Matrix Metalloproteinases in Joint Destruction in Rheumatoid Arthritis

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Abstract: The progressive destruction of articular cartilage is one of the hallmarks of rheumatoid arthritis (RA). Although different classes of proteolytic enzymes appear to be involved, cartilage degradation has been attributed mainly to the action of matrix metalloproteinases (MMPs), which are a family of enzymes that collectively degrade all components of the extracellular matrix. MMPs mediate irreversible matrix degradation and subsequent joint destruction in RA. This review describes the members of MMP family, the structure of MMPs, and the activation mechanism of proMMPs, and discusses the regulation of MMP expression and the pathologic implication in joint destruction in RA.

Key words: rheumatoid arthritis, matrix metalloproteinase, joint destruction, pannus, integrin

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic disabling disease characterized by systemic inflammation and joint tissue destruction. Degradation of articular cartilage is a common pathological feature in various arthritides including RA, and is mediated by the enhanced activity of proteolytic systems. For the cartilage destruction, there are intrinsic and extrinsic pathways. Chondrocytes themselves degrade cartilage extracellular matrix (ECM) intrinsically, whereas inflamed synovium, pannus tissue, and infiltrated inflammatory cells break down the ECM extrinsically. In both pathways, enzymatic digestion of ECM contributes to cartilage destruction.

Among several enzymes involved in the process, matrix metalloproteinases (MMPs) are thought to have an important role in joint destruction in RA. MMPs are a family of enzymes that collectively degrade all components of the ECM. While MMPs play a role in normal physiologic processes such as development and wound healing where MMP levels are generally low, up-regulation of MMP expression is found in arthritic diseases. MMPs mediate irreversible matrix degradation and subsequent joint destruction in RA. Proinflammatory cytokines, produced mainly by macrophages in the synovial pannus, induce the expression of MMPs from the synovium and cartilage in RA. These MMPs then degrade the matrix components of cartilage as well as bone and tendons.

In this review, we discuss an overview of the MMP family members, the regulation of MMP production, and the involvement of MMPs in joint destruction in RA.

The MMP Family

MMPs are zinc-containing, calcium-dependent proteinases that are active at neutral pH. To date, more than 20 MMPs have been identified in humans. On the basis of substrate specificity, sequence similarity, and domain organization, they are classified into six categories: collagenases, gelatinases, stromelysins, membrane-type MMPs, matrilysins, and other MMPs (Table 1). This chapter describes the first four categories of MMPs.

Collagenases

Collagenase 1 (MMP-1), collagenase 2 (MMP-8), and collagenase 3 (MMP-13) are in this group. These collagenases and membrane-bound MMP-14 are the only enzymes that can cleave the intact triple helix of collagen types I, II, and III. Collagenases can also digest a number of other ECM and non-ECM molecules.

Collagen is the most common protein of the vertebrate body and has a unique structure. Three coils of polypeptide form a rod-shaped triple-helical molecule with globular regions at each end. On leaving the cells, globular regions are proteolytically removed and the collagen molecules align to produce a characteristic staggered arrangement. Crosslinks form between the collagen molecules, which renders stability. These self-aligned collagen molecules form the collagen fibers that provide the strength and rigidity of ECM. Cartilage is composed mainly of collagen type II, which forms a fibrillar network with minor collagens types IX and XI. Of MMPs, therefore, collagenases play crucial roles in the maintenance of matrix homeostasis. Collagenases cleave collagen at a specific site three-fourths from the N-terminus of the collagen molecule, resulting in the opening of triple helix followed by its access to gelatinases and other proteinases. Thus, collagenase expression is a rate-limiting step for fibrillar collagen degradation.
Matrix Metalloproteinases in Joint Destruction

Table 1. The MMP family.

<table>
<thead>
<tr>
<th>MMP</th>
<th>Enzyme</th>
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<tbody>
<tr>
<td>MMP-1</td>
<td>Collagenase-1</td>
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<td>MMP-8</td>
<td>Collagenase-2</td>
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<td>MMP-9</td>
<td>Gelatinase B</td>
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<td>MMP-3</td>
<td>Stromelysin-1</td>
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<td>MMP-10</td>
<td>Stromelysin-2</td>
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<td>MMP-11</td>
<td>Stromelysin-3</td>
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<td>MMP-7</td>
<td>Matrilysin-1</td>
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<tr>
<td>MMP-12</td>
<td>Metalloelastase</td>
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<tr>
<td>MMP-19</td>
<td>Enamelysin</td>
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Gelatinases

Gelatinases (MMP-2, MMP-9) cleave denatured collagen (gelatin), collagen type IV, and laminin. These enzymes have three repeats of a type II fibronectin domain inserted in the catalytic domain (Fig. 1), which bind gelatin, collagen, and laminin.

Stromelysins

Stromelysins (MMP-3, MMP-10, MMP-11) degrade numerous components of the extracellular matrix, including gelatin, aggrecan (the major proteoglycan in cartilage), and fibronectin. While stromelysin 1 (MMP-3) and stromelysin 2 (MMP-10) both have similar substrate specificities, MMP-3 has a stronger proteolytic efficiency than MMP-10. Besides digestion of ECM components, MMP-3 activates a number of proMMPs including proMMP-1 and proMMP-13. Its action on a partially processed proMMP-1 is the critical step for generation of fully active MMP-1.

Membrane-type MMPs (MT-MMPs)

There are six MT-MMPs: four are type I transmembrane proteins (MT1-MMP, MT2-MMP, MT3-MMP, MT5-MMP) and two are glycosylphosphatidylinositol (GPI)-anchored proteins (MT4-MMP, MT6-MMP). With the exception of MT4-MMP, they are all capable of activating proMMP-2. MT-MMPs can also digest a number of ECM molecules, and MT1-MMP (MMP-14) has collagenolytic activity on collagen types I, II, and III.

Structure of MMPs

MMPs are composed of several domains that determine the function of each MMP family member (Fig. 1). The catalytic domain contains a zinc-binding motif and a unique “Met turn” structure composed of a 5-stranded β-sheet, 3α-helices, and bridging loops. The hemopexin-like domain is required for collagenase activity and for cell surface activation of MMP-9. Gelatinases also bind the cell surface via interactions between their fibronectin domains and integrin receptors, and they can bind MT1-MMP directly. MT-MMPs contain a transmembrane domain and a cytoplasmic tail at the carboxyl terminus, which anchors the enzymes in the cell membrane.

![Fig. 1. Domain structures of matrix metalloproteinases (MMPs).](image)

MMPs consist of a propeptide (grey), which maintains the enzyme in a latent state, a catalytic domain (white) with the active site, and, with the exception of matrilysins, a hemopexin-like domain (black). A hinge region connects the catalytic and hemopexin-like domains. Gelatinases have an insert of three fibronectin type II repeats in the catalytic domain. Membrane-type MMPs (MT-MMPs) contain a transmembrane domain and a cytoplasmic tail at the carboxyl terminus. Zn shows a zinc-binding site.
Activation of ProMMPs

An N-terminal signal sequence targets MMPs for secretion. Secreted MMPs contain a prodomain and are enzymatically inactive. This domain contains a conserved sequence, known as the “cysteine switch”, with a central cysteine residue that binds to zinc in the active site; proteolytic cleavage releases this inhibitory domain, activating the enzyme. Some MMPs (MT-MMPs and MMP-11) are activated intracellularly by a furin-dependent cleavage of the prodomain. In general, however, the activation of MMPs occurs extracellularly through the action of various proteinases and MMPs. Because MMPs can activate other MMPs, activation of these enzymes is tightly regulated through complex activation cascades.

Proteolytic activation by MT-MMPs is thought to be a critical step for MMP activation in diseases including RA. Other proteinases like the plasma-derived urokinase-type plasminogen activator/plasmin system can activate MMPs such as MMP-1 and MMP-3, resulting in activation cascades. Some serine proteinases are present in arthritic joints, thus providing an in vivo mechanism for activating latent MMPs and allowing them to degrade connective tissue.

Endogenous MMP Inhibitors

Endogenous MMP inhibitors include plasma α-macroglobulin and tissue inhibitors of metalloproteinases (TIMPs). TIMPs are specific inhibitors of MMPs that participate in controlling the local activities of MMPs in tissues. TIMPs bind MMPs in a 1:1 stoichiometry. TIMPs-1, -2, -3, and -4 have been identified in vertebrates. Under pathologic conditions associated with unbalanced MMP activities, changes of TIMP levels are thought to be critical because the inhibitors directly affect the level of MMP activity.

Regulation of MMP Gene Expression

Activator protein-1 and mitogen-activated protein kinase

Up-regulation of MMP gene expression is commonly seen in RA. Activator protein-1 (AP-1) is a pivotal transcriptional factor that regulates the production of MMPs. The upstream regulatory regions of MMP genes including MMP-1[10] and MMP-13[12] contain the AP-1 transcription factor binding sites in their proximal promoters. The AP-1 factor is composed of heterodimers of Fos and Jun proteins, or of Jun/Jun homodimers, which have been found in RA joints[19]. The synthesis and activation of AP-1 factor are regulated by mitogen-activated protein kinase (MAPK) signaling pathways[32]. Three major MAPK families have been identified: extracellular signal-regulated kinase (ERK), p38, and c-Jun NH₂-terminal kinase (JNK). MAPK cascades can be activated by proinflammatory cytokines like interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) [17]. Accumulating evidence indicates the significant role of AP-1 in arthritis. After intraperitoneal injection of double-stranded oligonucleotides that contain the AP-1 site into mice with collagen-induced arthritis (CIA), the nucleotides localize to the joint and reduce joint destruction without affecting cell infiltration. From the finding that both proinflammatory cytokine production and MMP gene expression are inhibited in the synovia, the AP-1 site may be targeted in the promoters of cytokines and MMPs. While a p38 MAPK inhibitor blocks MMP-13 expression in cultured chondrocytes, the inhibitor suppresses IL-1-mediated collagen degradation in cartilage explants. In the CIA model of RA, the p38 inhibitor significantly inhibits TNF-α and IL-6 production, reduces paw inflammation, and suppresses the formation of joint lesions, implicating p38 in the pathogenesis of arthritis[11]. Another MAPK family member, JNK, is also important for MMP regulation. While JNK is required for MMP-13 induction in chondrocytes and synoviocytes, inhibition of JNK by a specific inhibitor blocks bone destruction in adjuvant-induced arthritis [27].

Nuclear factor xB

Nuclear factor xB (NF-xB) is another key player in MMP synthesis. NF-xB pathway controls the expression of various inducible inflammatory genes like IL-1, IL-6, and TNF-α and several MMPs including MMP-1 and MMP-13[13,19,29]. NF-xB transcription factor is composed of p50 and p65 subunits that bind the inhibitor of NF-xB (IxB) in the cytoplasm. Upon phosphorylation by kinases that are activated by inflammatory signaling pathways, the IxB subunit is degraded. This releases p50 and p65 subunits, leading to their translocation to the nucleus and gene expression. Decreased MMP production in cells stimulated with proinflammatory cytokines in association with suppression of NF-xB pathway indicates that MMP induction by cytokines requires the NF-xB pathway.[42,43].

MMPs in RA

In RA joints, synovial cells, chondrocytes, and macrophages express active forms of MMPs, implicating their contribution to RA joint destruction. Expression of inducible MMPs (MMP-1, MMP-3, MMP-9, and MMP-13) in RA is strongly stimulated by proinflammatory cytokines including IL-1 and TNF-α that are mainly produced by activated macrophages. In contrast, MT1-MMP and MMP-2 are constitutively expressed in cells. Of these MMPs, serum proMMP-1 and proMMP-3 levels have been shown to correlate with disease activity and predict functional and radiographic outcome in early RA [25]. Synovial fluids from patients with RA contain high levels of proMMP-1, proMMP-2, proMMP-3, proMMP-8, proMMP-9, and TIMP-1. Recent studies suggest that the MMPs in synovial fluid could overcome the activity of TIMP once proMMPs are fully activated, leading to joint destruction in RA [28].
Cartilage Destruction by Pannus

RA is characterized by synovial membrane inflammation, leading to invasion of synovial tissue into the adjacent cartilage matrix in the form of a pannus, with proteolytic degradation of articular cartilage and bone as a consequence. Cartilage destruction takes place at the sites of cartilage-pannus contact. RA synovial fibroblasts are key effector cells and associated most strongly with the destruction. The cells exhibit features of stable cellular activation, leading to their attachment to the articular surface of cartilage including the point of cartilage-pannus junction and the release of MMPs. Reduced cartilage invasion of RA synovial fibroblasts by overexpression of TIMP supports the involvement of MMPs in the process. Of MMPs, MMP-1 probably plays the most important role because RA synovial fibroblasts that are transduced with MMP-1-inhibiting ribozymes cause a significantly decreased invasion into cartilage.

This chapter focuses on the mechanism of MMP induction in RA synovial fibroblasts at the cartilage-pannus junction.

MMP induction by proinflammatory cytokines in pannus

The observation that cartilage degradation by pannus takes place in the absence of vascular supply suggests that local factors at the cartilage-pannus junction are involved in the degradation processes. IL-1, found in the elevated levels in RA synovium and synovial fluid, is considered to be one of those factors. TNF-α has also been implicated in the activation of RA synovial fibroblasts. The proinflammatory cytokines can induce MMPs. Eventually, up-regulation and overexpression of MMPs are commonly found in rheumatoid joint destruction. At the point of cartilage-pannus junction, synovial fibroblasts express elevated collagenase levels, which are required for cartilage invasion by IL-1-stimulated RA synovial fibroblasts. Because macrophages constitute the major source of proinflammatory cytokines in the inflamed synovial tissue, these cells may modulate the invasion of RA synovial fibroblasts at the cartilage-pannus junction.

Integrin-mediated MMP induction in pannus

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

In RA synovium, both macrophage-like and fibroblast-like synoviocytes express α5, α1, α2, and α6 integrin subunits. RA synovial fibroblasts at the cartilage-pannus junction express integrin subunits α5, α6, and β1. In the presence of collagenase activity, integrins α5β1, α6β1, and α1β1 are able to bind fibronectin, one of the cartilage matrix components. The RGD sequence within the central cell-binding domain of fibronectin is recognized by integrins α5β1 and α6β1, while integrin α1β1 recognizes CS-1 and CS-5 in alternatively spliced IIICS domain of fibronectin. Enhanced accumulation of fibronectin is found on the inflamed synovial and pannus surfaces in the knee joints of patients with RA. Recent studies have shown that the COOH-terminal fibronectin fragment and synthetic peptide that can bind α1β1 integrin individually stimulate the production of MMP-1, MMP-3, and MMP-13 in association with MAPK activation in RA synovial fibroblast cultures. In addition, expression of MMP-1 and MMP-3 is induced in rabbit synovial fibroblasts plated on the central RGD-containing fragments of fibronectin. Proteolytic fragments of fibronectin are found to be increased in RA synovial fluid. Therefore, in addition to the proinflammatory cytokines, integrin stimulation may be another
local factor for cartilage destruction by RA synovial fibroblasts at the cartilage-pannus junction through MMP induction.

CONCLUSIONS

The critical roles of MMPs in joint destruction in RA are well recognized. At the cartilage-pannus junction, increased proinflammatory cytokines such as IL-1 and TNF-α activate MAPK and NF-κB pathways that contribute to up-regulated gene transcription of MMPs. Once the enzymes degrade cartilage ECM, the release of ECM fragments like fibronectin fragments could lead to further MMP induction via specific integrins in RA synovial fibroblasts, resulting in a chronic vicious cycle of damage with further joint destruction (Fig. 2).

References


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