Mod Rheumatol (2001) 11:34–39
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ORIGINAL ARTICLE

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Expression of membrane-type matrix metalloproteinases in synovial tissue from patients with rheumatoid arthritis or osteoarthritis

Received: April 30, 2000 / Accepted: September 19, 2000

Abstract We investigated the expression of membrane-type matrix metalloproteinase (MT-MMP) and matrix metalloproteinase (MMP) mRNAs in synovial tissue from patients with rheumatoid arthritis (RA, $n = 5$) or osteoarthritis (OA, $n = 5$) by Northern blot analysis. Northern analysis demonstrated strong expression of MT1-MMP, MT3-MMP, MMP-1, and MMP-3 and weak expression of MT2-MMP and MMP-8 in synovial tissue from patients with RA or OA. MT4-MMP was not detected. No significant difference was shown in the expression of MT-MMP mRNAs between RA and OA. Synovial tissue of RA or OA patients expressed MT-MMPs as well as MMPs. These results indicate that, in addition to MMPs, MT1-MMP, MT3-MMP, and probably MT2-MMP may play a role in the degradation of bone and cartilage matrix in RA and OA. Such information may provide a clue to the development of a novel therapeutic approach targeted on the prevention of joint destruction.

Key words Matrix metalloproteinases (MMPs) · Membrane-type matrix metalloproteinases (MT-MMPs) · Osteoarthritis (OA) · Rheumatoid arthritis (RA) · Synovial tissues

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Introduction

Several lines of evidence indicate that immune system mediators evoke an inflammatory response in rheumatoid arthritis (RA), resulting in bone and cartilage destruction, and osteoarthritis (OA) is known to be a degenerative disease of the articular cartilage.[1] It is known that the bone and cartilage destruction is mediated by synovial macrophages and fibroblasts as a consequence of metalloproteinase release induced by some inflammatory cytokines.[2,3] Indeed, explants from human synovial tissue from patients with RA have been found to release large amounts of collagenase,[4] and increased collagenase activity has been reported in synovial fluid from patients with RA.[5] It has also been suggested that some cells of the synovial tissue show the destructive processes through a self-perpetuating autocrine/paracrine network.[6] Elevated levels of inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and granulocyte macrophage colony-stimulating factor (GM-CSF), as well as matrix metalloproteinases (MMPs) secreted by synovial tissue, have been reported.[5,7–9]

MMPs, a family of enzymes that consist of 20 different members in humans, are capable of degrading some components of the extracellular matrix (ECM) under both physiological and pathological conditions.[10,11] They are secreted as latent proenzymes that undergo proteolytic cleavage of the amino terminal domain during activation, and their catalytic activity is inhibited by specific tissue inhibitors of metalloproteinase (TIMPs). Based on their structure and substrate specificity, the MMP family can be divided into five groups in humans: the collagenase group, which includes interstitial collagenase (MMP-1), neutrophil collagenase (MMP-8), and collagenase-3 (MMP-13), the stromelysin group, which includes stromelysin-1 (MMP-3), stromelysin-2 (MMP-10), stromelysin-3 (MMP-11), MMP-19, and enamelysin (MMP-20), the gelatinase group, which includes gelatinase A (MMP-2) and gelatinase B (MMP-9), another group which includes matrilysin (MMP-7) and macrophage metalloelastase (MMP-12), MMP-23, and MMP-

26, and the membrane-type matrix metalloproteinase (MT-MMP) group, which is a group of MMPs expressed on the cell surface as a membrane protein.[11–14] Six MT-MMPs (MT1, 2, 3, 4, 5, 6-MMP) have thus far been reported.[11–13] MT1-MMP has been shown to convert progelatinase A (proMMP-2) and procollagenase 3 (proMMP-13) to an active form.[15,16] MT1-MMP has also been reported to degrade ECM components.[17]

The expression of collagenases, stromelysins, and gelatinases in synovial tissue from patients with RA or OA has been extensively studied, and the expression of MT-MMPs in the synovial tissue and the potential difference in expression level by polymerase chain reaction (PCR) between RA and OA have been examined.[18,19] In the present study, we compared the expression level of MT-MMP and MMP mRNA in fresh synovial tissue from patients with RA or OA using Northern blot analysis.

Materials and methods

Patients

Table 1 summarizes the clinical features of patients with RA or OA from whom the synovial tissue was obtained. Five tissue samples from RA patients and five from OA patients, ranging in age from 54 to 76 years, were obtained at the time of total knee arthroplasty. Patients with RA were given two or three of the following types of medication in combination: prednisolone (5–7.5 mg/day); DMARD (lobenzarit disodium, 80 mg/day; bucillamine, 200 mg/day; sodium aurothiomalate, 10 mg/2weeks; actarit, 200 mg/day); NSAID (indomethacin, 50 mg/day; sulindac, 200 mg/day; diclofenac sodium, 50–100 mg); immunosuppressant (methotrexate, 2.5–7.5 mg/week; azathioprine, 100 mg/day). However, patients with OA were given only NSAIDs. Patients with RA or OA were diagnosed according to the criteria proposed by the American College of Rheumatology.[20,21]

RNA extraction

Synovial tissue was dissected and directly frozen in dry ice within 10 min of excision. Total RNA was isolated from 300–600 mg frozen tissue. In the presence of liquid nitrogen, the frozen synovial tissue was homogenized using a pestle, and transferred to a stainless steel tube which was frozen in dry ice. The tissue was then quickly ground with a hammer. After evaporation of the liquid nitrogen, the sample powder was transferred to a plastic tube, the extraction reagent (Isogen: Wako Pure Chemicals, Tokyo, Japan) was added, and the homogenate was mixed thoroughly in a homogenizer. Further isolation was carried out according to the technical manual provided by the manufacturer. Total RNA was precipitated with isopropanol. The pellet was resuspended in 300 μl diethyl pyrocarbonate (DEPC)-treated water.

Preparation of cDNA

In this study, single-stranded cDNA was synthesized from the total RNA isolated from the synovial tissue of several RA patients by reverse transcription using a RNA PCR kit (Takara Biomedicals, Tokyo, Japan). MT1, 2, 3, 4-MMP, MMP-1, MMP-8, and MMP-3 cDNA probes for Northern blot analysis were amplified by PCR with Extaq DNA polymerase (Takara Biomedicals, Tokyo, Japan). Amplification was performed by 30 cycles of denaturation (1 min, 94°C), annealing (2 min, 59–63°C), and extension (2 min, 72°C) in a DNA Cycler 2400 (Perkin Elmer, Norwalk, CT, USA). The resulting fragment was purified by gel electrophoresis and cloned into pBluescript SK+ (MT1-MMP) or Unizap XR (MT2, 3, 4-MMP, MMP-1, MMP-8, and MMP-3) and subsequent gel purification. Each primer was sequenced by the dideoxy chain termination method with a dye terminator FS kit (Perkin Elmer, Norwalk, CT, USA), using an automated DNA sequencer (377; Applied Biosystems, Foster City, CA, USA). The entire cDNA sequence of probes was

Table 1. Clinical features of patients with rheumatoid arthritis (RA) and osteoarthritis (OA)

Patient	Age/sex	Duration of disease (years)	RF	ESR (mm/h)	Medication	Surgical procedure
RA						
1	65/F	35	+	32	7.5 mg pred./day, DMARD, IS	TKA
2	64/F	7	+	40	5 mg pred./day, NSAID, DMARD	TKA
3	71/F	15	+	31	5 mg pred./day, NSAID, DMARD, IS	TKA
4	54/F	19	+	87	5 mg pred./day, DMARD, IS	TKA
5	63/F	10	+	109	5 mg pred./day, DMARD, IS	TKA
OA						
1	67/F	17	−		NSAID	TKA
2	76/M	10	−		NSAID	TKA
3	72/F	20	−		NSAID	TKA
4	66/F	6	−		NSAID	TKA
5	58/F	10	−		NSAID	TKA

RF, rheumatoid factor; ESR, erythrocyte sedimentation rate; IS, immunosuppressant; DMARD, disease modifying antirheumatic drug; NSAID, nonsteroidal anti-inflammatory drug; pred, prednisolone; TKA, total knee arthroplasty
The ESR of patients with OA was within the normal range

confirmed in both strands using universal oligonucleotide primers. MT1-MMP cDNA was 296 bp in length, starting at nucleotide position 404 and terminating at position 699. MT2-MMP cDNA was 1863 bp in length, starting at nucleotide position 1658 and terminating at position 3520. MT3-MMP cDNA was 987 bp in length, starting at nucleotide position 1174 and terminating at position 2160. MT4-MMP cDNA was 1623 bp in length, starting at nucleotide position 683 and terminating at position 2305. We used full-length cDNA probes in MMP-1 (2.5 kbp), MMP-8 (1.4 kbp), and MMP-3 (1.5 kbp). A 1.8 kbp β-actin cDNA fragment (Clontech, Palo Alto, CA, USA) was used as a control.

Northern blot analysis

After the quantitation of the concentration and purity of the total RNA, 20 μg of total RNA were separated on a 1.2% GTG agarose (FMC Bioproducts, Rockland, ME, USA) gel containing formaldehyde and transferred to a nylon membrane (Hybond N^+, Amersham, UK) using a capillary blotter (Taitec Corporation, Tokyo, Japan). After linking, the nylon membrane was stared at −20°C until used. The membrane was prehybridized in hybridization buffer (Clontech Laboratory, Polo Alto, CA, USA) at 68°C for 30 min. cDNA probes were labeled with [α^{32}P] dCTP using a randomprime cDNA labeling kit (Takara Biomedicals, Tokyo, Japan) and purified by co-precipitation of 100 μg salmon testis DNA. The radioactivity of the cDNA probes were adjusted to approximately equal levels (about 8 million cerenkov counts per cDNA). Hybridization was performed in a prehybridization solution containing the labeled probe (10 ng/ml) at 68°C for 1 h. Unbound cDNA probe was removed by washing the membrane several times at room temperature in 2 × SSC, 0.05% SDS, and twice at 50°C for 40 min in 0.1 × SSC, 0.1% SDS. The Northern blot membranes were exposed for 2.5 h, or longer in some instances (MT2, 4-MMP, and MMP-8), and analyzed with a FujiX Bio-Imaging Analyzer BAS 2000 (Fuji Photo Film Co., Tokyo, Japan). The labeled probe from the previous hybridization was removed by washing the membrane for 10 min in boiling water containing 0.5% SDS. One membrane was successively hybridized with different cDNA probes in the following order: MT1, 2, 3, 4-MMP, and another one made in same manner was successively hybridized with MMP-1, MMP-8, MMP-3, and β-actin cDNA probes.

Data analysis

Differences in the mRNA expression level of MT-MMPs and MMPs in patients with RA or OA were compared by Dunnett's test.

Results

To examine the differences in the levels of expression between RA and OA, the expression of MT1, 2, 3, 4-MMP, MMP-1, MMP-8, and MMP-3 in synovial tissue from five patients with RA and five patients with OA were analyzed by Northern blotting. The clinical characteristics of the patients are summarized in Table 1.

The transcripts of MT1-MMP (4.5 kb), MT2-MMP (4.5 kb), and MT3-MMP (12, 10, and 3.5 kb) were detected in most RA and OA synovial tissue, while no mRNA expression of MT4-MMP was observed (Fig. 1). The cDNA probe for MT4-MMP was verified to be working using a tumor tissue as the positive control (data not shown). The mRNA expression of MT2-MMP was observed in all samples under the overexposure condition. A semiquantitative assessment of the expression level was carried out by densitometric analysis using FujiX Bio-Imaging Analyzer BAS 2000 (Table 2). The mRNA levels of MT1-MMP,

Table 2. Expression of MT1-MMP, MT2-MMP, MT3-MMP, and MT4-MMP mRNA in synovial tissue from patients with RA and OA

Patient	MT1-MMP	MT2-MMP	MT3-MMP	MT4-MMP
RA				
1	1035	76	2185	
2	928	152	3272	
3	787	98	2112	
4	1227	145	4041	
5	447	192	1173	
Mean ± SD	885 ± 293	133 ± 46	2557 ± 1114	ND
OA				
1	1263	129	3589	
2	1044	154	3343	
3	1424	117	3395	
4	1241	178	4135	
5	1116	237	2565	
Mean ± SD	1218 ± 146	163 ± 48	3405 ± 565	ND

The mRNA expression level of MT-MMPs was determined by densitometric scanning in PSL units (FujiX Bio-Imaging Analyzer BAS 2000) and was normalized to 1000 PSL of β-actin expression. No significant difference was found between RA and OA in MT1-MMP, MT2-MMP, and MT3-MMP
ND, not detected

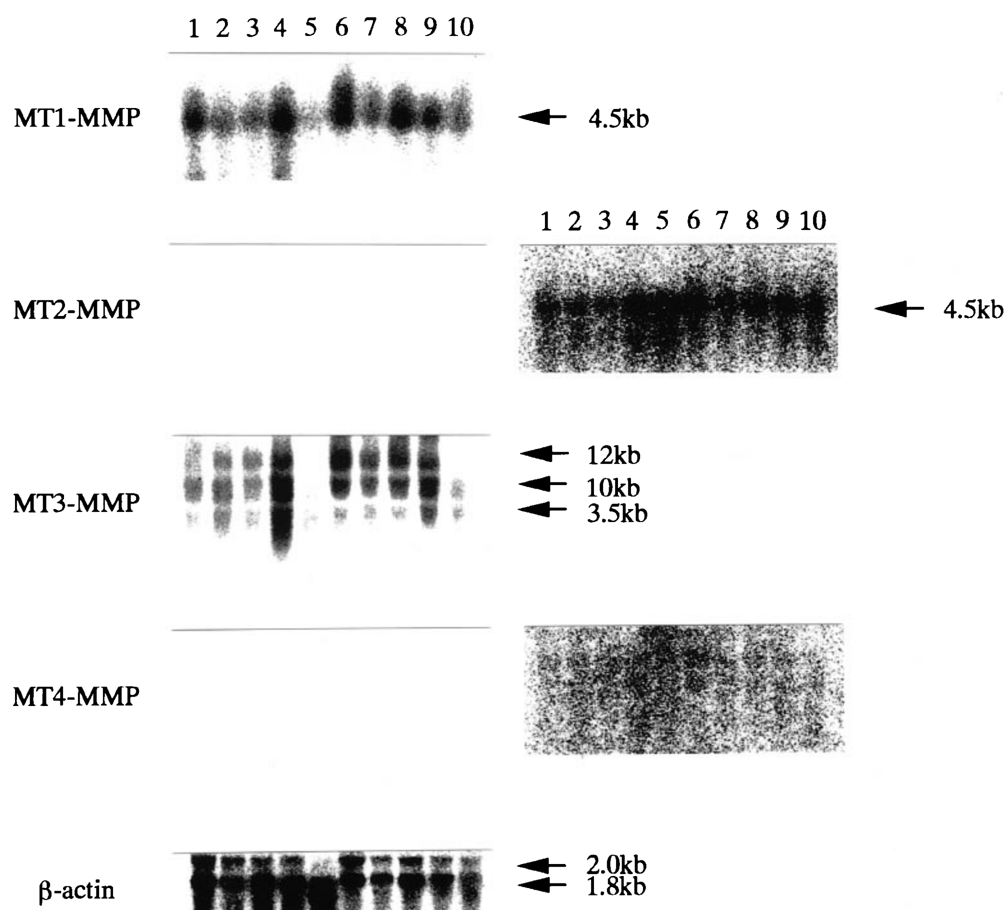

Fig. 1. Northern blot analysis of MT1-MMP, MT2-MMP, MT3-MMP, and MT4-MMP mRNA in synovial tissue from patients with RA and OA. Each lane contains 20 μg total RNA. Lanes 1–5, total RNA extracted directly from five different patients with RA; lanes 6–10, total RNA extracted directly from five different patients with OA. Most of the membranes were exposed for 2.5 h (*left panel*). To facilitate comparison of the signal intensity, membranes for MT2-MMP and MT4-MMP were overexposed (*right panel*). β-actin was used as a control for loading equal amounts of RNA

MT2-MMP, and MT3-MMP were not statistically different in RA or OA patients, although the expression level seemed to be slightly lower in RA patients.

Transcripts of MMP-1 (1.8 kb), MMP-8 (3.3 kb), and MMP-3 (2.5 and 1.6 kb) were detected in synovial tissue from patients with RA and OA by Northern blot analysis (Fig. 2). The mRNA expression of MMP-8 was much weaker than that of other MMPs, and was only detectable in a small number of samples under the overexposure condition. Table 3 shows the results of a semiquantitative assessment of the expression levels of MMPs. The mRNA level of MMP-1 in patients with RA was higher than that of OA patients, although the difference did not reach statistical significance. The mRNA level of MMP-3 in patients with RA was significantly higher than that of patients with OA ($P < 0.05$, Dunnett's test).

Table 3. Expression of MMP-1, MMP-8, and MMP-3 mRNA in synovial tissue from patients with RA and OA

Patient	MMP-1	MMP-8	MMP-3
RA			
1	232	1	762
2	2134	12	5563
3	1139	9	5587
4	1023	10	3600
5	808	7	3291
Mean ± SD	1067 ± 691	8 ± 4	3761 ± 1989*
OA			
1	2005	12	2784
2	33	3	121
3	556	6	2061
4	311	6	963
5	23	7	398
Mean ± SD	586 ± 824	7 ± 3	1266 ± 1128

The mRNA expression level of MMPs was determined by densitometric scanning in PSL units (FujiX Bio-Imaging Analyzer BAS 2000) and was normalized to 100 PSL of β-actin expression. The expression level of MMP-3 was significantly higher in patients with RA than in patients with OA

* $P < 0.05$, Dunnett's test

Discussion

The present study demonstrated that MT1-MMP and MT3-MMP were strongly expressed in synovial tissue from patients with both RA and OA. MT2-MMP was only weakly expressed, however, and MT4-MMP was not detected.

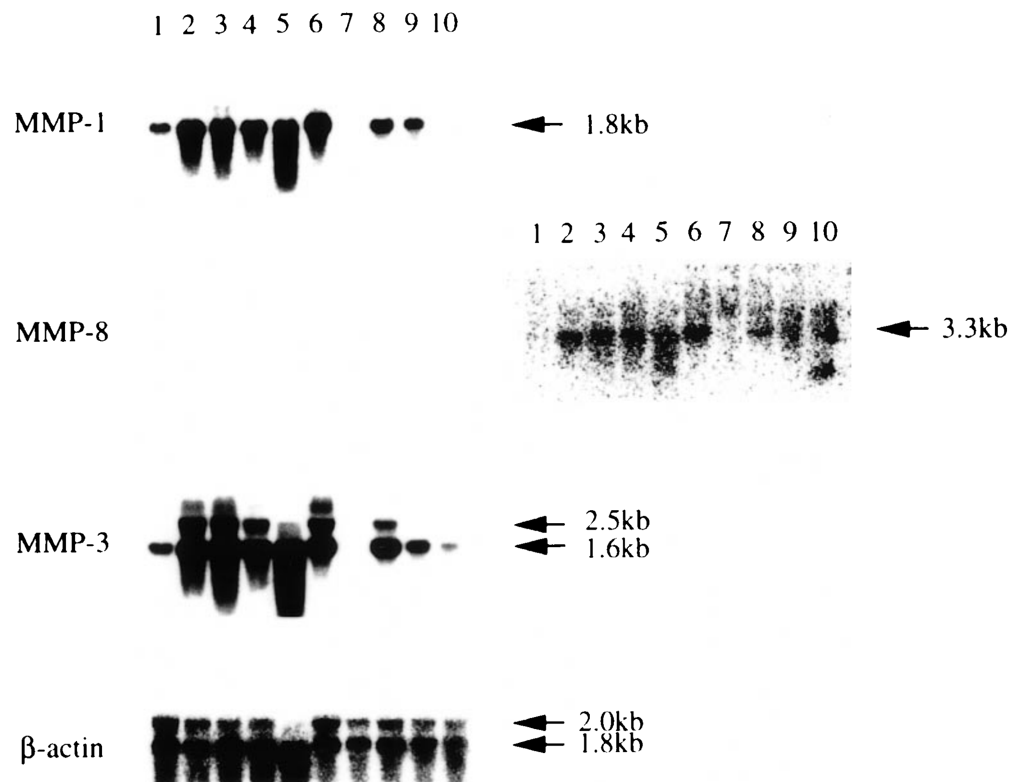

Fig. 2. Northern blot analysis of MMP-1, MMP-8, and MMP-3 mRNA in synovial tissue from patients with RA and OA. Each lane contains 20 μg total RNA. lanes 1–5, total RNA extracted directly from five different patients with RA; lanes 6–10, total RNA extracted directly from five different patients with OA. Most of the membranes were exposed for 2.5 h (*left panel*). To facilitate comparison of the signal intensity, membrane for MMP-8 was overexposed (*right panel*). β-actin was used as a control for loading equal amounts of RNA

These findings suggested that, in addition to MMPs, MT1-MMP and MT3-MMP in the synovial tissue might be involved in progressive joint destruction in both RA and OA.

The expression of MT1-MMP has been reported in several types of cell, such as chondrocytes,[22] osteoblasts,[23] osteoclasts,[24] and synovial fibroblasts.[25] Imai et al.[22] showed that expression of MT3-MMP mRNA and protein was found only in advanced OA cartilage, and speculated that MT3-MMP accelerates cartilage degradation through progelatinase A activation. They also reported that MT2-MMP is not detectable in OA cartilage. Yamanaka et al.[19] showed the expression of MT-MMPs in synovial tissue from patients with RA and OA using the RT-PCR method. In this study, we compared the level of expression of MT-MMPs and MMPs in synovial tissue from patients with RA and OA using Northern blot analysis.

MT1, 2, 3-MMPs are known to contribute to the activation of progelatinase A (proMMP-2) to MMP-2 in vitro.[17,26] MT1-MMP is also known as activator of procollagenase-3 (proMMP-13).[16] MT1-MMP is also known to degrade the ECM components, including types I, II, and III collagen, fibronectin, vitronectin, laminin, and gelatin, directly.[17] MT3-MMP has been reported to cleave type III collagen and fibronectin.[27] These observations and our findings suggest that synovial MT1-MMP and MT3-MMP might be the mediators which lead to progressive joint destruction, not only by activating proMMPs, but also by acting directly on ECM components of the cartilage.

It has previously been reported that MMP-1, MMP-8, and MMP-3 are expressed in destructive joint diseases such as RA or OA.[28,29] Beekman et al.[30] demonstrated that MMP activity in synovial fluid was highest in RA, followed by OA, and lowest in control subjects. Our observations suggested that, in contrast to MMP-1 and MMP-3 which are preferentially expressed in RA, expression of MT-MMP mRNA is almost equal in RA and OA. There may be a connection between MMPs and joint destruction other than that of MT-MMPs.

The present study has some limitations. Normal synovial tissue was not available, and therefore, it is not possible to conclude that the expression of MT-MMP mRNA in synovial tissue in RA or OA theoretically represents a pathological condition. In addition, patients with RA were treated with prednisolone, immunosuppressive drugs, and/or DMARDs, which may have down-regulated the expression of MT-MMP mRNA. Such possibilities have to be evaluated in future studies.

Conclusions

Our findings suggest that MT1-MMP, MT3-MMP, and probably MT2-MMP may be involved in joint destruction in RA and OA. Further investigations on the function and regulation of MT-MMPs in synovial tissue will be useful in elucidating the pathogenesis of joint diseases such as RA and OA.

Acknowledgments The authors would like to thank Drs. K. Kitano and H. Shirafuji (Discovery Research Laboratories II, Pharmaceutical Discovery Research Division, Takeda Chemical Industries Ltd.) for encouragement throughout this work.

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