

Review Article

Recent Findings on the Role of Gelatinases (Matrix Metalloproteinase-2 and -9) in Osteoarthritis

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Received 18 December 2011; Accepted 27 May 2012

Academic Editor: Zoe Dailiana

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Several studies dealing with the pathomechanisms of OA refer to MMP-1, -3, -7, -8, and -13 whereas a smaller number of investigations have pointed out the pathogenic role of gelatinases in OA. These gelatinases are best known for their involvement in pulmonary, myocardial, and neoplastic disease but they are emerging as important proteases implicated in the OA progression. This paper highlights the role of the gelatinases as emerging factors in OA pathogenesis through the regulation of subchondral bone resorption and microvascular invasion. The most significant new findings over the last year that add to our knowledge of the activity of these proteins in OA have been reported.

1. Introduction

Hereditary, mechanical, and biological factors participate in the causation of osteoarthritis (OA) that is finally characterized by a net loss of the articular cartilage, resulting in pain, deformity, loss of motion, and decreased function [1]. Changes in the normal homeostasis of articular cartilage and subchondral bone during OA are caused by the combination of (1) chondrocyte death, (2) increased degradation, and (3) decreased production of extracellular matrix (ECM).

The matrix metalloproteinases (MMPs) are a family of Zn²⁺-dependent endopeptidases that regulate the degradation of ECM and play a pivotal role in many physiological and pathological processes of different tissues [2–4]. Indeed, the timely breakdown of ECM is essential for embryonic development, morphogenesis, reproduction, and tissue resorption and remodeling [5].

MMPs are categorized into the following groups: collagenases (MMP-1, MMP-8, and MMP-13), gelatinases (MMP-2 and MMP-9), stromelysins (MMP-3, MMP-10, and MMP-11), matrilysin (MMP-7), metalloelastase (MMP-12), and membrane-type matrix metalloproteinases (MT-MMP 1, 2, 3, and 4) [6].

Several studies dealing with the pathomechanisms of OA refer to MMP-1, -3, -7, -8, and -13 [7–12] whereas a smaller number of investigations have pointed out the pathogenic role of gelatinases in OA. Indeed, the ECM of articular cartilage is primarily composed of type II collagen and aggrecan that are not the primary substrates of gelatinases. These gelatinases are best known for their involvement in pulmonary [13], myocardial [14], and neoplastic disease [15] but they are emerging as important proteases implicated in the OA progression.

In this paper, we summarize the present state of knowledge of gelatinases' role in OA. The most significant new findings over the last year that add to our knowledge of the activity of these proteins in OA have been reported. As far as we know, no previous articles to comprehensively cover this topic are available in the literature.

2. Materials and Methods

We performed a literature search of the MEDLINE/PubMed, Excerpta Medica/EMBASE databases for articles published during the past 30 years (1981–2011). Our purpose was to identify all English-language literature included under the

key-words *metalloproteinase-2, metalloproteinase-9, MMP-2, MMP-9, gelatinase A, and gelatinase B* alone or combined with *osteoarthritis*. The contents of 166 pertinent abstracts or full-text articles were identified during our literature search. Then, abstracts, case reports, and letters to the editor were excluded thus leaving 101 articles to be finally considered for this paper.

We have cited articles that meet accepted quality standards for design and reporting [16]. Review articles and book chapters are also cited to provide readers with more details and references. No attempt was made to solicit unpublished data or to retrieve additional information from any of the authors of the studies.

3. Structure and Function of Gelatinases

MMP-2 (gelatinase A, 72 kDa type IV collagenase) is a matrix metalloproteinase which was first described and purified from highly metastatic murine tumors [17, 18] and cultured human melanoma cells [19]. MMP-2 is abundantly expressed in fibroblasts, endothelial, and epithelial cells [20–22] and it is secreted as proenzyme and activated at the cell surface. Its activation is mediated by the membrane-type metalloproteinase-1 (MT-MMP 1) [23, 24]. MMP-2 activation involves tissue inhibitor of MMP (TIMP)-2 as a bridging molecule between MT-MMP 1 and pro-MMP-2. Thus, net activity of MT-MMP 1 and MMP-2 depends on TIMP-2 concentration [25].

MMP-2 participates in ECM degradation with a wide range of substrates. Indeed, it is able to degrade type I, IV, V, VII, and X collagens, laminin, elastin, fibronectin, and proteoglycans [26–28]. Normal adult articular chondrocytes express significant amount of MMP-2 both *in vivo* and *in vitro* suggesting this metalloproteinase is involved in physiological collagen turnover of human adult articular cartilage [29].

MMP-9 (gelatinase B, 92 kDa type IV collagenase) was first purified from human macrophages [20]. Its expression is limited to osteoclasts, macrophages, trophoblasts, hippocampal neurocytes, and migrating keratinocytes [22]. In particular MMP-9 and cathepsin K are considered the most abundant proteases in osteoclasts [30]. MMP-9 is controlled by growth factors, chemokines, and other stimulatory signals [31]. It is secreted as an inactive precursor form named pro-MMP-9 that forms a tight complex with TIMP-1 and TIMP-3. The complex of pro-MMP-9 and TIMP-1 [24, 32], the plasmin, and the complex plasminogen/MMP-3 are activators of pro-MMP-9 [26, 33].

Gelatinase B has been shown to dissolve extracellular matrix, initiating and promoting new vessel formation [34]. Furthermore, this enzyme is known to cleave native type IV, V, VII, and X collagens and elastin, as well as the products of collagens types I, II, and III after proteolysis by collagenases (Table 1) [3].

In vivo the MMP-9 is poorly expressed in normal adult chondrocytes suggesting that this gelatinase is hardly involved in physiological collagen turnover [35].

4. Gelatinases in OA

It was demonstrated that the expression of both MMP-2 and MMP-9 is enhanced in osteoarthritic cartilage (Figure 1) [36]. Also the MT-MMP 1, which activates the MMP-2, was found highly expressed in the chondrocytes during OA [37]. Duerr et al. evaluated the quantitative expression levels and the distribution of MMP-2 and MMP-9 both in normal and osteoarthritic cartilage and in cultured articular chondrocytes [29, 35]. They found that in osteoarthritic cartilage degradation, MMP-9 is expressed at a very much lower level than MMP-2. Accordingly, Wang et al. reported minimal changes in the cartilage expression of MMP-9 in an experimental model of secondary OA [38]. Indeed, this study showed that the experimentally induced cartilage damage led to OA-like lesions with disarrangement of cellular disposition, cell-free areas, coagulation necrosis, pyknotic nuclei, and local loss of extracellular matrix accompanied by absent immunopositive expression of MMP-3, MMP-9, TIMP-1, and aggrecan.

Current data suggest that during OA, the activity of gelatinases is higher on the subchondral bone rather than on cartilage ECM [39]. Indeed, MMP-2 is capable of cleaving type I and other fibrillar collagens [40] that are uncommon in the ECM of articular cartilage but are present in the ECM of subchondral bone. Using a specific gelatinase inhibitor, Hill et al. showed that both the gelatinases participate in the degradation of the organic matrix of bone [41]. Mansell and Bailey investigating the cancellous bone metabolism during OA, reported an increased potential for collagen degradation in presence of increased levels of both pro- and active MMP-2 [42]. What is evident from this study is that OA cancellous bone is metabolically active compared with normal tissue. Such differences in turnover might result in altered joint morphology, which in turn might exacerbate the osteoarthritic process (Figure 1). Osteoclasts constitutively express MMP-2, and synthesize MMP-9, MMP-3, and TIMP-1 in response to IL-1 α stimulation, and during OA the increased levels of osteoclast-derived MMPs might contribute to osteoclast lacunar resorption [43]. This hypothesis concurs with the demonstration of higher plasma levels of MMP-9 in patients with rapidly destructive hip OA in comparison patients with OA or normal controls [44]. The higher detected amount of MMP-9 could be explained by the wide number of osteoclasts, which are one source of MMP-9, observed around necrotic bone in subchondral areas in rapidly destructive hip OA [45, 46]. Nor should be excluded the enhanced production of MMP-9 by synovial cells of patients with this kind of OA [47]. A direct route into the bloodstream via the subchondral microcirculatory system and an indirect route from synovial fluid into circulation could explain the higher plasma levels of MMP-9 in OA [48].

The work of Bellido et al. strengthens the role of the subchondral bone as a key player in the puzzle of OA development [49]. They found raised subchondral MMP-9 levels in patient suffering from OA demonstrating a clear increase in local bone resorption with a decreased thickness of the subchondral plate. The subchondral plate that separates articular noncalcified cartilage from the bone

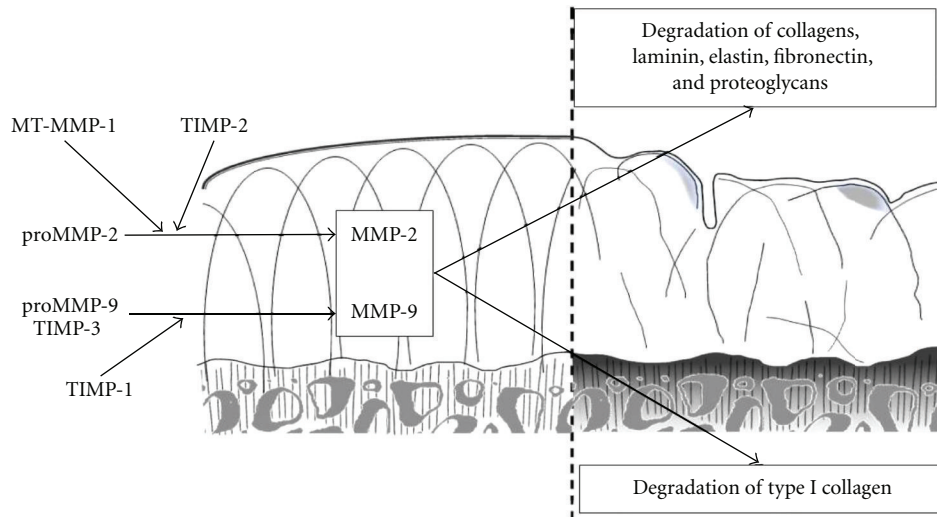


FIGURE 1: The activation of gelatinases and their activity during osteoarthritis. Normal (left) and osteoarthritic (right) cartilage and subchondral bone.

TABLE 1: Metalloproteinase-2 and metalloproteinase-9 chromosome location, biological effects and substrates of action.

Gene	Biological effects	Main substrates
<i>MMP-2</i> 16q13-q21	(1) Adipocyte migration (2) Apoptosis (amion epithelial cells) (3) Conversion of vasodilator to vasoconstrictor (4) ECM degradation (5) Enhanced collagen affinity (6) Epithelial cell migration (7) Generation of vasoconstrictor (8) Increased bioavailability of IGF1 and cell proliferation (9) Increased bioavailability of TGF- β (10) Mesenchymal cell differentiation with inflammatory phenotype (11) Neural apoptosis leading to neurodegeneration (12) Neurite outgrowth	(13) Aggrecan
		(14) Collagen type I
		(15) Collagen type III
		(16) Collagen type IV
		(17) Collagen type VII
		(18) Collagen type X
		(19) Collagen type XI
		(20) Decorin
		(21) Elastin
		(22) Fibrinogen
		(23) Gelatin
		(24) Laminin
<i>MMP-9</i> 20q11.2-q13.1	(29) Bioavailability of TGF- β (30) Generation of angiostatin-like fragment (31) ECM degradation (32) Enhanced collagen affinity (33) Hypertrophic chondrocytes apoptosis and recruitment of osteoclast (34) Pro-inflammatory (35) Reduced IL-2 response (36) Tumor cell resistance (37) Thymic neovascularization	(25) Plasminogen
		(26) proMMP-9
		(27) proMMP-13
		(28) Vitronectin
		(38) Collagen type I
		(39) Collagen type IV
		(40) Collagen type VII
		(41) Collagen type X
		(42) Collagen type XI
		(43) Collagen type XVIII
		(44) Elastin
		(45) Fibronectin
		(46) Gelatin
		(47) Laminin
		(48) proMMP-2
		(49) proMMP-9
		(50) Vitronectin

TABLE 2: Articles dealing with the gelatinases over the last year.

Authors	Type of study	Main results
De Ceunink et al. [64]	Review	MMP-2 and MMP-9 useful as OA biomarkers
Kim et al. [58]	Human	MMP-9 is involved in activation of MMP-13
Kim et al. [59]	Human	MMP-9 is associated with VEGF expression in OA
Li et al. [61]	Human	MMP-9 expression correlates with vascular invasion in severe OA
Sanchez et al. [62]	Human	The expression of MMP-9 increases by cyclic compression on osteoblasts from osteoarthritic subchondral bone
Guo et al. [63]	Human	TGF- β 1 protects articular cartilage downregulating

marrow cavity consists of calcified cartilage and subchondral lamellar bone layers [50]. Any impairment in subchondral bone quality makes this organ not able to receive and properly distribute loads from and/or to the articular cartilage. Thus, changes at subchondral bone may aggravate cartilage damage. Indeed, the authors observed a direct correlation between subchondral structural parameters and cartilage damage evaluated with the Mankin's scale. The presence of subchondral bone resorption pits composed by MMP-producing cells derived from bone marrow has been previously evidenced together with their contribution to cartilage degradation through the invasion of this tissue [51].

The type II collagen, the most abundant collagen expressed in articular cartilage, is natively degraded by MMP-1, -8, -13, and -14 producing fragments. However, denatured and partially degraded collagen II is further degraded by gelatinases and stromelysins thus obtaining a C-terminal peptide fragment, named CTX-II. This fragment is used as an urinary marker of cartilage degradation because it was found to correlate with cartilage loss in animal models of OA [52] and increased CTX-II levels in patients with OA compared with controls were reported [53]. Correlations of CTX-II with clinical assessment [54] and X-rays [55] or MRI [56, 57] evaluation of human OA have been found.

5. Recent Findings

Recent articles confirm that the gelatinases influence OA onset and progression regulating the subchondral bone remodeling (Table 2). In particular, a predominant role of MMP-9 emerged during, last year. Among various MMPs, the total MMP-9 level is positively correlated with the total MMP-13 level in OA [58], and it has been hypothesized that this gelatinase might be involved in the activation of pro-MMP-13 through yet unknown mechanisms. Notably, MMP-13 has long been considered as the major enzyme involved in cartilage erosion during OA, thus MMP-9 might play a role, at least cooperatively, in joint degradation.

High levels of VEGF and gelatinases were confirmed in osteoarthritic fluid but MMP-2 and -9 levels were not significantly associated with VEGF expression [59]. Differently, it was previously demonstrated that the MMP-9 levels correlated with synovial fluid VEGF levels and with the pattern of vascularity found in the synovial membrane tissue of patients with arthritis. Moreover, synovial membrane

explants stimulated with VEGF increased supernatant MMP-9 levels by 2-fold from baseline [60]. Recently, Li et al. [61] demonstrated that higher MMP-9 expression was found in case of severe OA in comparison with early OA and this expression correlated in a direct manner with vascular invasion. These findings together suggest a possible relationship among gelatinases and the angiogenesis noted with OA development, and it is tempting to speculate that MMP-9 may be therapeutic target for angiogenesis inhibition.

New regulatory mechanisms of gelatinase expression have been proposed. The increase of gelatinases in OA might be induced by abnormal mechanical pressure applied to the articulation. Indeed, cyclic compression on osteoblasts from osteoarthritic subchondral bone increases the expression of genes coding for MMP-9. Conversely, MMP-2 was not modulated by compression, suggesting that this is not a mechanosensitive gene [62]. An interesting article demonstrated that TGF- β 1 protects articular cartilage by downregulating the expression of MMP-9 of chondrocytes and synoviocytes in OA, which may delay the biological behavior of this disease. The authors found a negative correlation between the expression of MMP-9 protein and TGF- β 1 protein, and between the expression of MMP-9 mRNA and TGF- β 1 mRNA in the specimens of osteoarthritic cartilage [63].

Based on the demonstration that high CTX-II levels are predictive of OA progression [53–56], De Ceuninck et al. [64] recently highlighted the role of this degradation fragment of gelatinases not only as a diagnostic, but also as a prognostic reliable biomarker of OA. Conversely, Kim et al. [58] addressed the role of MMP-9 as possible biomarker for differentiating between OA and other articular cartilage diseases.

6. Conclusions

Collagenases have long been considered as the major enzymes involved in OA occurrence and progression. This paper highlights the role of the gelatinases as important factors in OA pathogenesis through the regulation of subchondral bone resorption. New intriguing regulatory mechanisms of gelatinases expression and further data about the relationship between these proteins and microvascular invasion commonly found in OA have been demonstrated over the last year. Experimental strategies that modify the

expression and/or the activity of MMPs might consider the gelatinases as promising targets for the treatment of OA disease.

Conflict of Interests

The authors declare that they have no conflict of interests.

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