

REPORT

What are the roles of metalloproteinases in cartilage and bone damage?

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A role for metalloproteinases in the pathological destruction in diseases such as rheumatoid arthritis and osteoarthritis, and the irreversible nature of the ensuing cartilage and bone damage, have been the focus of much investigation for several decades. This has led to the development of broad spectrum metalloproteinase inhibitors as potential therapeutics. More recently it has been appreciated that several families of zinc dependent proteinases play significant and varied roles in the biology of the resident cells in these tissues, orchestrating development, remodelling, and subsequent pathological processes. They also play key roles in the activity of inflammatory cells. The task of elucidating the precise role of individual metalloproteinases is therefore a burgeoning necessity for the final design of metalloproteinase inhibitors if they are to be employed as therapeutic agents.

There have been major advances in the understanding of the pathogenesis of arthritic diseases, and the role of the matrix metalloproteinases in the irreversible degradation of articular cartilage and bone has been extensively documented.^{1–4} However, attempts at the development of metalloproteinase inhibitors as potential therapeutic agents in degradative diseases in general have met with little success, and a number of reviews have considered the problem.^{5–7} This short overview will present the current state of our knowledge and the future directions that therapies based on metalloproteinase inhibition might usefully take.

THE METZINCINS

The Human Genome Project has identified more than 550 genes encoding proteases, of which over 185 use a zinc driven hydrolytic mechanism, the zincins.⁸ The metzincin super family of zinc endopeptidases includes several families of enzymes that are involved in the regulation of the extracellular environment, governing cell–extracellular matrix (ECM) and cell–cell interactions in fundamental ways. Notably the matrix metalloproteinases (MMPs; 23), the disintegrin metalloproteinases (ADAMs; 21) and the disintegrin metalloproteinases with thrombospondin type 1 like repeats (ADAM TSs; 19) are secreted by many cell types, either as soluble proteins which interact with the ECM, or cells sequestered by direct binding, or through transmembrane domains (fig 1). Other families, such as the astacins and the pappalysins (PAPPS) will not be discussed here. The MMPs, ADAM TSs and many of the ADAMs have the common feature of an HEXGHXXGXXH zinc binding motif followed by a methionine within the catalytic domain. Currently, representative three dimensional structures of different proteinase families bearing this motif show comparable overall topology, despite low sequence similarity. This includes a substrate binding cleft, which subdivides the

enzyme moiety into an upper and a lower subdomain. A common five stranded beta-sheet and two alpha-helices are always found in the upper subdomain with a further C-terminal helix in the lower subdomain. The catalytic sites of the metalloproteinases, especially the MMPs, have been targeted for the development of low molecular weight synthetic inhibitors with a zinc chelating moiety. Inhibitors able to fully differentiate between individual enzymes have not been identified thus far, although a reasonable level of discrimination is now being achieved in some cases.⁷ Each family does, however, have other unique domains with numerous roles, including the determination of physiological substrate specificity, ECM, or cell surface localisation (fig 1). It has been postulated that a better understanding of the specificity incurred by the interactions mediated by these domains, including the potential for allosteric effects on the catalytic domain, may provide novel targets for inhibitors that do show individual specificities.⁹

CARTILAGE DEGRADATION AND METALLOPROTEINASES

Degradation of the ECM of cartilage, which is a feature of arthritic diseases, is orchestrated by both MMPs and ADAM TSs which degrade two major structural components of cartilage extracellular matrix, the proteoglycan aggrecan and type II collagen.¹⁰ Pathological cleavage of aggrecan at Glu 373/Ala 374 (the 'aggrecanase' site) was identified as the major site of aggrecan degradation in human joint disease by analysis of synovial fluid samples from a range of human joint pathologies including osteoarthritis. This is also a major site of aggrecan cleavage in response to inflammatory stimuli and can be identified with antibodies to the neoepitopes generated. Several members of the ADAM TS family of enzymes (ADAM TS1, 4, 5, 8, 9, and 15) are known to be capable of cleaving aggrecan at the Glu 373/Ala 374 and other sites, but ADAM TS4 and ADAM TS5 (aggrecanase-1 and aggrecanase-2, respectively) seem to be the most active aggrecanases. Which aggrecanase is responsible for aggrecan degradation during human articular cartilage destruction, however, remains unclear. Gene targeted deletion of the catalytic domains of ADAM TS1, ADAM TS4, or ADAM TS5 in mouse models of arthritis have indicated the overwhelming importance of ADAM TS5 in the loss of aggrecan from joint cartilage in this species. There are also convincing data for the involvement of MMPs in aggrecan cleavage, but at rather lower levels. The specific products of MMP activity have been detected in vivo and in vitro.¹ Furthermore, it has been proposed that MT4 MMP, MMP 17 may regulate ADAM TS4 activity.¹¹

Abbreviations: ADAM, disintegrin metalloproteinase; ADAM TS, disintegrin metalloproteinase with thrombospondin repeats; ECM, extracellular matrix; MMP, matrix metalloproteinase; MT, membrane type; TACE, tumour necrosis factor α converting enzyme; TIMP, tissue inhibitor of metalloproteinases

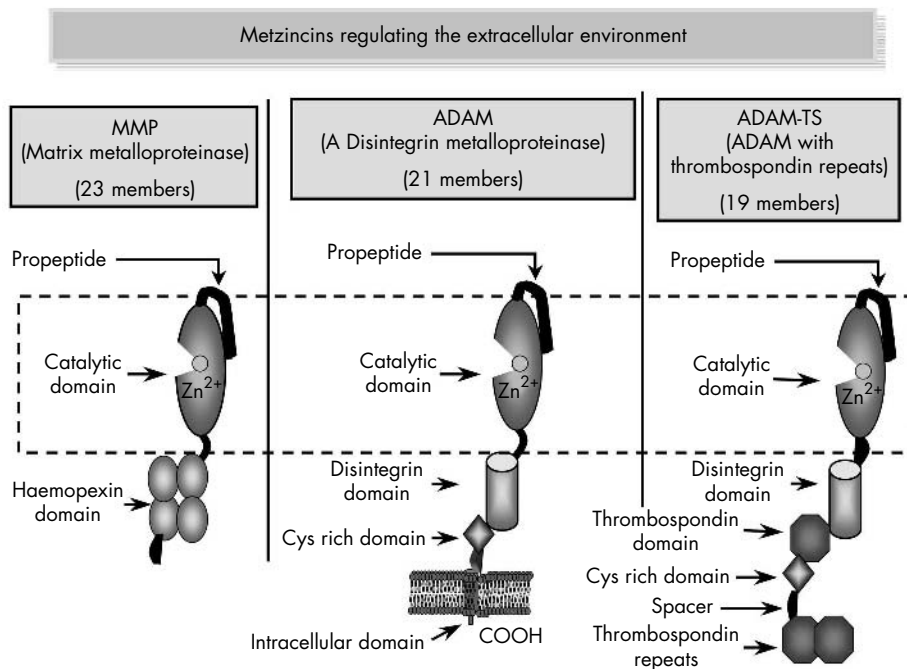


Figure 1 Three metzincin families, matrix metalloproteinases (MMPs), disintegrin metalloproteinases (ADAMs), and disintegrin metalloproteinases with thrombospondin repeats (ADAM TSs) have been identified as having roles in the biology and pathology of cartilage and bone. They have in common the zinc containing catalytic domain which is rendered inactive in the presence of an N-terminal propeptide. Activation is usually effected by the proteolytic removal of the propeptide at cellular sites specific to individual proteinases. Most of these metalloproteinases have other domains conferring specificity with respect to substrate cleavage or cellular or ECM localisation. Some members of the MMP family and all the ADAM family are membrane associated.

The collagenolytic MMPs (MMP 1, MMP 2, MMP 8, MMP 13, and MMP 14) are all produced by chondrocytes as well as by cells in the synovium, but their expression in arthritic tissues seems to vary enormously in both models and human disease. Antibodies that recognise terminal amino acid sequences generated by proteolysis at specific sites in the core protein of type II collagen have been invaluable for identifying the proteinases responsible for cartilage breakdown both in vitro and in vivo. Preliminary results with quantitative assays of type II collagen neoepitopes suggest that they may be useful markers of joint disease in humans.¹ Long term studies correlating neoepitope concentration with clinical and radiographic disease are now required to validate the utility of neoepitopes as surrogate markers of cartilage degeneration and joint disease.

Both MMPs and ADAM TSs have been implicated in the breakdown of other matrix components such as cartilage oligomeric matrix protein (COMP),¹² which may also have utility as a marker of disease and of drug efficacy.

ADAM 10, ADAM 12, ADAM 15, and ADAM 17 have all been found in cartilage at the mRNA level but there are few detailed studies to date of their relation to disease states or their precise role in chondrocyte biology.¹³

BONE DEGRADATION AND METALLOPROTEINASES

Many MMPs have been associated with bone matrix turnover, involving virtually all the cell types present. Collagenolytic MMPs have a role in the actions of the bone forming cells, the osteoblasts, but the major collagenolytic proteinase in osteoclastic bone resorption appears to be the cysteine proteinase, cathepsin K which can function in the acidic phagolysosomal resorption zone of the osteoclast. However, MMPs do play a role in osteoclastic resorption in pathological conditions, including MMP 1, MMP 2, MMP 3,

MMP 9, MMP 12, MMP 13, and MMP 14.¹⁴ They degrade a number of the non-collagenous proteins of the bone matrix and clearly play roles beyond ECM cleavage as discussed below.

Very little work on the ADAM content of bone has been carried out, although a number are expressed at the mRNA level in both osteoclasts and osteoblasts. ADAM 12 may be important in the formation of osteoclasts.¹⁵

OTHER ROLES FOR METALLOPROTEINASES

MMPs can collectively degrade many components of the ECM and have been extensively analysed in that context.¹⁶ However, their role in the regulation of cell behaviour stretches far beyond an influence on ECM integrity. ECM fragments frequently have cellular effects not seen with the parent molecule.¹⁶ Fibronectin fragments influence cytokine and nitric oxide production by chondrocytes as well as the levels of MMPs produced. MMPs can also release growth factors from the ECM or the cell surface. They can modify both cell-cell and cell-ECM interactions by the proteolysis of cell surface growth factor and adhesion receptors, and they are key regulators of inflammatory responses, which can be proinflammatory or anti-inflammatory (table 1). Cleavage of chemokines, cytokines, and their receptors can activate or inactivate these critical signals and could potentially have dual effects. It has been proposed that the metalloproteinase and cytokine axes are intrinsically linked, where the balance of one can influence the other.¹⁷

METALLOPROTEINASE INHIBITORS

Synthetic inhibitors

The breakdown of cartilage and bone in arthritic diseases leads to structural damage and prevents joints from functioning normally. Protecting bone and articular cartilage

Table 1 Metalloproteinase regulation of inflammation

Proinflammatory actions	Anti-inflammatory actions
Tumour necrosis factor α	MCP-1
α -Defensin	MCP-2
Syndecan-1	MCP-3
CXCL1	MCP-4
L-selectin	Stromal derived factor-1 α
Transforming growth factor β	
Vascular endothelial growth factor	
Fibroblastic growth factor	
Insulin-like growth factor	

MCP, monocyte chemoattractant protein.

from damage consequently has major potential both therapeutically and economically. If joint destruction can be prevented or significantly reduced, the long term function of joints could be preserved, severe disability could be avoided, and patients would benefit from an improved quality of life. The development of synthetic inhibitors of zinc metalloproteinases as potential therapeutics has relied on the use of a peptide sequence, recognised by the targeted protease, to which have been grafted different chemical functionalities able to interact with the zinc ion of the active site. This strategy has allowed the identification of several potent peptidomimetic inhibitors of metalloproteinases. Based on the chemical structure of the zinc binding group, four different classes of zinc metalloprotease inhibitors have been developed, incorporating a hydroxamate, a carboxylate, a thiolate, or a phosphinyl moiety. The exploitation of hydroxamates proved to be very effective in developing potent inhibitors of metalloproteinases, but one drawback of this approach has been the poor selectivity displayed by this class of inhibitors.⁷ Despite knowledge of the three dimensional structure of several catalytic domains of metalloproteinases, the development of highly specific synthetic active-site-directed inhibitors of metalloproteinases, able to differentiate the different members of this protease family, remains a huge challenge. Due to the flexible nature of MMP active site, the development of specific MMP inhibitors will need to combine sophisticated theoretical and experimental approaches to identify the specific structural and dynamic features of each individual metalloproteinase that can be exploited to obtain the desired selectivity.

The majority of the clinical trials using synthetic metalloproteinase inhibitors have been in cancer and have proved disappointing, principally due to lack of efficacy, and also due to untoward side effects in some cases. In animal models metalloproteinase inhibitors have been relatively effective in preventing development and progression of early disease, but have had little effect on advanced disease. Hence their poor performance is unsurprising given the design of the trials that have been undertaken to date,⁶ many of which involved the use of MMP inhibitors as single agent therapies for patients with advanced disease. It is clear that a more detailed knowledge of the roles of the metalloproteinases in each disease and their spatiotemporal expression are needed to guide the development and use of inhibitors of clinical value. This would need to be coupled with the development of robust markers of efficacy, such as the use of the neoepitope antibodies to detect cleavage fragments as described above, and other approaches.¹⁸

Tissue inhibitors of metalloproteinases

Four mammalian tissue inhibitors of metalloproteinases (TIMPs) have been identified. They have many basic similarities but they exhibit distinctive surface structural features, biochemical properties, and expression patterns,¹⁹ suggesting that they have distinct physiological roles. The

TIMPs have six disulphide bonds and are folded into a three loop N-terminal domain and an interacting three loop C sub-domain. Metalloproteinase inhibitory activity resides within a ridge structure at one edge of the N-terminal domain. Most of the biological functions of these proteins thus far are attributable to the N-terminal domains, although the C sub-domains mediate interactions with the catalytic domains of some MMPs and with the haemopexin domains of MMP 2 and MMP 9. The TIMPs are secreted proteins, but are localised at the cell surface in association with membrane bound proteins, including metalloproteinases. Uniquely, TIMP 3 is sequestered in the ECM, probably by binding to heparan sulphate and chondroitin sulphate proteoglycans. TIMPs 1–4 all inhibit active forms of the MMPs with binding constants in the low pM range, although TIMP 1 is a poor inhibitor of a number of the MT MMPs and MMP 19. TIMP 3 inhibits some of the ADAMs, notably ADAM 17. TIMP 1 inhibits ADAM 10 and TIMP 2, TIMP 3, and TIMP 4 inhibit ADAM 28. TIMP 3 inhibits the aggrecan degrading ADAM TS 1, TS 4, and TS 5. Because of their pivotal role in metalloproteinase regulation, the TIMPs are an important factor in the regulation of cytokine and chemokine activity as well as cell surface molecules and not just the ECM.

The concept that TIMPs might be modified and exploited as novel therapies in degradative diseases is currently under investigation. We have demonstrated that the engineering of metalloproteinase specific “designer” TIMPs is a viable option using the basic OB fold as a skeleton on which to place specific interacting residues.²⁰ Figure 2 shows the consensus modifications required for optimal TACE inhibition.²⁰ Coupled with the use of gene therapy approaches aimed at delivering TIMPs to specific sites in the joint,^{19–21} this may prove fruitful as an efficient way to abrogate deleterious metalloproteinase activity.

NOVEL APPROACHES FOR METALLOPROTEINASE INHIBITION

Alternative strategies for the development of metalloproteinase inhibitors are being considered.⁹ The production of specific antibody fragments developed from phage library screens could be promising. Such proteins could target the

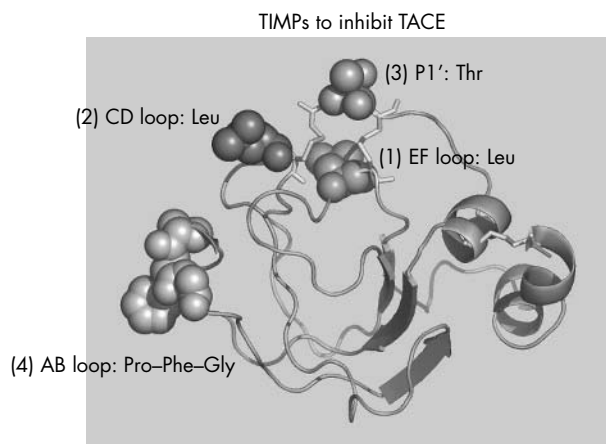


Figure 2 Modification of the metalloproteinase binding ridge of tissue inhibitors of metalloproteinases (TIMPs) to generate potent inhibitors of TACE. Based on any of the four TIMP basic scaffolds a tight binding inhibitor of the disintegrin metalloproteinase (ADAM) tumour necrosis factor α converting enzyme (TACE) can be made by the modification of four positions on the active site binding ridge. (1) a leucine residue on the EF β strand loop; (2) a leucine residue on the CD β strand loop; (3) a threonine residue at the P1' binding site; and (4) a proline-phenylalanine-glycine triad at the tip of the AB β strand loop. Adapted from Lee *et al.*²⁰

active site in a more specific way than chemical inhibitors. They may also be used to define exo sites on the MP that determine their substrate specificity or extracellular location. For instance, it is known that the specificity for ADAM TS4 for aggrecan and other substrates is determined by extra-catalytic domain interactions with the catalytic domain. Similarly, the haemopexin domain of the collagenase, MMP 1, is essential for the specificity of the catalytic domain cleavage of collagen. Gelatinase A, MMP 2 is localised to specific extracellular collagenous sites by its fibronectin-like domain and MT1 MMP; MMP 14, requires the haemopexin domain for cell surface clustering as part of its collagenolytic capacity and ability to mediate proMMP 2 activation. The haemopexin domain also determines its binding to CD44. Further understanding of the nature of these interactions will allow the development of either specific chemical antagonists of binding or of fragment antibodies that target these interactions.

Several other strategies may potentially downregulate metalloproteinases. Both the intracellular signalling pathways and the downstream transcription factors which induce gene expression are being intensively studied. Blockade of mitogen activated protein kinase, MAPK, pathways, nuclear factor (NF)- κ B or activator protein (AP)-1 have all been shown to have some efficacy in vitro or in animal models of arthritis.²²⁻²³ The use of biological reagents to block inflammatory cytokines also reduces metalloproteinase expression in many cases. The tetracyclines, which are rather weak inhibitors of MMP catalytic activity, also influence on their synthesis and have been successfully trialled in rheumatoid arthritis.²⁴ n-3 Polyunsaturated fatty acids reduce expression and activity of collagenases and aggrecanases, as well as a number of proinflammatory mediators in osteoarthritic cartilage and have been strongly promoted as a dietary supplement. Gene therapy, either overexpressing TIMPs or using cytokines/growth factors, has been successful in animal models and may have future uses once the problems of the secure and efficient delivery of genes into target cells and tissues have been overcome.¹⁹⁻²¹

CONCLUSIONS

The identification of specific MPs that need to be targeted in arthritis should be correlated with the design of the inhibitors that selectively reduce the binding and cleavage of key substrates whilst not interfering with the cleavage of others. This requires a precise understanding of the roles of individual proteinases, with respect to not only ECM degradation, but also modulation of cytokine and growth factor function. This must be coupled with a clearer knowledge of the expression of these enzymes in diseased cartilage and precise identification of the enzymes that must be targeted in each specific situation.

Promising preclinical data indicate that metalloproteinase inhibitors will be able to block cartilage destruction in human disease, but they should clearly be linked with agents boosting reparative mechanisms. Clinical trials will only be successful if sound procedures to monitor efficacy are in place. The use of imaging techniques to follow cartilage integrity and the identification of robust surrogate markers of efficacy may allow the effective evaluation of metalloproteinase inhibitors based therapies for arthritis.

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