

## MECHANISM OF CARTILAGE DESTRUCTION IN OSTEOARTHRITIS

NAOKI ISHIGURO\*, TOSHIHISA KOJIMA\* and A. ROBIN POOLE†

*\*Department of Orthopaedic Surgery, Nagoya University, School of Medicine, Nagoya, Japan*

*†Joint Diseases Laboratory, Shriners Hospitals for Children, Division of Surgical Research, Department of Surgery, McGill University, Montreal, Canada*

### ABSTRACT

Osteoarthritis (OA) is one of the most common diseases among the elderly. For many years, OA was considered a normal result of the aging process with few treatment options. Remarkable progress in understanding OA cartilage has been achieved in recent years. The application of technical advances to clinical studies of chondrocytes and cartilage tissue metabolism will provide important new insights concerning the pathophysiology of OA and identify new therapeutic strategies to regulate and inhibit the degenerative process of OA. However, many problems remain unresolved. In this review we try to focus on recent advances in the field of cartilage metabolism and molecular markers to facilitate the determination of a patient's prognosis and the need for cartilage protective treatment.

Key Words: osteoarthritis, matrix metalloproteinase, aggrecan, type II collagen, aggrecanase

### WHAT IS OSTEOARTHRITIS?

Osteoarthritis (OA) is a clinical classification for a combination of pathological conditions involving the progressive degeneration of articular cartilage, remodelling of sub-chondral bone, and synovitis that is usually limited to the affected joint. OA is considered the cumulative result of mechanical and biological events that induce an imbalance between the degradation and synthesis within articular joint tissues. However, in patients with rheumatoid arthritis, more severe destruction throughout the cartilage and bone tissue in the affected joint tends to occur. OA has been demonstrated to be a complex process that includes multiple changes in joint components such as cells, matrix and molecular production<sup>1)</sup>.

OA has been variously described as a part of an age-related change or disease. The incidence of OA is twice as high in women than in men and increases with age, especially after 60<sup>2)</sup>. Estrogen decline is considered one of the main factors inducing cartilage degeneration in elderly women<sup>3)</sup>. It is thought that the changes that lead to the development of OA are slow and steady. Following joint trauma, there is an increased incidence of OA that probably results from accelerated degeneration during a period of more than 10 years. Idiopathic OA may only primarily involve one or two major joints and then become generalized. The clinical presenta-

---

Address for correspondence:

Naoki Ishiguro, M.D., Department of Orthopaedic Surgery, Nagoya University, School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466, Japan

Tel: 81-52-741-2111 Ext. 2240

Fax: 81-52-744-226

tion in idiopathic OA may only become apparent more than 15 years after the onset of changes.

Familial OA is another clinical entity within OA. It presents very early, often following the cessation of growth as a consequence of alterations in cartilage matrix structure, leading to clinical manifestations of joint degeneration. Several cases have been reported in patients with a mutation in the type II collagen or other cartilage molecules that cause fragility of the cartilage matrix<sup>4</sup>.

The disease process of OA affects not only the articular cartilage, but also the entire joint structure including the subchondral bone, ligaments, capsule, synovial membrane and periarticular muscles. Clinical manifestations include joint pain, tenderness, limitation of movement, crepitus, effusion and varying degrees of inflammation, and finally induce disability in many patients. In Japan and other economically advanced countries, OA is considered one of the most common and important diseases to be eradicated in the near future.

Numerous biological and inflammatory changes and etiological agents are implicated in the etiopathogenesis of OA. The disease process involves a disturbance in the normal balance of degradation and regeneration in articular cartilage and subchondral bone<sup>5</sup>. Once cartilage degradation has begun, the synovial cells and inflammatory macrophages phagocytose the breakdown products released into the synovial fluid, and then secrete pro-inflammatory cytokines such as TNF- $\alpha$  and IL-1 $\beta$ <sup>6</sup>. This mechanism is considered to accelerate cartilage destruction. Under normal conditions, the homeostasis of articular cartilage is tightly regulated by chondrocytes and synovial cells.

## WHAT HAPPENS TO OA CARTILAGE?

Loss of articular cartilage in OA is characterized radiographically by joint-space narrowing. The initial loss of articular cartilage in early OA is classically considered to be a focal process that may progressively involve all the joint compartments, inducing biological alterations in the molecular composition of articulating surfaces. In the early stage of OA, degeneration is commonly observed at the articular surface in the form of fibrillation. The clonal formation of chondrocytes is often observed in early OA. In the advanced stage of OA where alterations in cartilage matrix turnover are markedly depressed, degenerative changes may involve all the articular cartilage. Progressive loss of cartilage then occurs. Apoptosis of chondrocytes is predominantly observed in advanced OA, particularly at and close to the articular surface<sup>7</sup>.

Superficial fibrillation is associated with the degeneration of type II collagen of collagen fibrils. This degradation induces a loss of the mechanical properties and function of cartilage tissue that the native collagen fibrils possess and maintain. Tensile properties are normally much higher at the articular surface than deeper in the cartilage<sup>8</sup>.

Proteoglycans are major components of articular cartilage, and it is thought that their loss may result in the destruction of cartilage. Aggrecan is the predominant proteoglycan in articular cartilage. It consists of a core protein to which glycosaminoglycan chains are covalently attached, showing the physiological property of a GAG chain<sup>1</sup>. The loss of aggrecan molecules in cartilage tissue imposes an increasing load on collagen fibrils and induces collagenase accessibility to these fibrils<sup>9</sup>. The destruction of the collagen network in cartilage tissue causes damage to the major proteoglycan, aggrecan. Importantly, it takes a much longer time for the metabolic turnover of collagen fibrils than that of aggrecan<sup>10</sup>.

Cartilage destruction in OA is regarded as the consequence of a loss of cartilage homeostasis. Ordinarily, anabolic and catabolic pathways regulate the synthesis and destruction of extra-

cellular matrix (ECM) maintaining a balance between the two. It is well known that chondrocytes can synthesize the ECM matrix such as proteoglycans, collagen, fibronectin, integrins and other adhesive proteins which are needed to maintain high tensile strength and low compressibility under load. The maintenance of normal cartilage ECM to resist mechanical forces could be the key in preventing the initiation of the pathological process in OA.

Abnormal loading to the ECM can induce ECM cleavage as well as alter the synthesis of ECM molecules. Pathological changes in cartilage ECM in OA are likely to result in a disturbance of the normal balance between mechanical loading and direct cytokine/growth factor signals, causing changes in gene expression.

### WHAT IS RESPONSIBLE FOR DEGRADATION OF THE CARTILAGE MATRIX IN OA?

Previous studies have shown that matrix metalloproteinases (MMPs) play an important role in the degradation of the matrix in OA and rheumatoid arthritis (RA)<sup>11</sup>. Matrix metalloproteinases (MMPs) comprise more than 20 proteinases, each of which is the product of a different gene, and some MMPs are produced abundantly by chondrocytes and synovial cells in arthritic joints (Table 1). The structural similarity shared by all MMPs includes a catalytic zinc-binding domain with a conserved sequence motif His Glu xxx xxx His<sup>16</sup>. MMPs are divided into five subgroups, in terms of substrate specificity: collagenase, stromelysin, gelatinase, membrane type MMPs and others. Interstitial collagenase (MMP-1) digests types I, II, and III collagen, while 72 kDa type IV gelatinase (MMP-2) cleaves a number of substrates, including gelatins, types IV, V, VII, X, and XI collagen, fibronectin, and proteoglycans. Stromelysin-1 (MMP-3) has been proven to digest proteoglycans, types IV, VII, IX, and XI collagen, and also fibronectin<sup>11</sup>. Stromelysin-1 also activates other MMPs<sup>17</sup>, and is thus considered to play a significant role in matrix degradation<sup>18,19</sup>. In addition, 92 kDa type IV gelatinase (MMP-9) has various substrates, such as type I, III, IV and V collagen, gelatin, proteoglycans, and elastin. It has been shown that MMP-1 (collagenase-1), MMP-3 (stromelysin-1), MMP-2 and MMP-9 (gelatinases), MMP-8 (neutrophil collagenase), and MMP-13 (collagenase-3) play important roles in cartilage matrix destruction in arthritic joints<sup>20, 21</sup>.

Four membrane type-MMPs (MT-MMPs) have been identified in cartilage tissue, MT1-MMP (MMP-14), MT2-MMP (MMP-15), MT3-MMP (MMP-16), and MT4-MMP (MMP-17)<sup>22</sup>. It is well known that these membrane-associated members of the MMP family (except MMP-14) are expressed at low levels by chondrocyte and synovial cells<sup>23</sup>. MT1-MMP was demonstrated to be the most predominant in cartilage tissue and the most clearly regulated by cytokines and growth factors<sup>23</sup>. Moreover, MT1-MMP shows the ability to initiate the activation of proMMP-2<sup>24</sup> and proMMP-13<sup>25</sup>. These two enzymes are especially considered to mediate the breakdown of the type II collagen network in cartilage tissue<sup>26</sup>.

Among MMPs, MMP-1, -8, and -13, initially described as collagenase -1, -2, and -3, are now thought to be differentially regulated and to engage in cartilage destruction separately in OA<sup>27</sup>. The other two MMPs (MMP-2 (gelatinase A) and MT1-MMP (MMP-14)) showed activity as collagenases against type II collagen *in vitro*. An expressed soluble form of MT1-MMP has also been shown to behave as a collagenase<sup>28</sup> and an activator of proMMP-2<sup>29</sup>.

Thus, it is likely that these MMPs are responsible for cartilage destruction in patients with OA and RA<sup>30</sup>. Their presence may reflect disease activity at the level of proteolysis. Imbalances in proteinase/inhibitor concentrations favoring proteolysis have been found in osteoarthritic cartilage.

Table 1. Matrix metalloproteinases

MMP No.	Enzyme	Size	Substrates
<b>I. Collagenase</b>			
MMP-1*	Tissue Collagenase	55 kDa	Type I, II, III, VIII, X Collagen
MMP-8*	Neutrophil Collagenase	75 kDa	Type I, II, IIIa, X Collagen
MMP-13*	Collagenase-3	65 kDa	Type I, II, III, X Collagen
MMP-18	Xenopus Collagenase-4	60?kDa	Type I Collagen, Gelatin
<b>II. Gelatinase</b>			
MMP-2*	Gelatinase A	72 kDa	Denatured collagen, Type IV, V, VII Collagen
MMP-9*	Gelatinase B	95 kDa	Denatured collagen, Type IV, V, VII Collagen, Elastin
<b>III. Stromelysin</b>			
MMP-3*	Stromelysin-1	57 kDa	Proteoglycan, Type IX, IV, VII Collagen, Fibronectin, Gelatin, Laminin
MMP-10	Stromelysin-2	57 kDa	Proteoglycan, Type III, IV, VII Collagen, Fibronectin, Gelatin
<b>IV. Membrane-type Matrix Metalloproteinase (MT-MMP)</b>			
MMP-14*	MT1-MMP	63 kDa	Gelatin, Type I, II, III Collagen, Proteoglycan, Fibronectin, Vitronectin Laminin
MMP-15*	MT2-MMP	64 kDa	Gelatin, Type I, III Collagen, Proteoglycan, Fibronectin, Laminin
MMP-16*	MT3-MMP	72 kDa	Gelatin, Type I, II, III Collagen, Proteoglycan, Fibronectin, Vitronectin Laminin
MMP-17*	MT4-MMP	70 kDa	–
MMP-24	MT5-MMP	63 kDa	Proteoglycan
MMP-25	MT6-MMP	–	Gelatin (12)
<b>V. Others</b>			
MMP-7*	Matrilysin	28 kDa	Proteoglycan, Type IX, IV, VII Collagen, Fibronectin, Gelatin, Laminin, Elastin
MMP-11	Stromelysin-3	51 kDa	Proteoglycan, Fibronectin, Elastin
MMP-12	Metalloelastase	54 kDa	Elastin, Fibronectin
MMP-19	RASI-1	57 kDa	Proteoglycan
MMP-20	Enamelysin	54 kDa	Amelogenin
MMP-22	C-MMP	42 kDa	Type I Collagen, Casein, Gelatin
MMP-23		43 kDa	Gelatin
MMP-26	Matrilysin-2	–	Type IV Collagen, Fibronectin, Gelatin (13)

\*: These proteins were identified in OA and /or RA joints tissue. There was a report that MT2-MMP was not observed in joint tissue<sup>14)</sup>. It was considered that MT2-MMP, MT4-MMP may not be an important factor in joint destruction because of their negligible production in arthritic joints<sup>15)</sup>.

Other proteinases, classified as disintegrin and metalloprotease with thrombospondin repeats family (ADAM-TSx) that can digest the aggrecan core protein were identified and named aggrecanase-1 and -2 respectively<sup>31</sup>. These two aggrecanases are regulated in a different manner by cytokines and growth factors compared to MMPs<sup>32</sup>. The aggrecanase generated cleavage sites in the aggrecan molecules were identified as distinct from those generated by MMPs (Asu341-Phe342)<sup>33</sup>. Moreover, it had been recently revealed that aggrecanase-1 can secondly cleave the aggrecan molecule at an MMP site (Glu373-Ala374)<sup>34</sup>.

Tissue inhibitors of metalloproteinases (TIMP)-1, TIMP-2, TIMP-3 and TIMP-4 have the ability to inhibit MMPs, since these TIMPs play an important role in the regulation of MMP activity, they are crucial in controlling the destruction of tissue. Previous studies have shown that TIMPs, once activated, can inhibit MMPs, and it is now widely accepted that the balance between MMPs and TIMPs is very important in maintaining joint cartilage homeostasis<sup>35</sup>. Recently, it was reported that TIMP-3 can inhibit aggrecanase activity *in vitro*<sup>36</sup>. Interestingly, it has been shown that both TIMP-1 and TIMP-2 each have growth-promoting activity<sup>37</sup>. Such activity may influence matrix turnover in OA cartilage. It is known that MMP-7 (matrilysin), which shows broad substrate cleavage activity, is also increased in OA<sup>38</sup>.

It is noteworthy that the level of latent MMP synthesis in OA cartilage greatly exceeds the up-regulation of gene expression for TIMP-1, -2, -3, and -4. Although TIMP-1 mRNA expression by chondrocytes in OA cartilage was higher than that by chondrocytes derived from normal cartilage, the amount and TIMP isoform produced by OA chondrocytes is insufficient to inhibit the level of MMPs. In addition, once activated and not effectively inhibited by TIMPs, MMPs degrade both the endogenous and newly synthesized ECM molecules. The degradation of newly synthesized ECM molecules has a particularly serious effect on cartilage. As these ECM molecules are synthesized by chondrocytes to replace the ECM molecules lost by MMP degradation, uncontrolled degradation may sometimes induce a total loss of cartilage integration.

Significant up-regulation of MMP gene expression is seen as an early OA change in animal models and human patients<sup>39</sup>. This suggests that MMP gene expression is essential for the development of OA<sup>40</sup>. The activity of MMPs is considered low in articular cartilage with strict regulatory control of synthesis and activation. Pro-inflammatory cytokines such as IL-1 $\beta$  and TNF- $\alpha$ , produced by activated synoviocytes or by chondrocytes, up-regulate MMP gene expression<sup>1</sup>. MMPs are produced by chondrocytes and synovial cells as latent pro-enzymes<sup>1</sup>. Thus, several activation pathways have been implicated in the OA process. These activation events may be targets for therapeutic intervention. Chondrocytes synthesize the plasminogen activator (PA) that can produce plasmin. It is well known that the PA forms found in urine (uPA) and tissue (tPA) are both produced by chondrocytes<sup>41</sup>. The uPA form may be more critical in terms of cartilage breakdown because plasmin (known as a general MMP activator) can convert pro-MMP-3 to an active form of MMP-3<sup>1</sup>. That active form in turn can activate procollagenases. The plasmin/PA pathway is regulated by plasminogen activator inhibitors (PAI). In OA cartilage, plasmin concentration is significantly higher than normal, and a notably higher level of activated MMPs is observed<sup>1</sup>. Other activation pathways have been implicated in the membrane-type MMPs (MT1-MMP; MMP-14) which were shown to activate MMP-2 (gelatinase A) and collagenase-3<sup>25</sup>. Significantly increased MMP activity has also been measured in human cartilage sampled from patients in an early stage of OA. These results have strongly suggested that a therapeutic intervention designed to inhibit specific MMP activity could be useful for OA therapy.

## WHAT HAPPENS TO THE RESIDUAL MOLECULE IN OA?

The integrity of cartilage tissue is dependent on the complex network of type II collagen, proteoglycans, and accessory proteins such as fibronectin. These molecules are synthesized and integrated into the residual ECM by chondrocytes. The loss of ECM in cartilage is associated with an increased cleavage of type II collagen by collagenase and an aggrecan cleavage along with the degradation of small proteoglycans. The principal proteoglycan component of cartilage by mass is the molecule aggrecan<sup>1)</sup>, which is composed of a core protein attached with many chondroitin sulphate and keratan sulphate glycosaminoglycan (GAG) chains. Aggrecan is the major proteoglycan in articular cartilage since it is stabilized to hyaluronan by link protein and forms Aggrecan aggregates to regulate the diffusion of aggrecan from cartilage ECM. Loss of aggrecan with proteolytic degradation mediated by activated MMPs and aggrecanase causes a significant loss of mechanical properties in cartilage.

Although the loss of aggrecan in articular cartilage is essential for the progression of OA, the final cartilage damage is inflicted by the loss of the collagen network<sup>42)</sup>. Collagenases such as MMP-1, -8, and MMP-13 can only degrade the helical domain of type II collagen<sup>27)</sup>. A type II-specific collagenase was found in cultured chondrocyte lysates after stimulation with TNF $\alpha$  and IL-1 $\beta$ . MMP-3 also degrades type IX and type XI collagen. These collagen molecules interact to produce hybrid fibrils with the major type II collagen fibril in cartilage<sup>1)</sup>. Such type II collagen degradation starts with activated collagenases secreted by chondrocytes in a latent form<sup>43)</sup>. However, the final dissolution of cartilage collagen may be mediated by the activity of gelatinases that are also produced by chondrocytes. It is noteworthy that gelatinase activity has been observed to increase in the fibrillated area of OA cartilage. As progressive damage to the cartilage increases, denaturation and cleavage of type II collagen by collagenase have been reported<sup>44)</sup>.

It has been demonstrated that excessive cleavage of aggrecan occurs in OA cartilage in the core protein. The cleavage sites in the interglobular domain (IGD) located between the G1 and G2 domains have been particularly well investigated by many researchers<sup>45)</sup>. There are two principle sites of cleavage, the MMP site where various MMPs such as MMP-3, MMP-8 and MMP-13 can cleave, and the aggrecanase site where cleavage can also be produced by aggrecanase-1, and -2 which are membrane proteinases and members of the ADAMTS family<sup>46,47)</sup>. The cleavage neoepitopes of aggrecan, VDIPEN and NITGE, generated by MMPs and aggrecanase, respectively, appeared differently in the progression of arthritis (Figure 1)<sup>48)</sup>. Again, aggrecanase can cleave aggrecan molecules at the MMP site<sup>34)</sup>. Both types of cleavage are commonly observed in OA cartilage. The relative contribution of these two different families of proteinases in aggrecan degradation remains controversial.

There are many changes in the contents of other matrix molecules in OA. For example, aggrecan synthesis is upregulated, and an altered chondroitin sulfate structure is demonstrable by immunoassay or immunohistochemistry in the early stage of OA. Cartilage oligomeric protein is altered in distribution, and tenascin, osteonectin and fibronectin are all increased. The mature articular chondrocytes normally do not express type X collagen, which is synthesized by hypertrophic chondrocytes in the growth plate. However, in OA, type X was newly synthesized by chondrocytes<sup>49)</sup>.

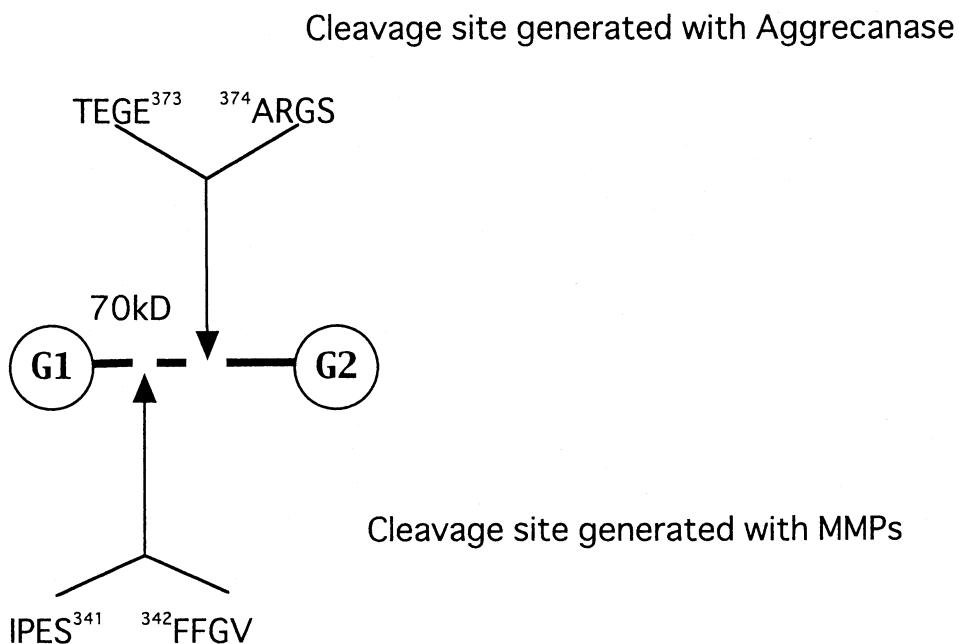


Figure 1: Schematic figure of ADAM-TS4/5 and MMP cleavage sites in inter-globular domain of the human aggrecanase core protein. The figure was modified based on the schema originally presented in Ref. 44.

### ANALYSIS OF SYNOVIAL FLUID TO RECOGNIZE CARTILAGE DESTRUCTION

The degradation of a collagen network in cartilage can be detected using antibodies to the collagenase-generated cleavage site in type II collagen. It has been well reported that this epitope is increased in joint fluids following resection of the anterior cruciate ligament in an animal model<sup>50</sup>. These results indicate that the measurement of a type II collagen cleavage epitope in synovial fluid provides a reasonable explanation of the cartilage degeneration process in OA<sup>51,52</sup>. Furthermore, other assays to detect type II collagen degradation products involving the telopeptide-cross-link complex will soon become commercially available<sup>53</sup>. Regeneration of type II procollagen can be detected by measurement of the C-propeptide of this molecule.

Proteoglycan aggrecan degradation products bearing aggrecan-cleavage sites can be detected in synovial fluid and by the measurement of keratan sulphate, chondroitin sulphate and protein epitopes of aggrecan by immunoassay. Moreover, it is thought that the presence of sulphated glycosaminoglycan (predominantly chondroitin sulphate) is also largely reflective of aggrecan degradation in cartilage. The glycosaminoglycan can be detected by chemical analysis of its constituent disaccharides of chondroitin-6 sulphate ( $\Delta$ di-C6S) and chondroitin-4 sulphate ( $\Delta$ di-C4S)<sup>54</sup>. The ratios of  $\Delta$ di-C6S/ $\Delta$ di-C4S and concentration of  $\Delta$ di-C6S may provide valuable indications of cartilage destruction, since adult human articular cartilage contains mainly  $\Delta$ di-C6S that changes to  $\Delta$ di-C4S in OA<sup>55</sup>.

Analyses of OA cartilages have demonstrated an increased concentration of a chondroitin sulphate 846 epitope present on the largest fully functional aggrecan molecules<sup>56</sup>. The 846

epitope is not present in normal articular cartilages (that are rich in KS), and is absent from KS epitope-containing fragments but present in larger molecules bearing these chondroitin sulphate disaccharides. Thus, the 846 epitope is associated more with the synthesis of new molecules that lack the KS epitope<sup>56</sup>. Moreover, it has been suggested that this epitope, which is markedly increased in OA synovial fluid over levels in peripheral blood, may be reflective of aggrecan synthesis<sup>57</sup>. Although the KS epitope present in body fluids is thought to be reflective of aggrecan degradation, proteoglycan degradation products bearing an antigenic KS epitope have reportedly increased or decreased in the serum of OA patients<sup>58</sup>. An 846 epitope, present in the proteoglycan aggrecan on some of the chondroitin sulfate chains of molecules, is released from cartilage and correlates with the synthesis of aggrecan, which shows a markedly increased concentration in OA synovial fluid.

No significant relationships were reported among the concentration of proteinases in OA joints, the presence of inhibitors, and the structural matrix components of cartilage matrix, which may be involved in either degradative and/or synthetic processes. Furthermore, the levels of proteinases and their inhibitors (measured by immunoassay and not as activities) did not change significantly as joint damage progressed (Figure 2), although a loss of proteoglycan chondroitin sulphate components and evidence of reduced matrix synthesis (846 epitope and CPII) were noted in advanced OA<sup>52</sup>.

It was observed that the correlation between MMP-1 and MMP-3 concentrations suggests that there may be some co-ordination of synthesis, secretion and activity between those proteinases in OA. A striking correlation of TIMP-1 with both these metalloproteinases has also been reported. Increases in the production of inhibitors would be thought to favour matrix assembly and repair such as that mediated by the synthesis of matrix molecules. This observation suggested that even in a pathology, the production of these proteinases may be coupled with the production of this inhibitor. It was apparent that the concentrations of MMPs and TIMPs were not identifiably correlated with the release of proteoglycans or fragments thereof bearing the KS epitope or  $\Delta$ di-C4S or  $\Delta$ di-C6S. One reasonable explanation for this lack of correlation is that the degradation of these proteoglycans may be mediated not by MMPs, but by aggrecanase. The proteoglycan fragments such as  $\Delta$ di-C6S,  $\Delta$ di-C4S and KS epitope observed in synovial fluid in OA have the characteristics of degradation products of proteoglycan aggrecan molecules released from the original 'resident' matrix. Thus, the disease process may be characterized as an ongoing attack on the original 'resident' molecules.

Measurements of these molecules are proving useful in assessing disease progression and the response to therapy designed to control inflammation, arrest cartilage degradation, and promote synthesis and cartilage repair. In this way, assays may be used in clinical trials to investigate chondroprotection and assess the effects of new therapies.



## RECENT ADVANCES IN OSTEOARTHRITIS

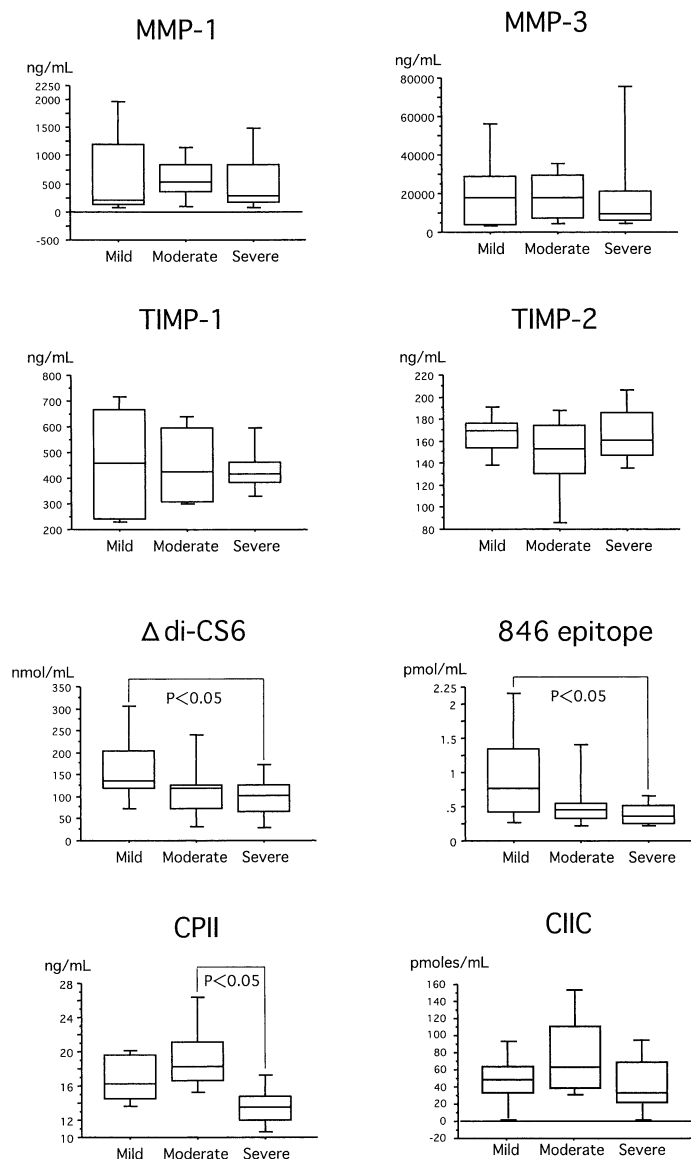


Figure 2: Concentrations of proteinases, inhibitors, proteoglycan components, CPII and HA in synovial fluid in patient groups classified by radiographic and MR imaging assessments. Significant differences are indicated. The 34 OA patients were divided according to the severity of joint destruction into three groups of mild ( $n=10$ ), moderate ( $n=10$ ) and severe change ( $n=14$ ). In the mild group, plain radiographs showed no obvious abnormalities. MR images showed a degeneration of menisci classified as stage 0 or 1 using the method of Mink et al<sup>59</sup>, and no sign of subchondral bone involvement. In the moderate group, plain radiographs showed slight abnormalities including small osteophytes and subchondral sclerosis. MRI revealed a degeneration of either meniscus classified into stage 2 and evidence of limited of subchondral bone involvement. In the severe group; plain radiographs showed obvious abnormalities including osteophytes, and pronounced subchondral sclerosis. MRI identified the degeneration of either meniscus classified as stage 3 or 4 and subchondral bone changes. The data are expressed as box plots. From the bottom each horizontal line represents either a 10 percentile (bar), 25 percentile, 75 percentile (box) or 90 percentile (bar). The median is shown in each box. The figures were referred from Ref. 52.

## REFERENCES

- 1) Poole, A.R.: Cartilage in health and disease. In: *Arthritis and Allied Conditions: A Textbook of rheumatology*. 14th Edition. Edited by Koopman, W.J., Williams & Wilkins, Baltimore (2000).
- 2) Felson, D.T.: Epidemiology of the rheumatic diseases. In: *Arthritis and Allied Conditions: A Textbook of Rheumatology*. 14th Edition. Edited by Koopman, W.J., Williams & Wilkins, Baltimore (2000).
- 3) Massart, F., Reginster, J.Y., and Brandi, M.L.: Genetics of menopause-associated diseases. *Maturitas*, 40, 103–16 (2001).
- 4) Ala-Kokko, L., Baldwin, C.T., Moskowitz, R.W., and Prockop, D.J.: Single base mutation in the type II procollagen gene (COL2A1) as a cause of primary osteoarthritis associated with a mild chondrodysplasia. *Proc. Nat. Acad. Sci.*, 6565–6568 (1990).
- 5) Westacott, C.J., Webb, G.H., Warnock, M.G., Sims, J.V., and Elson, C.J.: Alteration of cartilage metabolism by cells from osteoarthritic bone. *Arthritis Rheum.*, 40, 1282–1291 (1997).
- 6) Carson, J.P., Fernandes, J.C., Martel-Pelletier, J., Tardif, G., Mineau, F., Geng, C., and Pelletier, J.P.: Chondroprotective effect of intraarticular injection of interleukin-1 receptor antagonist in experimental osteoarthritis: suppression of collagenase-1 expression. *Arthritis Rheum.*, 39, 1535–1544 (1996).
- 7) Hashimoto, S., Ochs, R.L., Komiya, S., and Lotz, M.: Linkage of chondrocyte apoptosis and cartilage degradation in human osteoarthritis. *Arthritis Rheum.*, 41, 1632–1638 (1998).
- 8) Kempson, G.E., Muir, H., Pollard, C., and Tuke, M.: The tensile properties of the cartilage of human femoral condyles related to the content of collagen and glycosaminoglycans. *Biochim. Biophys. Acta.*, 297, 465–472 (1973).
- 9) Felson, D.T., Lawrence, R.C., Dieppe, P.A., Hirsch, R., Helmick, C.G., Jordan, J.M., Kington, R.S., Lane, N.E., Nevitt, M.C., Zhang, Y., Sowers, M., McAlindon, T., Spector, T.D., Poole, A.R., Yanovski, S.Z., Ateshian, G., Sharma, L., Buckwalter, J.A., Brandt, K.D., and Fries, J.F.: Osteoarthritis: new insights. Part 1: the disease and its risk factors. *Ann. Intern. Med.*, 133, 635–646 (2000).
- 10) Poole, A.R., Kojima, T., Yasuda, T., Mwale, F., Kobayashi, M., and Laverty, S.: Composition and structure of articular cartilage: a template for tissue repair. *Clin. Orthop.*, 391 Suppl, S26–33 (2001).
- 11) Vincenti, M.P., Clark, I.M., and Brinckerhoff, C.E.: Using inhibitors of metalloproteinases to treat arthritis. Easier said than done? *Arthritis Rheum.*, 37, 1115–26 (1994).
- 12) Uria, J.A. and Lopez-Otin, C.: Matrilysin-2, a new matrix metalloproteinase expressed in human tumors and showing the minimal domain organization required for secretion, latency, and activity. *Cancer Res.*, 60, 4745–4751 (2000).
- 13) Velasco, G., Cal, S., Merlos-Suarez, A., Ferrando, A.A., Alvarez, S., Nakano, A., Arrivas, J., and Lopez-Oti, C.: Human MT 6-matrix metalloproteinase: identification, progelatinase A activation, and expression in brain tumors. *Cancer Res.*, 60, 877–882 (2000).
- 14) Yamanaka, H., Makino, K., Takizawa, M., Nakamura, H., Fujimoto, N., Moriya, H., Nemori, R., Sato, H., Seiki, M., and Okada, Y.: Expression and tissue localization of membrane-type 1, 2, and 3 matrix metalloproteinases in rheumatoid synovium. *Lab. Investigat.*, 80, 677–687 (2000).
- 15) Pap, T., Shigeyama, Y., Kuchen, S., Frenihough, J.K., Simmen, B., Gay, R.E., Bilingham, M., and Gay, S.: Differential expression pattern of membrane-type matrix metalloproteinases in rheumatoid arthritis. *Arthritis Rheum.*, 43, 1226–1232 (2000).
- 16) Frederick, Woessner Jr.: Matrix metalloproteinases and their inhibitors in connective tissue remodeling. *FASEB Journal*, 2145–2154 (1991).
- 17) Ito, A. and Nagase, H.: Evidence that human rheumatoid synovial matrix metalloproteinase 3 is an endogenous activator of procollagenase 1. *Arch. Biochem. Biophys.*, 267, 211–216 (1998).
- 18) Okada, Y., Takeuchi, N., Tomita, K., Nakanishi, I., and Nagase, H.: Immunolocalization of matrix metalloproteinase 3 (stromelysin) in rheumatoid synovioblasts (B cells): correlation with rheumatoid arthritis. *Ann. Rheum. Dis.*, 48, 645–53 (1989).
- 19) Ishiguro, N., Ito, T., Obata, K., Fujimoto, N., and Iwata, H.: Determination of stromelysin-1, 72 and 92 Kda type IX collagenase, tissue inhibitor of metalloproteinase-1 (TIMP-1), and TIMP-2 in synovial fluid and serum from patients with rheumatoid arthritis. *J. Rheumatol.*, 23, 1599–1604 (1996).
- 20) Cole, A.A., Chubinskaya, S., Schumacher, B., Huch, K., Cs-Szabo, G., Yao, J., Mikecz, K., Hasty, K.A., and Kuettner, K.E.: Chondrocyte matrix metalloproteinase-8. Human articular chondrocytes express neutrophil collagenase. *J. Biol. Chem.*, 271, 11023–11026 (1996).
- 21) Reboul, P., Pelletier, J.P., Tardif, G., Cloutier, J.M., and Martel-Pelletier, J.: The new collagenase, collagenase-3, is expressed and synthesized by human chondrocytes but not by synoviocytes. A role in osteoarthritis. *J Clin Invest.*, 97, 2011–2019 (1996).

- 22) Pap, T., Shigeyama, Y., Kuchen, S., Fernihough, J.K., Simmen, B., Gay, R.E., Billingham, M., and Gay, S.: Differential expression pattern of membrane-type matrix metalloproteinase in rheumatoid arthritis. *Arthritis Rheum.*, 43, 1226–1232 (2000).
- 23) Buttner, F.H., Chubinskaya, S., Margerie, D., Huck, K., Elechtenmacher, J., Cole, A.A., Kuettner, K.E., and Bartnick, E.: Expression of membrane type 1 matrix metalloproteinase in human articular cartilage. *Arthritis Rheum.*, 40, 704–709 (1997).
- 24) Imai, K., Ohta, S., Matsumoto, T., Fujimoto, N., Sato, H., Seiki, M., and Okada, Y.: Expression of membrane-type 1 matrix metalloproteinase and activation of progelatinase A in human osteoarthritic cartilage. *Am. J. Pathol.*, 151, 245–256 (1997).
- 25) Knoper, V.C., Will, H., Lopez-Otin, C., Smith, B., Atkinson, S.J., Stanton, H., Hembry, R.M., and Murphy, G.: Cellular mechanisms for human procollagenase-3 (MMP-13) activation. Evidence that MT1-MMP (MMP-14) and gelatinase A (MMP-2) are able to generate active enzyme. *J. Biol. Chem.*, 271, 17124–17131 (1996).
- 26) Mitchell, P.G., Magna, H.A., Reeves, L.M., Lopresti-Morrow, L., Yocum, S.A., Rosner, P.J., Geoghegan, K.F., and Hambor, J.E.: Cloning, expression, and type II collagenolytic activity of matrix metalloproteinase-13 from human osteoarthritic cartilage. *J. Clin. Invest.*, 97, 761–768 (1996).
- 27) Shlopov, B.V., Lie, W.-R., Mainardi, C.L., Cole, A.A., Chubinskaya, S., and Hasty, K.A.: Osteoarthritic lesions. Involvement of three different collagenases. *Arthritis Rheum.*, 40, 2065–2074 (1997).
- 28) d'Ortho, M.P., Will, H., Atkinson, S., Butler, G., Messent, A., Gavrilovic, J., Smith, B., Timpl, R., Zardi, L., and Murphy, G.: Membrane-type matrix metalloproteinases 1 and 2 exhibit broad-spectrum proteolytic capacities comparable to many matrix metalloproteinases. *Eur. J. Biochem.*, 250, 751–757 (1997).
- 29) Will, H., Atkinson, S.J., Butler, G.S., Smith, B., and Murphy, G.J.: The soluble catalytic domain of membrane type 1 matrix metalloproteinase cleaves the propeptide of progelatinase A and initiates autoproteolytic activation. Regulation by TIMP-2 and TIMP-3. *Biol. Chem.*, 271, 17119–17123 (1996).
- 30) Malesud, C.J. and Goldberg, V.M.: Future directions for research and treatment of osteoarthritis. *Front. Biosci.*, 4, D762–71 (1999).
- 31) Tortorella, M.D., Malfait, A.M., Deccico, C., and Arner, E.: The role of ADAM-TS4 (aggrecanase-1) and ADAM-TS5 (aggrecanase-2) in a model of cartilage degradation. *Osteoarthritis. Cartilage.*, 9, 539–552 (2001).
- 32) Bluteau, G., Conrozier, T., Mathieu, P., Vignon, E., Herbage, D., and Mallein-Gerin, F.: Matrix metalloproteinase-1, -3, -13 and aggrecanase-1 and -2 are differentially expressed in experimental osteoarthritis. *Biochim. Biophys. Acta.*, 1526, 147–158 (2001).
- 33) Mercuri, F.A., Doege, K.J., Arner, E.C., Pratta, M.A., Last, K., and Fosang, A.J.: Recombinant human aggrecan G1-G2 exhibits native binding properties and substrate specificity for matrix metalloproteinases and aggrecanase. *J. Biol. Chem.*, 274, 32387–32395 (1999).
- 34) Westling, J., Fosang, A.J., Last, K., Thompson, V.P., Tomkinson, K.N., Hebert, T., McDonagh, T., Collins-Racie, L.A., LaVallie, E.R., Morris, E.A., and Sandy, J.D.: ADAMTS4 cleaves at the aggrecanase site (Glu373-Ala374) and secondarily at the matrix metalloproteinase site (Asn341-Phe342) in the aggrecan interglobular domain. *J. Biol. Chem.*, 277, 16059–16066 (2002).
- 35) Pelletier, J.-P., Mineau, F., Faure, M.P., and Martel-Pelletier, J.: Imbalance between the mechanisms of activation and inhibition of metalloproteinases in the early lesions of experimental osteoarthritis. *Arthritis Rheum.*, 33, 1466–1476 (1990).
- 36) Kashiwagi, M., Tortorella, M., Nagase, H., and Brew, K.: TIMP-3 is a potent inhibitor of aggrecanase 1 (ADAM-TS4) and aggrecanase 2 (ADAM-TS5). *J. Biol. Chem.*, 276, 12501–12504 (2001).
- 37) Gomez, D.E., Alonso, D.F., Yoshiji, H., and Thorgeirsson, U.P.: Tissue inhibitor of matrix metalloproteinase: structure, regulation and biological function. *Eur. J. Cell Biol.*, 74, 111–122 (1997).
- 38) Ohta, S., Imai, K., Yamashita, K., Matsumoto, T., Azumano, I., and Okada, Y.: Expression of matrix metalloproteinase-7 (matrilysin) in human osteoarthritic cartilage. *Lab. Invest.*, 78, 79–87 (1998).
- 39) Mauviel, A.: Cytokine regulation of metalloproteinase gene expression. *J. Cell Biochem.*, 53, 288–295 (1993).
- 40) Woessner, J.F. Jr.: Imbalance of proteinases and their inhibitors. In: *Osteoarthritic Disorders*. Edited by Kuettner, K.E., Goldberg, V.M., American Association of Orthopaedic Surgeons, Rosemont, IL (1995).
- 41) Martel-Pelletier, J., Faure, M.P., McCollum, R., Mineau, F., Cloutier, J.M., and Pelletier, J.P.: Plasmin, plasminogen activators and inhibitor in human osteoarthritic cartilage. *J. Rheumatol.*, 18, 1863–1871 (1991).
- 42) Poole, A.R.: An introduction to the pathophysiology of osteoarthritis. *Front Biosci.*, 4, D662–670 (1999).
- 43) Hollander, A.P., Pidoux, I., Reiner, A., Rorabeck, C., Bourne, R., and Poole, A.R.: Damage to type II collagen in ageing and osteoarthritis starts at the articular surface, originates around chondrocytes and extends into the cartilage with progressive degeneration. *J. Clin. Invest.*, 96, 2859–2869 (1995).
- 44) Hollander, A.P., Heathfield, T.F., Webber, C., Iwata, Y., Bourne, R., Rorabeck, C., and Poole, A.R.: Increased damage to type II collagen in osteoarthritic articular cartilage detected by a new immunoassay. *J. Clin. Invest.*, 93, 1722–1732 (1994).

- 45) Poole, A.R., Alini, M., and Hollander, A.H.: Cellular biology of cartilage degradation. In: *Mechanisms and Models in Rheumatoid Arthritis*. Edited by Henderson, B, JCW Edwards, ER Pettipher. Academic Press, London (1995).
- 46) Tortorella, M.D., Burn, T.C., Pratta, M.A., Abbaszade, I., Hollis, J.M., Liu, R., Rosenfeld, S.A., Copeland, R.A., Decicco, C.P., Wynn, R., Rockwell, A., Yang, F., Duke, J.L., Solomon, K., George, H., Bruckner, R., Nagase, H., Itoh, Y., Ellis, D.M., Ross, H., Wiswall, B.H., Murphy, K., Hillman, Jr. M.C., Hollis, G.F., Newton, R.C., Magolda, R.L., Trzaskos, J.M., and Arner, E.C.: Purification and cloning of aggrecanase-1: a member of the ADAMTS family of proteins. *Science*, 284, 1664–1666 (1999).
- 47) Hurskainen, T.L., Hirohata, S., Seldin, M.F., and Apte, S.S.: ADAM-TS5, ADAM-TS6, and ADAM-TS7, novel members of a new family of zinc metalloproteases. General features and genomic distribution of the ADAM-TS family. *J. Biol. Chem.*, 274, 25555–25563 (1999).
- 48) Tortorella, M.D., Malfait, A.M., Deccico, C., and Arner, E.: The role of ADAM-TS4 (aggrecanase-1) and ADAM-TS5 (aggrecanase-2) in a model of cartilage degradation. *Osteoarthritis Cartilage*, 9, 539–552 (2001).
- 49) von der Mark, K., Kirsch, T., Nerlich, A., Kuss, A., Weseloh, G., Glekert, K., and Stos, H.: Type X collagen synthesis in human osteoarthritic cartilage. Indication of chondrocyte hypertrophy. *Arthritis Rheum.*, 35, 806–811 (1992).
- 50) Stoop, R., Buma, P., van der Kraan, P.M., Hollander, A.P., Billinghamurst, R.C., Meijers, T.H., Poole, A.R., and van den Berg, W.B.: Type II collagen degradation in articular cartilage fibrillation after anterior cruciate ligament transection in rats. *Osteoarthritis Cartilage*, 9, 308–315 (2001).
- 51) Price, J.S., Till, S.H., Bickerstaff, D.R., Bayliss, M.T., and Hollander, A.P.: Degradation of cartilage type II collagen precedes the onset of osteoarthritis following anterior cruciate ligament rupture. *Arthritis Rheum.*, 42, 2390–2398 (1999).
- 52) Ishiguro, N., Ito, T., Oguchi, T., Kojima, T., Iwata, H., Ionescu, M., and Poole, A.R.: Relationships of matrix metalloproteinases and their inhibitors to cartilage proteoglycan and collagen turnover and inflammation as revealed by analyses of synovial fluids from patients with rheumatoid arthritis. *Arthritis Rheum.*, 44, 2503–2511 (2001).
- 53) Christgau, S., Garnero, P., Fledelius, C., Moniz, C., Ensig, M., Gineyts, E., Rosenquist, C., and Qvist, P.: Collagen type II C-telopeptide fragments as an index of cartilage degradation. *Bone*, 29, 209–215 (2001).
- 54) Shinmei, M., Miyauchi, S., Machida, A., and Miyazaki, K.: Quantitation of chondroitin 4-sulphate and chondroitin 6-sulphate in pathologic joint fluid. *Arthritis Rheum.*, 35, 1304–1308 (1992).
- 55) Bayliss, M.T.: Proteoglycan structure in normal and osteoarthritic cartilage. In: *Articular Cartilage Biochemistry*. Edited by Kuettner, K.E., Schleyerbach, R., Hascall, V.C., Raven Press, New York, 295–308 (1986).
- 56) Rizkalla, G., Reiner, A., Bogoch, E., Poole, A.R.: Studies of the articular cartilage proteoglycan aggrecan in health and osteoarthritis. Evidence for molecular heterogeneity and extensive molecular changes in disease. *J. Clin. Invest.*, 90, 2268–2277 (1992).
- 57) Poole, A.R., Ionescu, M., Swan, A., and Dieppe, P.A.: Changes in cartilage metabolism in arthritis are reflected by altered serum and synovial fluid levels of the cartilage proteoglycan aggrecan. Implications for pathogenesis. *J. Clin. Invest.*, 94, 25–33 (1994).
- 58) Thonar, E.J.-M.A., Lenz, M.E., and Klintworth, G.K.: Quantitation of keratan sulfate in blood as marker of cartilage metabolism. *Arthritis Rheum.*, 28, 1367–1376 (1985).
- 59) Mink, J.H., Levy, T., Crues, J.V. 3d.: Tears of the anterior cruciate ligament and menisci of the knee : MR imaging evaluation. *Radiology*, 167, 769–774 (1988).