Cartilage destruction by matrix degradation products

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Abstract The progressive destruction of articular cartilage is one of the hallmarks of osteoarthritis and rheumatoid arthritis. Cartilage degradation is attributed to different classes of catabolic factors, including proinflammatory cytokines, aggrecanases, matrix metalloproteinases, and nitric oxide. Recently, matrix degradation products generated by excessive proteolysis in arthritis have been found to mediate cartilage destruction. These proteolytic fragments activate chondrocytes and synovial fibroblasts via specific cell surface receptors that can stimulate catabolic intracellular signaling pathways, leading to the induction of such catalysts. This review describes the catabolic activities of matrix degradation products, especially fibronectin fragments, and discusses the pathologic implication in cartilage destruction in osteoarthritis and rheumatoid arthritis. Increased levels of these degradation products, found in diseased joints, may stimulate cartilage breakdown by mechanisms of the kind demonstrated in the review.

Key words Cartilage destruction · Fibronectin fragment (FN-f) · Integrin · Matrix metalloproteinase (MMP) · Mitogen-activated protein kinase

Introduction

The extracellular matrix of cartilage is primarily composed of the large proteoglycan aggrecan and fibrils containing type-II collagen.1 Type-II collagen, composed of a triple helix of three identical α chains, forms fibrils stabilized by intermolecular crosslinks.2 The fibrils provide tensile strength and serve to constrain the swelling of aggrecan that endows cartilage with its compressive stiffness.3,4 Progressive destruction of cartilage, which results from an imbalance between the anabolic and catabolic processes, is a common feature of rheumatoid arthritis (RA) and osteoarthritis (OA). Proteoglycan loss that is observed in the development of early OA5,6 results in a reduction in cartilage stiffness.7,8 Degradation and loss of type-II collagen, which are observed in RA and OA,9,10 result in an irreversible loss of tensile properties and structural integrity.9 It is well known that proinflammatory cytokines including interleukin-1β (IL-1β) and tumor necrosis factor α (TNFα) have been shown to promote cartilage degradation by stimulating the production of matrix metalloproteinases (MMPs).11

The importance of understanding cell–matrix interactions at the level of regulation of matrix turnover is becoming very apparent. In addition to the proinflammatory cytokines, there is an increasing body of evidence that degradation products of cartilage matrix are another amplifier or catalyst in diseased joints, including RA and OA. This review focuses on the mechanism of cartilage destruction induced by matrix degradation products, especially by fibronectin fragments (FN-fs).

Structure of fibronectin

Fibronectin (FN) is an adhesive dimeric glycoprotein of 450kDa found in the extracellular matrix of many tissues, including normal cartilage12 and synovial membrane.13 It is also present in such body fluids as synovial fluid and plasma. As shown in Fig. 1, FN consists predominantly of three types of homologous repeating segments (designated I, II, and III). Significant protein heterogeneity results from the alternative splicing of a single RNA.14,15 The glycoprotein regulates functions of cellular adhesion and spreading, cell motility, cell growth, and differentiation and opsonization.16 FN contains amino (NH2)-terminal heparin-, gelatin-, cell- and carboxyl (COOH)-terminal heparin-binding domains. The central cell-binding region has an Arg-Gly-Asp (RGD) sequence in domain III10, recognized by several cell surface integrin family members.17 Several sites in the heparin-binding domain that are COOH-terminal to the central
Several peptides from domain III12–14 support cell attachment with varying affinities. The IIICS, or the variable (V) region, contains the integrin-binding sites, CS-1 and CS-5. Fibronectin fragments in OA and RA

Elevated levels of FN are found in OA cartilage and in OA synovial fluid. While FN is ubiquitous within active rheumatoid synovium, enhanced accumulation of FN is found on the inflamed synovial and pannus surfaces in the knee joints of patients with RA. Fibronectin is readily degraded into fragments by proteinases. Thus, activation of extracellular proteolysis in OA and RA may lead to the fragmentation of FN, indicating that FN-fs could be generated in vivo within cartilage and synovial fluid. Indeed, increased levels of FN-fs of 30–200kDa are found in cartilage and synovial fluid from patients with OA and RA. In OA synovial fluids, FN-fs of 100–200kDa are found at approximately 1µM. The levels of FN-fs in OA cartilage are suggested to be similar to those in OA synovial fluids. The FN-f concentrations that have been used in in vitro studies are less than 1µM, similar to the levels in in vivo diseased joints. Since FN-fs can penetrate into cartilage tissue in vitro, FN-fs in OA and RA cartilages may include the fragments from synovial fluid.

**Proteoglycan degradation by fibronectin fragments**

Increased aggrecan degradation is commonly observed in OA and RA. Homandberg et al. demonstrated for the first time that NH2-terminal heparin-, NH2-terminal gelatin-, and central cell-binding FN-fs enhance proteoglycan loss from bovine cartilage and decrease proteoglycan synthesis. In addition, COOH-terminal heparin-binding FN-f can promote loss of proteoglycan in bovine cartilage. Degradation of aggrecan that occurs early in cartilage damage is caused by aggrecanases and MMPs. Neoepitope antibodies specific for aggrecanase- or MMP-degraded aggrecan fragments distinguish between these activities in vivo. Anti-ITEGE373 and anti-A7RGSV antibodies identify aggrecanase cleavage site in the aggrecan interglobular domain, whereas anti-DIPEN and anti-F24FGVG antibodies detect MMP cleavage site in the same region. Both sites of aggrecanase cleavage are found in OA and RA cartilage. Treatment with NH2-terminal gelatin-binding FN-f results in the generation of aggrecanase-derived ITEGE373 neoepitope in porcine cartilage. Amino acid sequencing of aggrecan from cartilage with treatment with NH2-terminal heparin-binding FN-f also confirms cleavage at the aggrecanase site in bovine cartilage. However, there is no direct evidence on aggrecanase induction by FN-f in chondrocytes. In contrast to aggrecanase-derived neoepitope, levels of MMP-derived DIPEN neoepitope are unchanged in the FN-f-treated cartilage.
Type-II collagen degradation by fibronectin fragments

Matrix metalloproteinases are a family of zinc-dependent enzymes that mediate the turnover of extracellular matrix proteins. Upregulation of MMPs has been implicated in numerous pathologic processes, including OA and RA. The MMP family is classified into gelatinases, which degrade type-IV collagen and other basement membrane proteins; collagenases, which degrade the stromal fibrillar collagens (types I, II, and III); and others, which degrade additional matrix components. Of MMPs, collagenases are particularly important because of their ability to cleave fibrillar collagen, the most abundant component of the extracellular matrix. MMP-1 (collagenase-1) is expressed ubiquitously and is found in various cells, including fibroblasts, chondrocytes, and multiple tumor cells. MMP-8 (collagenase-2) is expressed mainly in neutrophils. MMP-13 (collagenase-3) exhibits the broadest substrate specificity of the collagenases, with the highest activity against type-II collagen, the main collagen in cartilage. Furthermore, MMP-13 degrades types I, III, IV, X, and XIV collagen, fibronectin, and aggrecan core protein. While MMP-13 expression is restricted to bone development and bone maintenance under normal physiologic conditions, it is upregulated under pathologic conditions like in OA chondrocytes, rheumatoid synovium, and tumor cells. MMP-2 and MMP-9 are widely expressed and are best known as gelatinases.

Werb et al. demonstrated for the first time that treatment of cultured rabbit synovial fibroblasts with the central cell-binding FN-f stimulates expression of MMP-1 and MMP-3. The FN-f can induce MMP-13 in human chondrocytes. NH2-terminal gelatin-binding FN-f enhances MMP-3 release in porcine chondrocytes. NH2-terminal heparin-binding FN-f enhances MMP-3 and gelatinase expressions. While treatment with COOH-terminal heparin-binding FN-f results in increased production of MMP-3 and MMP-13 in bovine cartilage, the FN-f stimulates production of MMP-1, MMP-2, MMP-9, and MMP-13 in human cartilage. The COOH-terminal heparin-binding FN-f also induces MMP-1, MMP-3, and MMP-13 in RA synovial fibroblasts. In association with MMP production, the immunohistochemistry for detection of type-II collagen cleavage by collagenase has demonstrated that COOH-terminal heparin-binding FN-f enhances collagenase-mediated cleavage of type-II collagen in human cartilages. Matrix metalloproteinase-13 is a candidate collagenase responsible for the cleavage of type-II collagen because MMP-13 inhibitor can suppress the FN-f-induced collagen cleavage.

Nitric oxide production by fibronectin fragments

Nitric oxide (NO) is a short-lived free radical that is synthesized enzymatically from L-arginine by a family of NO synthase (NOS) isoenzymes. Nitric oxide is produced by a variety of cells, including chondrocytes. Inducible NOS (iNOS) is expressed in response to bacterial endotoxin and proinflammatory cytokines such as IL-1. Once synthesized, iNOS generates large amounts of NO. Inducible NOS is strongly expressed in synovium and cartilage of patients with inflammatory joint diseases. NO acts principally as a proinflammatory and destructive mediator. The pathogenic role of NO in arthritis is certainly supported by the observation that inhibitors of NOS can suppress the development of disease in animal models, such as adjuvant arthritis and streptococcal cell wall arthritis. Of FN-fs, NH2-terminal heparin-binding FN-f has been shown to stimulate NO production in association with iNOS induction in human normal chondrocyte monolayer cultures. Another FN-f, COOH-terminal heparin-binding FN-f, also causes increased NO production in RA cartilage.

Cytokine production by fibronectin fragments

The early phase of cartilage degradation is associated with enhanced release of proinflammatory cytokines. In human cartilage NH2-terminal heparin-binding FN-f stimulates a pulsed release of TNFα and IL-1β, followed by a decrease in a few days. Enhanced release of IL-6 occurs earlier and continues for three weeks. IL-1α release shows a lag period.

Although cell responses to FN-fs and proinflammatory cytokines including IL-1 are qualitatively similar, the involvement of cytokines in FN-f effects is controversial. The cytokine release by FN-f could partly account for the catabolic effects of NH2-terminal heparin-binding FN-f on MMP-3 production and proteoglycan synthesis in human cartilage because antibodies to these cytokines partially block the FN-f activities. Inhibition of FN-f effects with IL-1 receptor antagonist indicates that IL-1 could mediate type-II collagen cleavage by collagenase stimulated with COOH-terminal heparin-binding FN-f in bovine cartilage and MMP-3 synthesis enhanced by RGD-containing peptide of central cell-binding FN-f in rabbit chondrocytes. In contrast, MMP induction by NH2-terminal gelatin-binding FN-f in porcine chondrocytes and by COOH-terminal heparin-binding FN-f in RA synovial fibroblasts is not via an IL-1 autocrine loop. Nitric oxide production by NH2-terminal heparin-binding FN-f in human chondrocytes is also IL-1-independent.

**Receptors for fibronectin fragments**

Cell–matrix interactions control cell function and behavior by signal transduction through a variety of cell surface receptors. FN can bind several integrins and other cell surface protein ligands.

**Integrin**

Integrins are heterodimeric transmembrane proteins consisting of α and β subunits. Integrins bind extracellular matrix molecules and mediate cell adhesion, migration, and
invasion during development, tissue repair, tumor invasion, and metastasis. In concert with growth factor or cytokine receptors, integrins regulate cell proliferation, differentiation, and survival. Integrins also serve as cell surface receptors that transduce intracellular signals. Although the cytoplasmic domains of the integrin α and β subunits have no intrinsic enzymatic activity, integrin signaling is achieved by coupling signaling molecules to the cytoplasmic and transmembrane domains of the integrin subunits. Integrins activate signaling pathways that are either common to all integrins or heterodimer-specific. The cytoplasmic domains of α subunits may trigger signaling events that are specific for each individual integrin heterodimer.

There is evidence that integrin regulates FN-f action. FN can bind α5β1 integrin through the cell-binding domain in III10 via RGD sequence (Fig. 1). Matrix metalloproteinase production by the central cell-binding FN-f is probably mediated by α5β1 integrin because anti-α5β1 integrin antibody and RGD-containing peptide induce MMP-1 and gelatinase in rabbit synovial fibroblasts. The cell-binding FN-f and anti-α5β1 integrin antibody can increase MMP-13 production in human chondrocytes. Recent studies using antisense oligonucleotides to α5 integrin subunit have also shown the involvement of α5-α5 integrin in cartilage proteoglycan degradation induced by NH2-terminal heparin-binding and NH2-terminal gelatin-binding FN-fs without cell-binding RGD sequence in addition to the central cell-binding FN-f. These two NH2-terminal FN-fs can be chemically cross-linked to α5 integrin subunit in chondrocytes. However, employment of α5β1 integrin by NH2-terminal heparin-binding FN-f remains to be investigated because blocking antibodies to α5 or β1 integrin subunit fail to inhibit the FN-f-stimulated NO production. Integrin α5β1 is the primary receptor involved in the assembly of dimeric fibronectin into the extracellular matrix.

Rheumatoid arthritis synovial fibroblasts at the cartilage–pannus junction express integrin subunits α4, α5, and β1. Integrin α4β1 recognizes CS-1 in alternatively spliced IIIICS domain of FN. Inhibition of MMP production with anti-α4 integrin antibody indicates that COOH-terminal heparin-binding FN-f, which contains CS-1 (Fig. 1), stimulates MMP-1, MMP-3, and MMP-13 in RA synovial fibroblasts via α4β1 integrin. Indeed, CS-1 peptide induces these MMPs in the cells. Since α4β1 integrin is newly expressed on articular chondrocytes in OA cartilage, the COOH-terminal heparin-binding FN-f may work via the integrin in OA chondrocytes.

Excessive amounts of RGD peptide are required to induce proteoglycan release in cartilage explant culture while the central cell-binding FN-f at the same level causes stronger release of proteoglycan. Compared with CS-1 peptide, the COOH-terminal heparin-binding FN-f can induce greater levels of MMPs. Thus, FN-f could activate integrins more effectively than synthetic peptides.

CD44

Another cell surface receptor that could mediate FN-f action is CD44, a principal hyaluronan receptor. The CD44 gene has 20 exons, 12 of which may be alternatively spliced to produce a number of different isoforms. Restricted expression of CD44 isoforms and post-translational glycosylation of the parent protein provide diverse functions of CD44. Of CD44 isoforms, CD44H is commonly expressed in human articular chondrocytes. Although CD44H is predominant, mRNA containing V3 exon of CD44 is also found in chondrocytes. The diversity of CD44 is further amplified by the differential use of glycosaminoglycan attachment sites on its extracellular domain. While chondroitin sulfate proteoglycan is attached to the membrane proximal portion of external domain of CD44H, heparan sulfate proteoglycan can bind CD44 at V3 in the membrane proximal extracellular domain of CD44v. Chondroitin sulfate and heparan sulfate proteoglycans employ identical or overlapping binding sites in the repeats III13 and III14 of COOH-terminal heparin-binding FN-f (Fig. 1). The COOH-terminal heparin-binding domain of FN is known to bind CD44. While MMP production is up-regulated by COOH-terminal heparin-binding FN-f in human articular cartilage, anti-CD44 antibody can block the enhanced MMP production. Suppression of the FN-f-stimulated MMP production by peptide V suggests that the peptide V domain, a binding site of COOH-terminal heparin-binding FN-f for cell surface heparan sulfate proteoglycan, is required for the FN-f-activated MMP induction. Thus, COOH-terminal heparin-binding FN-f may directly bind glycosaminoglycans on CD44 through the peptide V sequence, resulting in MMP induction.

CD44 is upregulated in articular cartilage from patients with OA and RA. Compared with normal cartilage, RA cartilage produces higher NO in response to the COOH-terminal heparin-binding FN-f. Anti-CD44 treatment using the monoclonal anti-CD44 antibody and the peptide V reveals that NO production enhanced by COOH-terminal heparin-binding FN-f is mediated by CD44 in RA cartilage. The inhibitory effects of anti-CD44 treatment are stronger in RA cartilage than in normal one, probably because CD44 is upregulated in RA cartilage and the proportion of CD44-positive chondrocytes is significantly higher than that in normal cartilage. These findings indicate that increased NO production by COOH-terminal heparin-binding FN-f in RA cartilage is associated with elevated levels of CD44 on chondrocytes under such pathologic conditions. Of interest, FN-fs themselves may upregulate CD44 on chondrocytes because NH2-terminal heparin-binding FN-f enhances CD44 expression in chondrocytes cultured in alginate beads, which allows abundant cartilage matrix deposition around chondrocytes like in vivo cartilage.
Intracellular signaling pathways activated by fibronectin fragments

Some FN-fs have been shown to activate the intracellular signaling pathways, mitogen-activated protein kinase (MAPK) and nuclear factor (NF)-κB pathways, leading to cartilage destruction.

Mitogen-activated protein kinase pathway

Activator protein-1 (AP-1), which includes members of the Jun and Fos families, is a pivotal transcriptional factor that regulates the production of cytokines and MMPs. The upstream regulatory regions of MMP genes contain the AP-1 recognition site. Another activator protein-1 can be activated by protein kinases that phosphorylate specific amino acid residues, especially by MAPK families. Three major MAPK families have been identified: extracellular signal-regulated kinase (ERK), p38 MAPK, and c-Jun NH₂-terminal kinase (JNK). All the three MAPK pathways are involved in the transcriptional regulation of Fos and Jun family genes.

The central cell-binding FN-f activates ERK, p38, and JNK, and increases the production of MMP-13 and gelatinases by human articular chondrocytes. Another fibronectin fragment, NH₂-terminal heparin-binding FN-f, stimulates NO production in association with the activation of ERK, p38, and JNK in human chondrocytes. Furthermore, collagenase induction leading to type-II collagen breakdown by COOH-terminal heparin-binding FN-f involves all the three MAPK pathways in human articular cartilage. The COOH-terminal heparin-binding FN-f also causes phosphorylation of ERK1/2, JNK, and p38 for MMP production in RA synovial fibroblasts and in human natural killer cells.

Individual MAPK pathways may play different roles in the production of individual MMPs in response to FN-f. In RA synovial fibroblasts ERK seems to be involved in MMP-1, MMP-3, and MMP-13 production with COOH-terminal heparin-binding FN-f stimulation, whereas p38 MAPK may contribute to MMP-3 induction by the FN-f. JNK seems to promote the production of MMP-1 and MMP-13 in the FN-f-stimulated RA synovial fibroblasts.

Different fragments of fibronectin may activate different isoforms of JNK. In human normal chondrocytes, NH₂-terminal heparin-binding FN-fs have been shown to individually activate ERK1/2 and p38. The NH₂-terminal heparin-binding FN-f activates JNK1 whereas the central cell-binding fragment induces the activation of JNK1/2. In contrast, the COOH-terminal heparin-binding FN-f activates JNK2.

Coupling of integrin receptors to MAPK pathways has been reported. Anti-α5β1 integrin antibody can activate ERK, p38, and JNK1/2 in human chondrocytes. In addition, CS-1 peptide, which binds α5β1 integrin, causes the phosphorylation of these three MAPKs in RA synovial fibroblasts. Thus, some integrin-binding FN-fs may employ integrin as a signaling receptor for MAPK activation. Upstream events in activation of MAPK cascades in association with FN-f stimulation remain to be clarified.

Nuclear factor-κB pathway

Nuclear factor-κB is another key regulator for MMPs. Activation of NF-κB is dependent on the phosphorylation and degradation of IκB, an endogenous inhibitor that binds to NF-κB in the cytoplasm. The released NF-κB then translocates to the nucleus, where it binds to specific NF-κB DNA binding sites and initiates gene expression including MMPs.

In contrast to MAPKs, NF-κB activation by FN-fs has rarely been studied. Phosphorylation of IκB by anti-α5β1 antibody suggests that the integrin-binding FN-fs could...
activate the NF-κB pathway. Our preliminary study using NF-κB inhibitor showed that NF-κB could be involved in collagenase production and type-II collagen cleavage by collagenase in human cartilage with treatment with COOH-terminal heparin-binding FN-f (Fig. 2). Further investigations will be required to clarify the involvement of NF-κB in catabolic actions of FN-fs.

Other degradation products of cartilage

Other fragments of matrix component can affect chondrocyte metabolism. For instance, fibrillar collagens and their degradation products influence cell-mediated proteolysis. Native and denatured forms of type-II collagen stimulate interstitial collagenase production by skin fibroblast. Human monocyes produce elevated levels of IL-1 when exposed to native type-II collagen. Synovial fluid mononuclear cells from patients with RA produce cytokines such as IL-1, IL-6, and TNFα in response to exposure to type-II collagen. The CB11 peptide of type-II collagen can stimulate increased IL-1 production by monocytes/macrophages. Jennings et al. have shown that mixed cleavage products of type-II collagen and those extracted from articular cartilage could induce proteolytic cartilage resorption at the level of proteoglycan degradation, and inhibit matrix synthesis. Cyanogen bromide-cleaved fragments of type-II collagen can cause increased cleavage of type-II collagen by collagenase in chondrocyte pellet cultures. Furthermore, hyaluronan hexasaccharides induce proteoglycan loss, suppression of proteoglycan synthesis, decreased aggregation of aggrecan, and gelatinase activity. Hyaluronan fragments also stimulate NO production through iNOS activation in articular chondrocytes.

Conclusion

Increased fragments from matrix degradation could play an important role in cartilage destruction in arthritis. These fragments activate chondrocytes and synovial fibroblasts, leading to the induction of MMPs, NO, and cytokines. Catabolic activities by FN-fs are probably mediated by cell surface receptors such as integrins that can stimulate catabolic intracellular signals, including MAPK (Fig. 3). Thorough understanding of the mechanism driven by matrix degradation products may contribute to prevention of cartilage destruction in OA and RA.

References

1. Poole AR. Cartilage in health and disease. In: Koopman WJ, editor. Arthritis and allied conditions: a textbook of rheumatology, 14th ed. vol 1. Baltimore: Lippincott, Williams and Wilkins; 2001. p. 226–84.
2. Eyre DR. Collagen cross-linking amino acids. Methods Enzymol 1987;144:115–39.
3. Kempson GE, Muir H, Poldur C, Tuke M. The tensile properties of the cartilage of human femoral condyles related to the content of collagen and glycosaminoglycans. Biochim Biophys Acta 1973;297:456–72.
4. Mow VC, Setton LA, Ratcliffe DS, Howell DS, Buckwalter JA. Structure-function relationships of articular cartilage and the effects of joint instability and trauma on cartilage function. In: Brandt KD, editor. Cartilage changes in osteoarthritis. Indiana School of Medicine/Ciba-Geigy; 1990. p. 22–42.
5. Carney SL, Billingham MEJ, Muir H, Sandy JD. Demonstration of increased proteoglycan turnover in cartilage explants from dogs with experimental osteoarthritis. J Orthop Res 1984;2:201–6.
6. McDevitt CA, Muir H. Biochemical changes in the cartilage of the knee in experimental and natural osteoarthritis in the dog. J Bone Joint Surg [Br] 1976;58:94–101.
7. Bonassar LJ, Frank EH, Murgio CG, Moore VL, Lark MW, et al. Changes in cartilage composition and physical properties due to stromelysin degradation. Arthritis Rheum 1995;38:173–83.
8. Kempson GE. Mechanical properties of articular cartilage. In: Freeman MAR, editor. Adult articular cartilage. Tunbridge Wells: Pitman Medical; 1979. p. 333–414.
9. Dodge G, Poole AR. Immunohistochemical detection and immunohistochemical analysis of type II collagen degradation in human normal, rheumatoid and osteoarthritic articular cartilages and in explants of bovine articular cartilage cultured with interleukin-1. J Clin Invest 1989;83:647–61.
10. Hollander AP, Pidoux I, Reiner A, Rorabeck C, Bourne R, Poole AR. Increased damage to type II collagen in osteoarthritic articular cartilage detected by a new immunoassay. J Clin Invest 1994;93:1722–32.
11. Arend WP, Dayer JM. Inhibition of the production and effects of interleukin-1 and tumor necrosis factor alpha in rheumatoid arthritis. Arthritis Rheum 1995;38:151–60.
12. Burton-Wurster N, Butler M, Harter S, Colombo C, Quintavalla J, Swartzendurber D, et al. Presence of fibronectin in articular cartilage in two animal models of osteoarthritis. J Rheumatol 1986;13:175–82.
13. Lavietes BB, Carson S, Diamond HS, Laskin RS. Synthesis, secretion, and deposition of fibronectin in cultured human synovium. Arthritis Rheum 1985;28:1016–26.
14. Hynes RO. Fibronectins. Berlin Heidelberg New York: Springer; 1990.
15. Schwarzbaumer JE. Alternative splicing of fibronectin: three variants, three functions. BioEssays 1991;13:527–33.
16. Ruoslahti E. Fibronectin and its receptors. Annu Rev Biochem 1988;57:375–413.
17. Yamada KM. Adhesive recognition sequences. J Biol Chem 1991;266:12809–12.
18. McCarthy JB, Chellberg MK, Mickelson DJ, Furcht LT. Localization and chemical synthesis of fibronectin peptides with melanoma adhesion and heparin binding activities. Biochemistry 1988;27:1380–8.
19. Drake SL, Klein DJ, Mickelson DJ, Oegema TR, Furcht LT, McCarthy JB. Cell surface phosphatidylinositol-anchored heparan sulfate proteoglycan initiates mouse melanoma cell adhesion to a fibronectin-derived, heparin-binding synthetic peptide. J Cell Biol 1992;117:1331–41.
20. Iida J, Skubitz AP, Furcht LT, Wayner EW, McCarthy JB. Coordinate role for cell surface chondroitin sulfate proteoglycan and \( \alpha \beta 1 \) in mediating melanoma cell adhesion to fibronectin. J Cell Biol 1992;118:1181–94.
21. Lories V, Cassiman JJ, van der Bergh E, David G. Differential expression of cell surface heparan sulfate proteoglycans in human mammary epithelial cell and fibroblasts. J Biol Chem 1992;267:1116–22.
22. Woods A, McCarthy JB, Furcht LT, Couchman JR. A synthetic peptide from the COOH-terminal heparin-binding domain of fibronectin promotes focal adhesion formation. Mol Biol Cell 1993:4:605–13.
23. Giuseppetti JM, McCarthy JB, Letourneau PC. Isolation and partial characterization of a cell-surface heparan sulfate proteoglycan from embryonic rat spinal cord. J Neurosci Res 1994;37:893–910.
24. Boehler EA, Garcia-Pardo A, Humphries MJ, McDonald JA, Carter WG. Identification and characterization of the \( \alpha \beta 1 \) lymphocyte adhesion receptor for an alternative cell attachment domain (CS-1) in plasma fibronectin. J Cell Biol 1989;109:1321–30.
25. Guan J-L, Hynes RO. Lymphoid cells recognize an alternatively spliced segment of fibronectin via the integrin receptor \( \alpha \beta 1 \). Cell 1990;60:53–61.
26. Mould AP, Humphries MJ. Identification of a novel recognition sequence for the \( \alpha \beta 1 \) in the COOH-terminal heparin-binding domain of fibronectin. EMBO J 1991;10:4089–95.
27. Mould AP, Wheldon LA, Koriyama A, Wayner EA, Yamada KM, Humphries MJ. Affinity chromatographic isolation of the melanoma adhesion receptor for the IICS region of fibronectin recognized by the integrin \( \alpha \beta 1 \). J Biol Chem 1990;265:4020–4.
28. Mould AP, Koriyama A, Yamada KM, Humphries MJ. The CS5 peptide is a second site in the IICS region of fibronectin recognized by the integrin \( \alpha \beta 1 \). J Biol Chem 1991;266:3579–85.
29. Miller DR, Mankin HJ, Shoji H, D’Ambrosia RD. Identification of fibronectin in preparations of osteoarthritic human cartilage. J Cell Biol 1984;104:267–75.
30. Jones KL, Brown M, Ali SY, Brown RA. An immunohistochemical study of fibronectin in human osteoarthritic and disease-free articular cartilage. Ann Rheum Dis 1987;46:809–15.
31. Homandberg GA, Wen C, Hui F. Cartilage damaging activities of fibronectin fragments derived from cartilage and synovial fluid. Osteoarthritis Cartilage 1998;6:231–44.
32. Xie DL, Meyers R, Homandberg GA. Fibronectin fragments in osteoarthritic synovial fluid. J Rheumatol 1992;19:1448–52.
33. Scott DL, Delamare JP, Walton KW. The distribution of fibronectin in the pannus in rheumatoid arthritis. Br J Exp Pathol 1981;62:362–68.
34. Shiozawa S, Ziff M. Immunoelectron microscopic demonstration of fibronectin in rheumatoid pannus and at the cartilage-pannus junction. Ann Rheum Dis 1983;42:54–63.
35. Xie DL, Homandberg GA. Fibronectin fragments bind to and penetrate cartilage tissue resulting in proteinase expression and cartilage damage. Biochim Biophys Acta 1993;1182:189–96.
36. Homandberg GA, Meyers R, Xie DL. Fibronectin fragments cause chondrolysis of bovine articular cartilage slices in culture. J Biol Chem 1992;267:3597–604.
37. Yasuda T, Poole AR. A fibronectin fragment induces type II collagen degradation by collagenase through an interleukin-1-mediated pathway. Arthritis Rheum 2002;46:138–48.
38. Homandberg GA, Meyers R, Williams JM. Intraarticular injection of fibronectin fragments causes severe depletion of cartilage proteoglycans in vivo. J Rheumatol 1993;20:1378–82.
(matrix metalloproteinase-13) production by human articular chondrocytes. Arthritis Rheum 2002;46:2686–76.

60. Xie D-L, Hui F, Meyers R, Homandberg GA. Cartilage chondrolysis by fibronectin fragments is associated with release of several proteinases: stromelysin plays a major role in chondrolysis. Arch Biochem Biophys 1994;311:205–12.

61. Yasuda T, Poole AR, Shimizu M, Nakagawa T, Julovi SM, Tamamura H, et al. Involvement of CD44 in induction of matrix metalloproteinases by a carboxyl-terminal heparin-binding fragment of fibronectin in human articular cartilage in culture. Arthritis Rheum 2003;48:1271–80.

62. Yasuda T, Shimizu M, Nakagawa T, Julovi SM, Nakamura T. Matrix metalloproteinase production by COOH-terminal heparin-binding fibronectin fragment in rheumatoid synovial cells. Lab Invest 2003;83:153–62.

63. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. N Engl J Med 1993;329:2002–12.

64. Nathan C. Nitric oxide as a secretory product of mammalian cells. FASEB J 1992;6:3051–64.

65. Stadler J, Stefanovic-Racic M, Billiar TR, Curran RD, McIntyre LA, Georgescu HI, et al. Articular chondrocytes synthesize nitric oxide in response to cytokines and lipopolysaccharide. J Immunol 1991;147:3915–20.

66. Sakurai H, Kohsaka H, Liu MF, Higashiyama H, Hirata Y, Kanno K, et al. Nitric oxide production and inducible nitric oxide synthase expression in inflammatory arthritis. J Clin Invest 1995;96:2357–63.

67. Ialenti A, Moncada S, Di Rosa M. Modulation of adjuvant arthritis by endogenous nitric oxide. Br J Pharmacol 1993;110:701–6.

68. McCarthy-Francis N, Allen JB, Mizej DE, Albina JE, Xie QW, Nathan CF, et al. Suppression of arthritis by an inhibitor of nitric oxide synthase. J Exp Med 1993;178:749–54.

69. Gemb a T, Valbracht J, Alsalameh S, Lotz M. Focal adhesion kinase and mitogen-activated protein kinases are involved in chondrocyte activation by the 29-kDa amino-terminal fibronectin fragment. J Biol Chem 2002;277:907–11.

70. Yasuda T, Kakinuma T, Julovi SM, Yoshida M, Hiramitsu T, Akiyoshi M, et al. COOH-terminal heparin-binding fibronectin fragment induces nitric oxide production in rheumatoid cartilage through CD44. Rheumatology (Oxford) 2004;43:1116–20.

71. Homandberg GA, Hui F, Wen C. Association of proteoglycan degradation with catabolic cytokine and stromelysin release from cartilage cultured with fibronectin fragments. Arch Biochem Biophys 1996;334:325–31.

72. Homandberg GA, Hui F, Wen C, Purple C, Bewsky K, Koeppe H, et al. Fibronectin-fragment-induced cartilage chondrolysis is associated with release of catabolic cytokines. Biochem. J 1997;321:751–7.

73. Bewsky KE, Wen C, Purple C, Homandberg GA. Fibronectin fragments induce the expression of stromelysin-1 mRNA and protein in bovine chondrocytes in monolayer culture. Biochem Biophys Acta 1996;1317:55–64.

74. Arner EC, Tortorella MD. Signal transduction through chondrocyte integrin receptors induces matrix metalloproteinase synthesis and synergizes with interleukin-1. Arthritis Rheum 1995;38:1304–14.

75. Johansson S, Svineng G, Wennerberg K, Armulik A, Lohikangas L. Fibronectin-integrin interactions. Front Biosci 1997;2:D126–46.

76. Hynes RO. Integrins: versatility, modulation, and signaling in cell adhesion. Cell 1992;69:11–25.

77. Giancotti FG, Mainiero F. Integrin-mediated adhesion and signaling in tumorigenesis. Biochem Biophys Acta 1994;1198:47–64.

78. Clark EA, Brugge JS. Integrins and signal transduction pathway: the road taken. Science 1995;268:233–9.

79. Schwarz MA, Scaller MD, Ginsberg MH. Integrins: emerging paradigms of signal transduction. Annu Rev Cell Dev Biol 1995;11:549–99.

80. Giancotti FG, Ruoslahti E. Integrin signaling. Science 1999;285:1028–32.

81. Yamada KM, Miyamoto S. Integrin transmembrane signaling and cytoskeletal control. Curr Opin Cell Biol 1995;7:681–9.

82. Giancotti FG. Integrin signaling: specificity and cell cycle progression. Curr Opin Cell Biol 1997;9:691–700.

83. Zhang Z, Vuori K, Reed JC, Ruoslahti E. The α5β1 integrin supports survival of cells on fibronectin and up-regulates Bcl-2 expression. Proc Natl Acad Sci USA 1995;92:6161–5.

84. Saxtry SK, Lakonishok M, Thomas DA, Muschler J, Horwitz AF. Integrin α subunit ratios, cytoplasmic domains, and growth factor synergy regulate muscle proliferation and differentiation. J Cell Biol 1996;133:169–84.

85. Yamada KM. Adhesive recognition sequences. J Biol Chem 1991;266:12809–12.

86. Damsky CH, Werb Z. Signal transduction by integrin receptors for extracellular matrix: cooperative processing of extracellular information. Curr Opin Cell Biol 1992;4:772–81.

87. Homandberg GA, Costa V, Ummadi V, Pichika R, Antiensense oligonucleotides to the integrin receptor subunit αβ5 decrease fibronectin fragment mediated cartilage chondrolysis. Osteoarthritis Cartilage 2002;10:381–93.

88. Homandberg GA, Costa V, Wen C. Fibronectin fragments active in chondrocytic chondrolysis can be chemically cross-linked to the αβ5 integrin receptor subunit. Osteoarthritis Cartilage 2002;10:938–49.

89. Dzamba BJ, Bullmann H, Akiyama SK, Peters DM. Substrate-specific binding of the amino terminus of fibronectin to an integrin complex in focal adhesions. J Biol Chem 1994;269:19646–52.

90. McKown-Longo PJ, Mosher DF. Interaction of the 70,000-mol-wt amino-terminal fragment of fibronectin with the matrix-assembling receptor of fibroblasts. J Cell Biol 1985;100:364–74.

91. Quade BJ, McDonald JA. Fibronectin’s amino-terminal matrix assembly site is located within the 29-kDa amino-terminal domain containing five type I repeats. J Biol Chem 1988;263:19602–9.

92. Schwarzrauer JE. Identification of the fibroectin sequences required for assembly of a fibrillar matrix. J Cell Biol 1991;113:1463–73.

93. Sottile J, Schwarzrauer J, Selegee J, Mosher DF. Five type I modules of fibronectin form a functional unit that binds to fibroblasts and Staphylococcus aureus. J Biol Chem 1991;266:12840–3.

94. Ishikawa H, Hirata S, Nishibayashi Y, Imura S, Kudo H, Ohno O. The role of adhesion molecules in synovial pannus formation in rheumatoid arthritis. Clin Orthop 1994;309:297–303.

95. Wayner EA, Garcia-Pardo A, Humphries MJ, McDonald A, Carter WG. Identification and characterization of the T lymphocyte adhesion receptor for an alternative cell attachment domain (CS–1) in plasma fibronectin. J Cell Biol 1989;109:1321–30.

96. Guan J-L, Hynes RO. Lymphoid cells recognize an alternatively spliced segment of fibronectin via the integrin receptor αβ1. Cell 1990;60:53–61.

97. Lapadula G, Iannone F, Zuccaro C, Grattagliano V, Covell M, Patella V, et al. Integrin expression on chondrocytes: Correlation with the degree of cartilage damage in human osteoarthritis. Clin Exp Rheumatol 1997;15:247–54.

98. Ostergaard K, Salter M, Petersen J, Bendtzen K, Hvalris J, Andersen CB. Expression of α and β subunits of the integrin superfamily in articular cartilage from macroscopically normal and osteoarthritic human femoral heads. Ann Rheum Dis 1998;57:303–8.

99. Aruffo A, Stamenkovic I, Melnick M, Underhill CB, Seed B. CD44 is the principal cell surface receptor for hyaluronan. Cell 1991;65:1303–13.

100. Screraton GR, Bell MV, Jackson DG, Cornelis FB, Georgescu HI, et al. Analysis of human articular chondrocyte CD44 isoform expression and function in health and disease. J Pathol 1996;179:10:938–49.

101. Salter DM, Godolphin JL, Gourlay MS, Lawson MF, Hughs DE, Dunne E. Expression and function of the lymphocyte homing receptor α4β1 in synovial tissue from patients with rheumatoid arthritis. Curr Opin Immunol 1998;10:938–49.

102. Bennett KL, Jackson DG, Simon JC, Tanczos E, Peach R, Modrell B, et al. CD44 isoforms containing exon V3 are responsible for the presentation of heparin-binding growth factor. J Cell Biol 1995;128:687–98.
