortalized chondrocytes, which unlike primary chondrocytes express significant levels of $\alpha2\beta1$, can utilize $\alpha2\beta1$ as a type II collagen receptor in addition to $\alpha1\beta1$ [40]. Similar to type VI collagen, cartilage matrix protein (matrilin-1) has been shown to use the $\alpha1\beta1$ chondrocyte integrin for cell adhesion [51], while adhesion to chondroadherin appears to be mediated by $\alpha2\beta1$ [52].

Cell signaling mediated by chondrocyte integrins

The binding of ECM ligands by cells can generate a multitude of complex intracellular signals which has been referred to as 'outside-in signaling' and which results in changes in gene expression and cell function [33, 34]. Integrin signaling is dependent on the formation of cytoskeletal complexes that contain a number of signaling molecules, many of which are activated by phosphorylation of specific tyrosine or serine and threonine residues. Studies on chondrocyte integrin signaling are just beginning to appear in the literature. Clancy [59] demonstrated the assembly of integrin signaling complexes containing F-actin, rho A, and FAK when monolayers of bovine chondrocytes were incubated with fibronectin-coated beads. Treatment with nitric oxide (NO) disrupted formation of the complex, suggesting that NO-induced matrix degradation may result from disrupted integrin signaling. Interestingly, ligation of the $\alpha5\beta1$ integrin with an activating antibody was shown to stimulate NO production as well as prostaglandin E_2, IL-6, and IL-8 by chondrocytes [60]. Autocrine production of IL-1β appeared to mediate the increased production of all of the inflammatory mediators studied. There is evidence for synergy between integrins and cytokines in chondrocyte-mediated cartilage catabolism. Treatment of cartilage explants with fibronectin fragments, which probably act via $\alpha5\beta1$, has been shown to stimulate MMP production and cartilage breakdown [61, 62]. Stimulation of MMP synthesis by RGD peptides or fibronectin fragments was enhanced by IL-1 and inhibited by the IL-1 receptor antagonist [44].

What is the function of chondrocyte integrins?

The functional role of integrins in cartilage has not been completely determined. Given what has been learned about integrins in other tissues, chondrocyte integrins might be expected to help regulate processes involved in cell survival, growth, and differentiation, and matrix remodeling. The studies cited above suggest a role for the $\alpha5\beta1$ integrin in matrix breakdown. Experiments using a chick sternal cartilage culture model have provided evidence that integrins are important in chondrocyte differentiation and survival [63]. Anti-β1 blocking antibodies were found to disrupt cell shape and size, to inhibit type X collagen deposition and sternal growth, and to cause a significant increase in apoptosis. Treating mouse limb bud cells with integrin-blocking antibodies was shown to inhibit chondrogenesis [64]. Similarly, blocking integrin-mediated ECM interactions with RGD peptides was found to inhibit epiphyseal chondrocyte differentiation [65]. Another study suggested that the proliferative response of rabbit sternal chondrocytes to fibroblast growth factor required fibronectin binding to the $\alpha5\beta1$ integrin

[66]. Chondrocyte integrins may work together with growth factor receptors to regulate matrix production. Proteoglycan synthesis by bovine chondrocytes was increased twofold in cells adherent to fibronectin relative to albumin, while a sevenfold increase was noted when the cells were further stimulated with IGF-I [59]. Potential synergy between IGF-I and β1 integrins was also suggested by experiments examining activation of cell signaling proteins including Shc [67].

Potential role of chondrocyte integrins as mechanotransducers

Cartilage is a tissue which is subjected in vivo to frequent and repetitive mechanical loads. There is abundant evidence that mechanical loading produces signals in chondrocytes mediated through the ECM, but the cell receptors responsible for transmitting mechanical signals from the matrix are not known. Given the ability of integrins to integrate the matrix with the cytoskeleton and cell-signaling proteins, these receptors are good candidates for mechanotransducers in cartilage. Early work in this area has provided evidence in support of this hypothesis. In chondrosarcoma cells, mechanical strain was found to increase α5 expression when the cells were adherent to plastic (most likely via fibronectin and vitronectin), while cells adherent to collagen type II increased expression of α2 [39]. Pressure-induced strain produces hyperpolarization of cultured chondrocytes, which was found to be inhibited by RGD peptides and antibodies to the α5 and β1 integrin subunits [68]. The hyperpolarization response appears to be mediated through autocrine signaling by integrin stimulated release of IL-4 [69]. These results fit with the common theme of integrin function in cartilage that is emerging – that integrins work together with growth factors and cytokines to regulate chondrocyte function.

Are integrins involved in the pathogenesis of OA?

At this time, the exact role that integrins might play in the development of the changes occurring in OA cartilage is not clear. The increased levels of certain integrins in OA cartilage, including α1β1 and α2β1 [6, 42], could be part of an attempt by the chondrocyte to repair damaged matrix and so do not necessarily implicate them in mediating matrix damage. There is some evidence that integrin signaling could be disrupted in OA chondrocytes. Mechanical stimulation of chondrocytes by pressure-induced strain, which produced an α5β1-mediated hyperpolarization response in normal chondrocytes, produced a depolarization response in chondrocytes from OA cartilage [70]. The same authors also found that pressure-induced strain increased aggrecan mRNA levels and decreased MMP-3 mRNA in normal but not OA chondrocytes [71]. The increase in aggrecan and decrease in MMP-3 mRNA in normal chondrocytes could be blocked by RGD-containing peptides, suggesting that the response was integrin-mediated and that for unknown reasons, OA chondrocytes were deficient in this response.

There is some evidence that integrins could have a pathogenic role in OA. Stimulation of the chondrocyte α5β1 integrin with activating antibodies resulted in increased production of mediators of cartilage destruction including IL-1 and NO [60]. Increased MMP production was noted after chondrocytes were treated with RGD peptides or fibronectin fragments [44] which likely work by stimulation of α5β1. Fibronectin fragments have been shown to be present in the synovial fluid and cartilage of patients with OA [72] and, as already noted, treatment of cartilage explants with fibronectin fragments results in cartilage breakdown [61, 62]. Therefore, inhibition of the signals generated when α5β1 is stimulated by fibronectin fragments may be a potential therapeutic target in OA. But care will need to be taken not to disrupt the α5β1-mediated signals that may be important for normal cartilage homeostasis.

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