Monitoring Cartilage Turnover

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In arthritic diseases, the stability of the extracellular matrix of articular cartilage is compromised by extensive proteolytic breakdown associated with alterations of synthesis of the proteins of the tissue leading to cartilage loss. This article reviews developments in assays of biochemical markers of cartilage matrix turnover and studies investigating their use. Because type II collagen and aggrecan are the most abundant proteins of the cartilage matrix, current biochemical markers are based mainly on immunologic reagents detecting their synthesis and degradation. Clinical studies indicate that some markers of type II collagen may be useful to predict disease progression in osteoarthritis and rheumatoid arthritis. Conversely, major achievements have been made in the development of immunoassays detecting the various fragments of aggrecan released by matrix metalloproteases or aggrecanases, but their use has been limited mostly to investigating cartilage turnover in ex vivo experiments. Because of the complexity of the mechanisms involved in arthritic joint damage, only a combination of different biochemical markers reflecting the various aspects of synthesis and degradation of matrix molecules will likely provide efficient cartilage turnover monitoring.

Introduction

Progressive loss of articular cartilage is a central feature of arthritic diseases including osteoarthritis (OA), rheumatoid arthritis (RA), and ankylosing spondyloarthritis (AS) [1]. In OA, no treatment exists to halt the progression of cartilage destruction, and monitoring the benefit of potential therapies is hampered by the lack of noninvasive techniques to adequately measure articular cartilage "health."

Change in joint space width (JSW) assessed by radiography remains the gold standard, but it does not allow the early detection of cartilage damage or the efficient monitoring of treatment efficacy, due to its poor sensitivity and relatively large precision error. Clearly, a need exists for improved techniques. MRI, which provides direct information on the alteration of the different joint tissues, is more sensitive than radiography in detecting cartilage loss [2]. Biochemical markers that reflect quantitative and dynamic variations in joint tissue remodeling have been proposed as a tool to detect early cartilage damage. A biochemical marker is generally considered a single molecule (or fragment thereof) of cartilage matrix that is released into biologic fluids during the process of tissue turnover. Several individual biochemical markers reflecting the synthesis and degradation of the cartilage have been proposed over the years, and the list is continuously expanded (Table 1).

In this paper, we review the most recent development in biologic markers of cartilage turnover and briefly discuss their utility in arthritic diseases, using the burden of disease, investigative, prognostic, efficacy of intervention, and diagnostic marker (BIPED) classification recently proposed by the Osteoarthritis Biomarkers Network [3•].

Biochemical Markers of Cartilage Synthesis and Degradation

Articular cartilage, a nonvascular tissue, is a multiphasic material comprising of two major phases: a fluid phase composed of water and electrolytes, and a solid phase composed of collagens, proteoglycans, glycoproteins, other proteins, and chondrocytes. Each phase contributes to its mechanical and physiologic properties. Type II collagen (CII) provides the major portion of the organic components (15%–22%), followed by aggrecan (4%–7%) and other noncollagenous proteins (0.5%–1%) including cartilage oligomeric matrix protein [4]. Because an imbalance in cartilage synthesis and degradation is central to cartilage loss in arthritis diseases, the development of biologic tools reflecting these metabolic processes for CII and aggrecan is currently under extensive investigation.

Markers of Type II Collagen Metabolism

Markers of type II collagen synthesis

CII is cartilage specific and forms the basic fibrillar structure of the extracellular matrix of cartilage. CII is a triple helical protein composed of three identical α 1 chains except at the two ends, in the linear N- and C-telopeptides (Fig. 1).

Following their synthesis, procollagen α chains are modified intracellularly by hydroxylation of proline and lysine residues, O-glycosylation of certain hydroxylysine residues, intrachain chain association, and interchain disul-

Table 1. Current available biochemical markers of cartilage turnover and their association with OA, RA, and spondylarthritis according to BIPED classification

Study	Cartilage matrix component	% of wet weight of cartilage	Assays	Body fluids or tissue	BIPED classification		
					OA	RA	Spondyl-arthritis
	CII	15–22	**Synthesis**				
Hinek et al. [6]			PIICP	S, SF, C	P, E, D*	D*	E, D*
Rousseau et al. [8]			PIIANP	S, SF	B, P, D*	E, D*	
Olsen et al. [12]			PIINP-I	S, C		D*	
			Degradation				
Christgau et al. [52]			CTX-II	U	B, P, E, D*	P, E, D*	E
Oestergaard et al. [20]			Preclinical CTX-II	S, SF, C	B, P, E	B, P	
Charni et al. [16••]			Helix-II	U, S, C	P, D*	P, D*	E
Downs et al. [31]			TIINE	U	B, P, E, D*	D*	
Poole et al. [53]			C2C	S, U, C, SF	P, E, D*	P	E
Billinghurst et al. [13]			Col2-3/4short	S, C, SF	P, D*		E
Deberg et al. [35]			Coll2-1	S, U	P, D*	D*	
Deberg et al. [35]			Coll2-1 NO_2	S, U	P, D*	D*	
	Aggrecan	4–7	**Synthesis**				
Otterness et al. [54]			Epitope 846	S, SF, C	E, D*	P, E, D*	B, E, D*
Sumer et al. [42]			G1/G2 domain	S, C		D*	
			Degradation				
			Aggrecanases neoepitope				
Pratta et al. [39]			ARGS/Keratan sulfate	C, SF			
Zeng et al. [41]			AGG-C1 neoepitope TEGE	S, SF		D*	
			MMP neoepitopes				
Sumer et al. [42]			AF-28/ G1 neoepitope	S, C		D*	
	Noncollagen/ nonaggrecan proteinases	0.5–1	**Synthesis**				
Harvey et al. [55]			Glycoprotein 39 (YKL-40)	S, SF	P, D*	P, E, D*	E
Moser et al. [56]			CD-RAP	S, SF		P	
			Degradation				
Saxne et al. [57]			COMP	S, SF	P, D*	P, E, D*	
Gemba et al. [50]			Fibronectin fragments	S, SF			
Schellekens et al. [58]			Autoantibodies anti-CCP	S, SF		P, D, E	

*Although most levels of most of markers have been found to be increased or decreased in patients with arthritis compared to age and sex matched healthy controls, a large proportion of patients have values within the normal range, suggesting limited diagnostic sensitivity when used alone.
The table shows human body fluid or tissue for which the application of the assay is documented in the literature. Provisional BIPED classification categories based on Bauer et al. [3•].
BIPED—burden of disease, investigative, prognostic, efficacy of intervention, diagnostic marker; C—cartilage explant; CCP—cyclic citrullinated peptide; CD-RAP—cartilage-derived retinoic acid–sensitive protein; CII—type II collagen; Col2-3/4short—C-terminus of 3/4 fragment short of type II collagen; C2C—C-terminus of 3/4 fragment long of type II collagen; Coll2-1—α-helix epitope of type II collagen; Coll2-1 NO_2—α-helix epitope of type II collagen nitrated; CTX-II—C-telopeptide of type II collagen; COMP—cartilage oligomeric matrix protein; Helix-II—helical peptide of type II collagen; MMP—matrix-metalloproteinase; PIIANP—type IIA procollagen amino propeptide; PIICP—procollagen II C-propeptide; PIINP—procollagen II amino propeptide; OA—osteoarthritis; RA—rheumatoid arthritis; S—serum; SF—synovial fluid; TIINE—type II collagen neoepitope; U—urine.

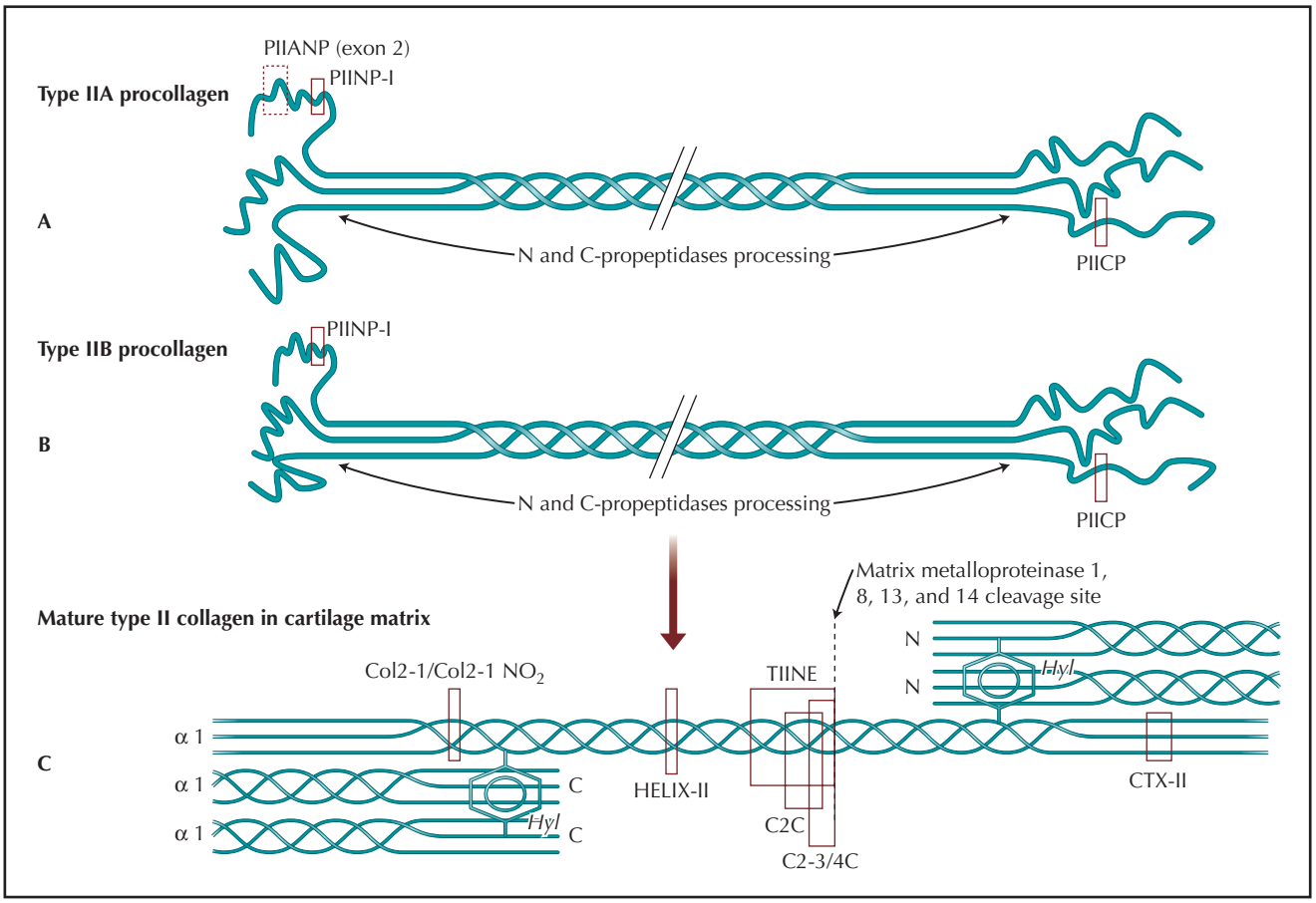

Figure 1. Schematic representation of the type II collagen molecule. Type II collagen is synthesized by the chondrocytes as a precursor to procollagen. Two forms of type II procollagen exist: **A**, procollagen IIA, which includes a peptide of 69 amino acid coded by exon 2 in the N-propeptide (*dotted line*), and **B**, procollagen IIB, in which this peptide is absent. The C-propeptide (PIICP) is common to procollagen IIA and IIB. Assays for type IIA procollagen N-propeptide (PIIANP), type IIA/IIB (PIINP-I), and PIICP have been developed. After removal of the N and C-propeptides, mature type II collagen molecules are linked together by pyridinoline cross-links in the linear N- and C-telopeptide regions. During the degradation of type II collagen by various enzymes, fragments bearing the neoepitopes C2 3/4C, C2C, TIINE (collagenases cleavages), Helix-II, unnitrosylated (Col 2-1) or nitrosylated (Col2-1 NO_2) (triple helix fragments), or CTX-II (C-telopeptide fragments) are released, **C**.

fide bond formation to form the triple helical structure. During the secretion in the extracellular cartilage matrix space, the procollagen triple helix is further modified by removal of the N and C-terminal propeptides by specific proteases, ordered arrangement of the mature fibrils, and cross-linking. The helices are intra- and intermolecularly cross-linked predominantly by hydroxylysyl pyridinoline cross-links within the telopeptides regions [5].

There are two splicing alternative forms of type II procollagen which differ by the presence (IIA) or absence (IIB) of a 69 amino acid sequence coded by exon 2 in the N-propeptide. Procollagen IIA is expressed mainly during development but can be re-expressed in OA cartilage, whereas the IIB variant is the major form of adult healthy cartilage. Because N- and C-propeptides are removed and released into biologic fluids during the secretion of CII, the measurement of these molecules provide a direct index of CII synthesis. The C-propeptide is identical in type IIA and IIB collagen (Fig. 1), and its measurement provides an index of total CII synthesis. An assay for PIICP was first developed by Hinek et al. [6], and it was shown that the measurement of PIICP in cartilage matrix correlated with CII synthesis assessed by ^{3}H incorporation [7]. More recently, assays measuring the N-propeptide have been developed.

Rousseau et al. [8] described a competitive enzyme-linked immunosorbent assay (ELISA) using a specific polyclonal antibody raised against recombinant exon 2 protein (Fig. 1). This assay specifically detects the synthesis of type IIA collagen. Compared to healthy sex and age-matched controls, decreased values of serum type IIA procollagen amino propeptide (PIIANP) levels [8] were found in patients with advanced knee OA. Using a modified version of the ELISA, increased levels of serum PIIANP in patients with early knee OA were shown to be associated with increased radiologic progression over 5 years [9••]. In both studies, combining PIIANP with a marker of type CII degradation (urinary CTX-II), allowed an improved prediction of radiologic progression [8,9••].

In the Genetics osteoARthritis and Progression (GARP) study, we recently analyzed a panel of eight different biochemical markers of bone, cartilage, and synovial

activity and their association with radiologic OA at different locations [10•]. Using principal component analyses, we found that PIIANP clustered together with cartilage oligomeric matrix protein and age and was associated with spine and hand OA but not knee or hip damage, suggesting that spine and/or hand may be a major contributor to circulating PIIANP. The changes of serum PIIANP were also investigated in a 12-month randomized trial of the anti–interleukin (IL)-6 antibody (tocilizumab) in 302 patients with early RA [11]. Compared to controls, there was a significant (12%) reduction of serum PIIANP after 12 weeks of treatment with tocilizumab, which was maintained up to 12 months. In patients receiving tocilizumab, the changes of PIIANP at 12 weeks were predictive of radiologic progression, more specifically loss of JSW, at 12 months. Thus, the data currently available suggest that in OA serum, PIIANP could be useful to predict disease progression in combination with CII degradation markers and could provide information on the burden of spine and hand. In RA, PIINAP could be useful to monitor the efficacy of disease-modifying therapies.

Olsen et al. [12] developed antibodies directed against a synthetic peptide of 15 amino acids, named PIINP-I, which is common to IIA and IIB N-propeptide (Fig. 1). This new marker of CII synthesis was increased in the supernatant of bovine cartilage explants activated by insulin growth factor-1, a strong anabolic agent. In explants stimulated by oncostatin M and tumor necrosis factor (TNF)-α, PIINP-I was also increased, probably because its detection by the antibody used in the assay is dependent on the cleavage by matrix-metalloproteases (MMP), which are upregulated by inflammatory cytokines. Increased PIINP-I levels may thus reflect the synthesis of new CII and/or increased degradation of matrix PIINP, complicating the biologic interpretation of this marker. In patients with advanced RA, plasma concentration of PIINP-I was significantly lower compared to healthy controls suggesting a deficient cartilage repair mechanism. Because the assays for procollagen II C-propeptide (PIICP), PIIANP, and procollagen II amino propeptide (PIINP)-I likely reflect different biologic aspects of CII synthesis, studies comparing the levels of these three markers in patients with arthritis would be very useful to evaluate their respective value alone or in combination.

Markers of type II collagen degradation

In arthritic diseases, the stability of collagen fibrils is compromised by their extensive proteolytic breakdown, leading to cartilage erosion and joint deterioration. Initial cleavage of CII molecules is attributed to the collagenase subfamily of MMPs, the collagenases 1, 2, 3, and membrane type 1-MMP (also known as MMP-1, MMP-8, MMP-13, and MMP-14, respectively) [13]. These collagenases preferentially cleave CII between Gly794 and Leu795, generating two fragments that are three fourths and one fourth the size of the collagen precursor [14]. Following this initial cleavage, the triple helix of CII fragments unwinds, providing a denatured substrate susceptible to further degradation by a variety of proteolytic enzymes. Antibodies recognizing different CII fragments have been developed (Fig. 1). Among these, Helix-II is a fragment arising from the degradation of the helical domain of CII. Helix-II, which is believed to reflect the degradation of the main part CII, appears to be released from cartilage degradation by enzymatic pathways in part different from those involved in the generation of CTX-II, a fragment of the C-telopeptides region [15]. Urinary Helix-II levels were found to be markedly increased in 90 patients with knee OA (56%) and in 89 patients with early RA (123%) compared to 162 healthy sex- and age-matched controls [16••]. We also found that serum Helix-II levels were decreased by a median of 18% ($P = 0.0015$) as early as 1 month after initiating anti-TNF (etanercept) treatment in 29 patients with AS, reaching a maximum median decrease of -33.4% ($P = 0.0079$) at month 12 [17].

Interestingly, in early RA [16••] and in hip OA [18], the combined measurement of Helix-II and CTX-II was more effective than either marker alone to identify patients with a rapidly destructive disease. This finding is probably because they reflect different mechanisms of cartilage degradation. Using a nonstimulated ex-vivo human cartilage explant model, which is responding to the endogenous proteinases present in vivo, we found that both cysteine proteases and MMPs are responsible for the release of Helix-II and CTX-II, although the individual enzymes were different. These data obtained with human cartilage explants differ in part from those reported by Sondergaard et al. [19] using bovine cartilage artificially stimulated by oncostatin M and TNF-α. Using nonselective inhibitors, they showed that inhibition of MMP activity reduces the liberation of CTX-II in the culture medium, whereas inhibition of cysteine proteases tends to increase its release, suggesting that CTX-II in this model is mainly generated by MMPs. It remains unclear, however, whether these findings are representative of physiologic situations of human OA cartilage. For this work, they used a new preclinical assay developed to measure only cross-linked dimers of CTX-II. This technique differs from the conventional competitive ELISA, which detects both monomers and dimers of CTX-II. The preclinical version of CTX-II assay was also used to assess cartilage degradation in a collagen-induced model of inflammatory arthritis [20]. Early increases of serum CTX-II showed a significant association with the histologic severity of cartilage damage.

In patients with OA, urinary CTX-II has been shown to be associated with the burden of the disease within one or multiple joints and higher levels found to be associated with more rapid radiologic progression [21]. In postmenopausal women with osteoporosis, urinary CTX-II decreased following treatment with the antiresorptive therapies including bisphosphonates, estrogens, selective estrogen modulators, and calcitonin [22] and more recently

with strontium ranelate [23]. In 2483 patients with knee OA participating in the large randomized trials of risedronate, we found a dose-dependent decrease of urinary CTX-II [24••]. Patients showed a normalization of their levels at 6 months and had significantly lower radiologic progression at 2 years [25]. Finally, in a small randomized clinical trial of oral calcitonin in patients with knee OA, researchers found a significant 20% reduction of urinary CTX-II after 84 days of treatment with the highest dose (1 mg/day) [26]. Together these data indicate that the bone anticatabolic drugs currently used in osteoporosis decrease CTX-II levels. The decrease of CTX-II could be attributed to a decrease of articular matrix degradation, a decrease of calcified cartilage turnover (a significant source of CTX-II), directly or indirectly by inhibition of subchondral bone turnover.

More recently, associations of CTX-II with MRI findings have been investigated. In patients with knee OA participating in a randomized trial of an investigational disease-modifying OA drug, we found that baseline and 3-month changes of urine CTX-II were associated with baseline and 3-month changes in MRI bone marrow abnormalities [27]. In a small study of 62 patients with knee OA, Bruyère et al. [28] reported that changes of CTX-II at 3 months were modestly associated with 12-month changes in medial cartilage thickness, although baseline values were not predictive. In a cross-sectional population-based study, we classified 201 subjects according to radiographic and MRI cartilage defects as normal (without radiographic or MRI alterations), preradiographic OA (MRI defects but no radiologic damage), and radiographic OA [29]. Compared to values measured in normal individuals, urinary CTX-II levels were increased in patients with radiographic OA, but not in preradiographic OA, suggesting that urinary CTX-II may not be sensitive enough to detect very early cartilage damage documented by MRI. Conversely, the urinary excretion of epitope C2C (Fig. 1), which detects the initial CII cleavage by collagenases, was increased in preradiographic OA but could not discriminate this group from patients with radiographic OA [29]. Clearly, the different CII degradation markers reflect the different biologic mechanisms of cartilage degradation that can be involved at various stages of disease.

The C-terminus of the 3/4 fragment resulting from collagenase cleavage can be readily identified in urine, making it a good candidate to quantify the extent of CII degradation in arthritic diseases. Otterness et al. [30] have generated the antibody 9A4 against the ^{888}GEPGDDGPS^{896} epitope. Also, 9A4 can detect the C-terminal neoepitope generated by collagenase digestion of type I and type III collagen. Specificity for CII was achieved by using a second capture antibody (5109) directed against a CII-specific sequence adjacent to that detected by 9A4 [30,31]. Using these two antibodies in a sandwich ELISA (type II collagen neoepitope [TIINE]), they were able to detect increased TIINE fragments in most OA and RA patients, and levels were associated with radiographic severity [31–33]. Urinary TIINE fragments were more recently measured by liquid chromatography–mass spectrometry/mass spectroscopy, which specifically detects a 45-mer CII degradation peptide. Using this technology, sequential measurements of urinary TIINE were reported to be associated with loss of JSW measured by radiography in a subgroup of 120 patients with knee OA participating in the randomized trial of doxycycline [34]. It should be emphasized, however, that baseline values of TIINE were not significantly associated with subsequent radiologic progression over 36 months.

Cartilage matrix molecules including CII can undergo post-translational modifications that may be mediated by an enzymatic process or can be spontaneous and age related. Measuring post-translational modified cartilage matrix proteins may lead to the development of biochemical markers offering valuable information on altered biologic processes related to OA or RA. Chondrocytes can express high levels of inducible and neuronal forms of nitric oxide (NO) synthetase which generated NO. NO can then react with superoxide radical to form peroxynitrite, a potent oxidizing radical that can in turn react with tyrosine residues of proteins to form nitrotyrosine. Two different assays recognizing a sequence—either un-nitrosylated (Coll 2-1) or nitrosylated (Coll 2-1 NO_2)—of the triple helix of CII have been developed [35]. Increased serum levels of Coll 2-1 and Coll 2-1 NO_2 have been reported in patients with knee OA. Changes at 1 year of their urinary levels (but not baseline values) were modestly related to more rapid disease progression over 3 years [36]. However, it remains unclear from these studies whether there is an additive value of investigating the nitrosylated over the non-nitrosylated forms of CII fragments in OA.

Markers of Aggrecan Turnover

Aggrecan, the large aggregating proteoglycan of cartilage, is one of the most abundant and widely studied proteoglycans, and one for which a clear relationship between structure and biologic function is known. In the extracellular matrix, aggrecan forms large link protein–stabilized aggregates with hyaluronan that are contained within the three-dimensional structure of CII. Aggrecan is highly hydrated because of its negatively charged long polysaccharide chains, thus providing the cartilage its ability to resist compressive loads. The amino terminal end of the aggrecan monomer core protein is composed of two globular domains called G1 and G2 that are separated by an interglobular domain [37] containing about 150 amino acid residues in length. The G2 region is followed by a long central glycoaminoglycan attachment region and by a COOH-terminal globular domain, G3 (Fig. 2).

One of the earliest events in cartilage degradation associated with arthritis is the loss of aggrecan molecules, which is believed to result from proteolytic cleavages

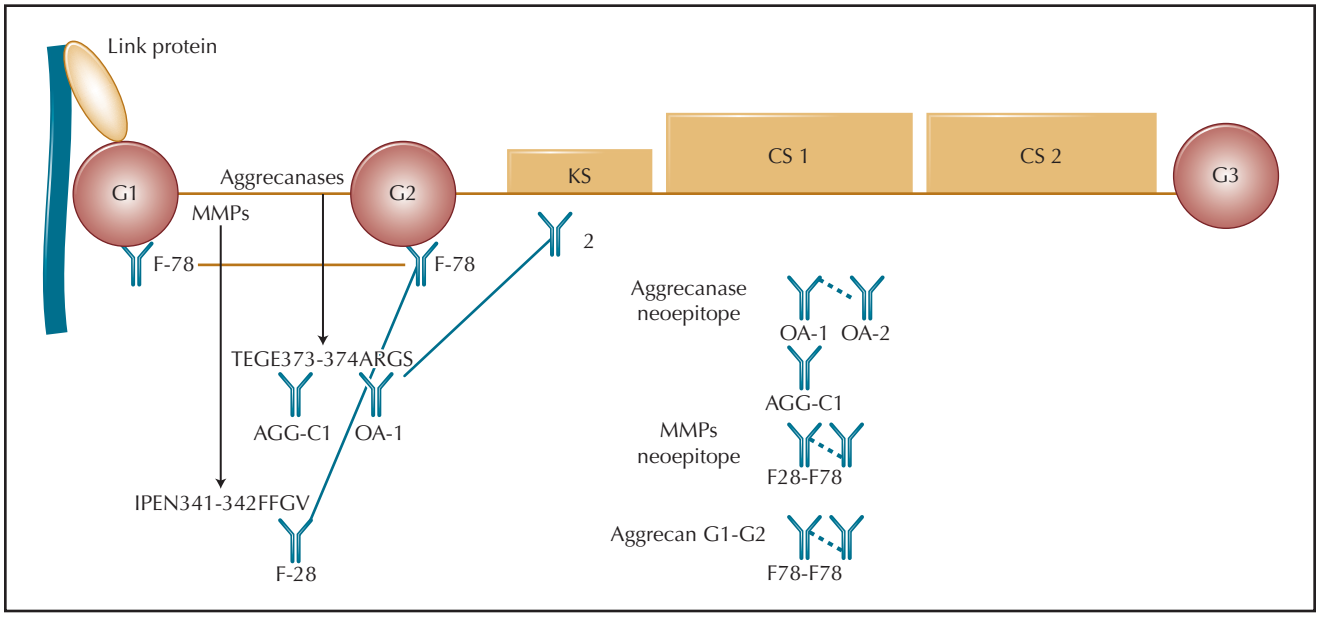

Figure 2. Schematic representation of the human aggrecan molecule with cleavage sites (*plain arrows*) by aggrecanases and matrix metalloproteinases (MMPs). Dotted lines link the couple of antibodies used in sandwich enzyme-linked immunosorbent assays to detect the fragments generated by MMPs and aggrecanase. OA-2 is developed by Biosource International (Camarillo, CA, USA). CS1 and CS2—chondroitine sulfated regions; IGD—interglobular domain; KS—Keratan sulfate region.

within the interglobular domain. Two major proteolytic cleavage sites have been identified in this domain: one between amino acids Asn^{341} and Phe^{342} and the other between Glu^{373} and Ala^{374}. MMPs have been shown to cleave aggrecan at the Asn^{341}-Phe^{342} site, whereas cleavage at the Glu^{373}-Ala^{374} site has been attributed to aggrecanases, including ADAMTS-5 and -4 (a disintegrin and metalloproteinase with thrombospondin motifs) [38].

Antibodies against various aggrecan neoepitopes generated by the activity of MMPs and aggrecanases have developed and were recently used in ELISAs (Fig. 2). Pratta et al. [39] developed a sandwich ELISA that quantifies the level of aggrecan fragments consisting of the N-terminal aggrecanase neoepitope ^{374}ARGSVIL and a keratan sulfate (KS) region, which is detected by a second commercially available antibody (Fig. 2). Using an ex-vivo model of human cartilage explant, they showed that these aggrecan fragments were released after stimulation with IL-1, a cytokine believed to induce aggrecan degradation through aggrecanase-dependent pathways [40]. They also detect the presence of this aggrecan neoepitope in human synovial fluids from patients with OA, but no data in serum were generated. The current version of this ELISA presents some limitations acknowledged by the authors (ie, the capture antibody detects a KS region that is not specific to aggrecan). Thus other KS-containing molecules may compete with the ARGSVIL-aggrecan fragments for the detection in the assay. This ELISA could also underestimate the true concentration of ARGSVIL-aggrecan fragments, because small peptides containing the ARGSVIL epitope but not KS (≤ 140 kDa) cannot be detected. This limitation may be particularly important when serum is analyzed, because large aggrecan fragments are likely to undergo further degradations.

Zeng et al. [41] generated a monoclonal antibody named AGG-C1 recognizing specifically the second aggrecanase-generated catabolic N-terminal neoepitope $TEGE^{373}$, which was used in a competitive ELISA. In a rabbit anterior cruciate ligament transaction OA model, this epitope was readily detectable in synovial fluid and serum. These promising but preliminary data suggest that this aggrecanase-generated neoepitope may be valuable to monitor aggrecan turnover in patients with arthritis.

Sumer et al. [42] were interested in developing an assay measuring MMP-mediated aggrecan degradation. They developed two sandwich ELISAs. The first one uses a monoclonal antibody (F-78) that detects a common repetitive epitope in the G1 and G2 domains of aggrecan. This assay is believed to reflect either the synthesis of newly aggrecan molecules and/or large degradation fragments, thus reflecting overall aggrecan turnover. The second sandwich ELISA uses the same format as the G1/G2 sandwich ELISA, except that it uses a different capture antibody (AF-28) and it recognizes the MMP cleavage neoepitope ^{342}FFGVG. Using this sandwich ELISA, the MMP-generated aggrecan fragments could be detected in human serum, whereas the competitive assay developed by Fosang et al. [43] with the same antibody AF-28 was not sensitive enough. The clinical values of these two new aggrecan assays were evaluated by measuring their serum levels in 20 patients with RA and 57 healthy controls. It was found that the levels of G1/G2 were decreased in patients with RA suggesting decrease aggrecan synthesis, whereas values of ^{342}FFGVG epitope were slightly but not significantly increased.

As discussed previously, markers of aggrecan turnover have mostly been used in ex-vivo cartilage explants models or in the synovial fluid of animals or patients with arthritis. Conversely, clinical data are still very limited, probably because the aggrecan fragment(s) of most relevance remain unclear. In their very elegant work, Struglics et al. [44] performed experiments to dissect the mechanisms involved in the generation of the major aggrecan fragments found in human cartilage and synovial fluid. They suggested that there are two main MMP- and aggrecanase-dependent pathways, which release aggrecan fragments of different sizes in cartilage and synovial fluid. However, it is possible that additional proteolytic mechanisms including those mediated by cysteine proteases may be active downstream in the lymph, serum, or urine. These mechanisms could further degrade aggrecan fragments resulting from primary cleavage by MMPs and aggrecanases. A detailed analysis of the different fragments of aggrecan in cartilage, synovial fluid, and serum is warranted to improve the development of clinically valuable markers of aggrecan turnover.

New Potential Biochemical Markers of Cartilage Turnover

Fragments of fibronectin mediating cartilage damage

In normal cartilage, fibronectin is a minor component of the extracellular matrix. However, fibronectin fragments are present in high concentrations in the cartilage of patients with RA or OA because of an increase in synthesis and accumulation of fibronectin [45]. Intact fibronectin molecules can be cleaved into smaller fragments, which have been found in cartilage and synovial fluid of OA and RA patients [46,47]. The degradation products of fibronectin (but not the intact molecule) can promote cartilage catabolism in human cartilage cultures. This suggests that fibronectin fragments may actually participate in the initiation and progression of arthritic diseases [48]. Indeed, fibronectin fragments have been shown to upregulate the expression of MMPs and aggrecanases [49], and they also induce the expression of proinflammatory factors such as IL-1β and inducible NO synthase [50]. Recently, Zack et al. [48] identified several fibronectin fragments containing either the N-terminus (272)VYQPQPHP or the C-terminus VRAA(271) sequences, which were detected following cytokine treatment of human cartilage extracts.

Fragments of CII mediating cartilage damage

Like fibronectin, peptides of CII can induce the cleavage of CII and aggrecan in articular cartilage. Yasuda et al. [51] reported that the CII peptides CB12-II (residues 195-218 of CII) and CB12-IV (residues 231-254 of CII), when present in sufficient concentration, can induce the cleavage of CII and aggrecan, respectively. Interestingly, these catabolic effects were accompanied by an increased expression of MMP-13, which is involved in both CII and aggrecan degradation. These observations suggest that specific sequences of CII can directly and differently stimulate specific catabolic responses of chondrocytes and promote CII degradation through a vicious circle mechanism.

The two above examples suggest that specific fragments of cartilage matrix molecules can be involved directly in the physiopathologic processes of articular degradation. Their assay in biologic fluids may potentially serve as biochemical markers of early cartilage damage.

Conclusions

Limitations in the current techniques for diagnosing and monitoring OA and RA have led to investigation of molecular markers that could reflect more precisely the turnover of cartilage matrix molecules. Many markers have been developed and used primarily to assess cartilage matrix turnover in ex vivo experiments. Although recent progress has been made towards the identification of the enzymatic pathways involved in the generation of the biochemical markers of synthesis and degradation of the cartilage matrix molecules, information about their detailed processing is still lacking. Consequently, the selection of an optimal marker or set of markers to investigate patients with arthritis is still unclear. Moreover, the level of biochemical markers is dependent not only on the rate of cartilage turnover, but also on the diffusion rate of the molecules between body compartments, the half-life of the markers in biologic fluids, and immunoassay performances. Therefore, extensive human clinical studies are required to establish the clinical value of any given immunoassay. Despite these limitations, recent clinical studies indicate that a combination of biochemical markers reflecting the synthesis and degradation of CII (and potentially aggrecan) may be useful to assess disease progression in OA and RA [21].

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