Commentary

Elucidation of the potential roles of matrix metalloproteinases in skeletal biology

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Abstract

Irreversible destruction of joint structures is a major feature of osteoarthritis and rheumatoid arthritis. Fibrillar collagens in bone, cartilage and other soft tissues are critical for optimal joint form and function. Several approaches can be used to ascertain the role of collagenases, matrix metalloproteinases, in proteolysis of joint collagens in arthritis. These approaches include identifying spontaneous genetic disorders of the enzymes and substrates in humans and animals, as well as engineering mutations in the genes that encode these proteins in mice. Insights gained from such studies can be used to design new therapies to interrupt these catabolic events.

Keywords: arthritis, bone remodeling, collagenases, collagens, skeletal development

Introduction

In a recent review in *Arthritis Research*, Murphy *et al*. emphasized the importance of degradative processes in joint tissue destruction in various forms of arthritis [1]. They presented aspects of the background biochemistry of the matrix metalloproteinases (MMPs) as 'major players' in the physiological turnover of the extracellular matrix and in the pathological destruction in disease. Specific inhibitors were introduced as potential therapy for arthritis, based on the potential roles of MMPs even though they were directed at downstream events.

Despite phenomenal advances in this area, it is still necessary to establish the importance of MMPs in processes such as normal embryonic development and adult tissue remodeling. This is essential to targeting a specific gene product whose function is thought to be critical in pathological events (e.g. degradation of bone and soft tissue extracellular matrices), in disorders such as osteoarthritis and rheumatoid arthritis. There are several approaches to establish that a MMP has the postulated biological function in arthritis. It is not sufficient only to demonstrate the presence of the MMP in tissue extracts or by immunohistochemistry in tissue sections, or to find elevated levels of mRNA in extracted RNA by northern hybridization, by quantitative PCR or by *in situ* hybridization in tissue sections. The identification in affected tissues of the specific cleavage product of a MMP-catalyzed reaction provides better evidence for an *in vivo* function. For example, using antibodies directed against the collagenase cleavage site in collagens, positive staining has been found for cleavage products of type I collagen in bone and of type II collagen in cartilage [2,3]. The expected effect of a specific enzyme inhibitor drug supports the role of the enzyme, although in the case of MMP inhibitors the specificity *in vivo* is yet to be established [4]. The demonstration of the expected phenotype that results from a spontaneous mutation in a MMP gene in humans or animals or from the targeted manipulation of a MMP gene in mice would provide the most compelling evidence.

The report of the mutation in the gene encoding MMP-2 (gelatinase A or 72 kDa gelatinase) in a human skeletal disorder is therefore of great interest [5,6]. There are several forms of osteolysis in humans that have a genetic basis. For example, a focal form (familial expansile osteolysis that maps to chromosome 18q21.2-21.3) appears to be explained by a mutation in the receptor activator of

MMP = matrix metalloproteinase; PCR = polymerase chain reaction; r/r = collagenase-resistant homozygote.

nuclear factor-κB gene that encodes constitutive activation and excessive osteoclastic bone resorption [7]. In a more generalized disorder of osteolysis, Whyte *et al.* recently identified the homozygous deletion of the gene on chromosome 8q24.2, *TNFRSF11B*, that encodes osteoprotegerin, a 'bone protector' [8].

More pertinent to the MMP field, Martignetti and colleagues described a form of multicentric osteolysis with tarsal and carpal bone resorption, accompanied by severe arthritis, osteoporosis, subcutaneous nodules and a distinctive facies, in several members of large, consanguineous Saudi Arabian families [5,6]. They localized the gene to 16q12-21 and demonstrated two family-specific mutations in the gene in the region that encodes MMP-2. In one family, there was a nonsense mutation that predicted the replacement of a tyrosine by a stop codon (Y244X). In another family, there was a missense mutation that predicted the substitution of an arginine by a histidine (R101H). In affected members of both families there was no MMP-2 activity detected in serum by gelatin zymography, in contrast to measurable levels in unaffected members. Although it is clear that affected individuals with this syndrome have decreased function of MMP-2, the clinical phenotype is not yet explained.

We still do not know precisely what role MMP-2 plays in human biological processes. *In vitro* MMP-2 can cleave type I and type IV collagens, but it is not known whether these are substrates for MMP-2 *in vivo*. In this regard, it should be appreciated that most of the MMPs were named after they were shown to act on a particular protein substrate *in vitro*, but such proteolysis may not be the biological function of the enzyme. Indeed, the null mutation in MMP-2 engineered in mice results in a very mild phenotype, manifested mainly by a decrease in bone length [9]. It has been speculated [10] that the function of MMP-2 might overlap with that of MMP-14 (MT1-MMP, a membrane-bound MMP); the engineered loss-of-function mutation of MMP-14 [11,12] is associated with skeletal defects considered to partially resemble those of the patients with the multicentric osteolysis syndrome. Most MMP-14$^{-/-}$ mice die when they are only a few weeks of age, however, and the pathogenesis and nature of the abnormalities in bone and cartilage in the mice that survive is not yet clearly established. Whatever the mechanisms to account for the abnormalities, the observations in multicentric osteolysis are important for the field since they comprise the first demonstration of a spontaneous mutation in a MMP gene resulting in a human disease. We look forward to descriptions of mutations and the resultant phenotype in other MMP genes.

Our group has also taken advantage of mouse models to further explore potential roles of MMPs *in vivo*. The first of these models is the collagenase-resistant (r) mouse, targeting mutations in *Col1a1* that encode amino acid substitutions around the collagenase cleavage site in the α1(I) chain of type I collagen [13–15]. The type I collagen extracted from the skin and tendons of homozygote (r/r) mice is not cleaved in the helical domain by MMP-13 or other collagenolytic MMPs. The r/r mice do not mount a normal osteoclast-mediated bone-resorptive response to parathyroid hormone, an inducer of bone resorption *in vivo* [16]. It had been hypothesized, based on published studies of osteoclast attachment and pit formation in *in vitro* assays, that collagenase produced by osteoblasts in remodeling bone acts on a layer of hypomineralized collagen on bone surfaces to permit osteoclasts to attach [17,18] and then resorb in the low-pH extracellular environment through action of the cysteine proteinase, cathepsin K [19]. There are other possible explanations, however, for the defect in bone resorption in the r/r mice, including a role for collagenase in osteoclast survival. The r/r mice also develop increased new bone, paradoxically in the presence of osteoblast and osteocyte apoptosis [20].

The second mouse model developed in our laboratory, descriptions of which have so far only appeared as abstracts [21,22], is the targeted disruption of the MMP-13 gene. The strategy employed involved the deletion of the critical zinc-binding region in the catalytic domain that resulted in markedly decreased transcription of the MMP-13 gene and no apparent translation. Recombinant MMP-13 lacking this catalytic domain has no enzymatic activity. The MMP-13$^{-/-}$ mice are fertile, and they grow and survive normally. The major developmental phenotype includes widened growth plates of long bones with increased chondrocyte proliferation and thickness of the hypertrophic zones. There are also striking alterations in bone remodeling seen as the mice mature. Bone resorption is reduced due to disordered osteoclast function, and bone deposition is increased ascribable to increased generation of osteoblasts.

Conclusion

MMP-13 has critical roles in embryonic development and remodeling of the skeleton in mice. These roles are reflected in the collagenase-mediated destruction of bone and cartilage in several forms of human inflammatory joint disease. Understanding precisely how MMP-13 functions will permit design of different approaches to dealing with these vents in arthritis.

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