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The Structure, Regulation, and Function of Human Matrix Metalloproteinase-13

*Matthew F. Leeman, Stephanie Curran, and Graeme I Murray**

Department of Pathology, University of Aberdeen, Aberdeen, UK

* Address correspondence to: Dr Graeme I Murray, Department of Pathology, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, UK. Tel. +44 (0)1224 553792; Fax +44 (0)1224 663002; email g.i.murray@abdn.ac.uk

ABSTRACT: Matrix metalloproteinase-13 (MMP-13) is a proteolytic enzyme that belongs to a large family of extracellular matrix-degrading endopeptidases that are characterized by a zinc-binding motif at their catalytic sites. MMP-13 has a key role in the MMP activation cascade and appears to be critical in bone metabolism and homeostasis. It also has an important role in tumor invasion and metastasis. This commentary provides a detailed overview of the regulatory mechanisms, structure, and function of human MMP-13 and highlights the key factors involved in the biology of this important molecule.

I. INTRODUCTION

The matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases collectively capable of degrading all components of the extracellular matrix (ECM).[1,2] There has been much recent research to characterize MMPs and define their biological roles. It is now apparent that these are a group of multifunctional proteins, with important roles in a wide variety of physiological and pathological processes, including normal cell growth, differentiation, and cell regulation.[3] These functions are in addition to their well-recognized and characterized role in tumor invasion and metastasis. Some of the regulatory functions may be distinct from the matrix-degrading capabilities of this group of enzymes.

MMP-13 has a central role in the modulation of MMP activity, and has key functions in the formation and remodeling of bone and tumor invasion, and therefore it is important to understand its structure, function, and regulatory mechanisms. The purpose of this commentary is to provide an overview of the molecular biology and biochemistry of human MMP-13 and highlight its important and unique properties.

MMP-13 (collagenase-3) is the third member of the collagenase subfamily of MMPs to be identified and has distinct properties compared with the other collagenases MMP-1 (interstitial collagenase) and MMP-8 (neutrophil collagenase). MMP-13 was first cloned from a breast cancer cDNA library.[4] Fluorescence *in situ* hybridization mapped the MMP-13 gene to chromosome 11q22-23.[5] This is a gene locus where several other MMPs (MMP-1, MMP-7, MMP-8, MMP-3, MMP-10, MMP-12, and MMP-20) are also localized, and it has been suggested that there is a common evolutionary ancestry for the MMP genes located on chromosome 11q.

Transcription of the MMP-13 gene results in a 2.7-kb mRNA that encodes a 471

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amino acid polypeptide. The gene comprises 10 exons and 9 introns spanning 12.5 kb.[6] The exon sizes range from 104bp (exon 9) to 1371 bp (exon 10), which also includes the entire 3'-untranslated region. The length of the final exon is one of the longest for any MMP and the large 3'-untranslated region could be important in posttranscriptional regulation. The intron sizes range between 92 bp (intron 1) and 2000 bp (intron 8) with the first intron being unusually short.

II. REGULATION OF MMP-13

A. Transcriptional Regulation of MMP-13

The nucleotide sequence and transcription factor binding domains of the 5'-flanking region of the MMP-13 gene has been analyzed in detail[6,7] (Figure 1). The transcription start site is 22 bp upstream from the ATG start codon.[7] The proximal region of the promoter contains a TATA box (TATAAA) at –32 to –37bp, an AP-1 consensus sequence at –50 to -56bp and an Ets/PEA-3 binding site at –77 to –83 bp.[7] The PEA-3 site is important for interacting with AP-1 sites to confer responsiveness to oncoproteins such as Ha-Ras. Interaction between these two sites is important for MMP transcription, the combination being termed an oncogene responsive unit.[8] Although three core motifs (AGGA/TCA) for hormone response elements (HREs) at –590, –891, and –920, have been identified these are only "half-sites" and are not in close proximity to each other.[7] In fact, Pendas et al.[6] describe an absence of either estrogen response elements or glucocorticoid response elements in the 1 kb of regulatory sequence that they analyzed.

There is an osteoblast specific element (OSE)-2 sequence 140 bp upstream from the start codon[6] (–140 bp) that was first proposed to play an integral role in osteoblast-specific osteocalcin expression.[9] Furthermore, there are five regions suitable for the CCAAT-binding proteins at –500, –617, –1218, –1240, and –1541 although these are most likely inactive.[7] Also of interest is the presence 523 nt upstream of the start site of a sequence with homology to the TGFβ-inhibitory element (TIE)[6]. TIEs are *cis*-transcriptional elements that bind those proteins that also recognize AP-1 binding sites.[10]

In normal and osteoarthritic chondrocytes, –514CAT, –406CAT, and –133CAT plasmid constructs had a higher level of transcriptional activity than the full –1599 length transcript, which suggests the presence of upstream repressor binding sites[7] in the region –515 to –1599. In COS-1 cells, a –56CAT construct (i.e., with AP-1 only) had eight-fold higher activity over basal levels after 12-*O*-tetradecanoylphorbol-13-acetate (TPA) stimulation,[6] whereas a construct containing AP-1 and PEA-3, -402CAT, led to only a four-fold increase in activity, suggesting the presence of an inhibitory element in this region.

Furthermore, a construct with a mutation in the PEA-3 site displayed only slightly decreased activity compared with wild type, indicating that this site plays no significant synergistic effect. This is not unexpected, because the distance between the AP-1 and PEA-3 sites is important for their synergistic effects.[8] As found by Tardif et al.,[7] the longest construct, -1004CAT, led to the least activity (2.5-fold over basal level), thereby suggesting the presence of more inhibitory elements further upstream from the transcriptional start site,[6] which contribute to the strict regulation of MMP-13 expression. Mutations in the AP-1 site demonstrated its importance for both basal activity (basal

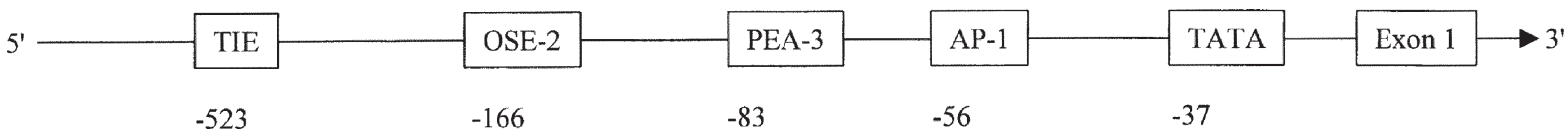

FIGURE 1: Structure of upstream regulatory region of the MMP-13 gene with the position of the major transcription factor binding sites identified. Transforming growth factor beta inhibitory element (TIE) bind proteins that also recognize AP-1 binding sites. Osteoblast-specific element (OSE-2) mediates induction of MMP-13 by cbfa1 in osteoblasts. The combination of PEA-3/AP-1 confers responsiveness to growth factors, oncogenes, and tumor promoters and is known as an oncogene-responsive element.

CAT activity in mutants was four times lower than wild type) and for inducibility by TPA (eight-fold increase for wild type compared with 1.5-fold for mutant) of the promoter.[6] Furthermore, the OSE-2 domain and AP-1 site cooperate in the upregulation of MMP-13 expression in normal differentiating osteoblasts during development, as mutations in either cancels any effect.[11] Core binding factor 1 (Cbfa1) is a transcription factor with a major role in bone formation that acts via a *runt* domain binding sequence. Cbfa1 recognizes the OSE-2 motif in the MMP-13 gene promoter region and has been linked to MMP-13 induction in osteoblasts and chondrocytes.[12] Indeed, knock-out mice deficient in Cbfa1 do not express MMP-13 during fetal development, highlighting its importance for MMP-13 induction *in vivo*.[12]

Bone morphogenetic proteins (BMPs) are important regulators of bone formation. Several BMPs (BMPs 2, 4, and 6) decrease MMP-13 mRNA expression by osteoblasts.[13] In particular, BMP-2 has been shown to suppress the rate of transcription of the MMP-13 gene and to decrease MMP-13 heterogeneous RNA and MMP-13 mRNA and levels.[14] Furthermore, noggin — a BMP inhibitor — prevents the reduction of collagenase transcripts, increases the level of MMP-13 heterogeneous nuclear RNA in osteoblasts, but does not alter MMP-13 mRNA decay rates.[13]

B. Growth Factors and Hormones

Parathyroid hormone (PTH) is a key factor in calcium homeostasis and increases MMP-13 mRNA, as detected by *in situ* hybridization, in a bone culture system[15] and also *in vivo*.[16] This increase occurs through both the AP-1 site and *runt* domain sequence.[17] Overexpression of c-Fos and c-Jun (which bind to the AP-1 site), osteoblast-specific factor and Cbfa1 (that interacts with the *runt* domain), all enhance the response to PTH in the wild-type promoter. It is reasonable to infer that these sites are cooperative, as the potentiation of PTH effects is lost if either site is mutated.[17] Recently, an interaction between cFos and cJun and Cbfa proteins, depending on the leucine zipper and the *runt* domain, respectively, has been identified.[18] It was shown that PTH-treated osteosarcoma cells required the interaction of AP-1 and Cbfa-1, along with a functional OSE-2 site, in order to enhance MMP-13 promoter transcriptional activity. MMP-13 mRNA and protein is significantly upregulated by 1α,25-dihydroxyvitamin D3, another key factor in calcium homeostasis, and transcriptional induction is mediated by the AP-1 binding site.[16]

The insulin-like growth factors (IGF-I and -II) inhibit MMP-13 synthesis, acting as autocrine repressors.[19] In fact, IGF-1 downregulates IL1 and oncostatin M induced MMP-13 mRNA expression in human articular chondrocytes.[20] Both platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF), on the other hand, stimulate MMP-13 transcription by stimulating AP-1 site binding of cFos, FosB, Fra-2, c-Jun, and JunB according to gel mobility shift analysis.[21,22] Again, these factors act through the AP-1 site in the promoter region of the MMP-13 gene.

Transforming growth factor-β1 (TGF-β1) provides an excellent example of how both the cellular environment and cell type influence MMP production. TGF-β1 accelerated the decay of collagenase mRNA (mRNA half-life decreased from 6.25 to 2 h) and decreased the level of MMP-13 heterogeneous nuclear RNA and rate of MMP-13 gene expression in osteoblast cells.[23] This is by a putative and as yet unidentified TGF-β1 binding site. Alternatively, it could be through binding at the AP-1 sites like other growth factors.

On the other hand, TGFβ1 strongly induces MMP-13 expression in human KMST fibroblasts.[24] This signaling pathway involves protein kinase C and tyrosine kinase activity; the effects of TGF-β1 are partially mediated by an AP-1 site, where c-Fos, c-Jun, and JunD play specific roles, as determined by electrophoretic mobility shift analysis. Furthermore, recently TGF-β1 has been shown to activate p38 MAPK (mitogen-activated protein kinase) in two cell lines, whereas inhibition of p38 prevents increased MMP-13 expression.[25]

Interference with plasmin furin (95%), and mannose-6-phosphate/insulin-like growth factor 2 receptor (M6P/IGF-2R) decreased the levels of latent TGFβ1 latent complex induced MMP-13 by inhibiting the activation of the TGF-β1 in chondrocytes.[26] Furthermore, based on the immunolocalization of furin and M6P/IGF-2R in different zones of normal and osteoarthritic cartilage, it is possible to conclude that furin convertase is an integral factor involved in the activation and activity of latent TGF-β1.

Hepatocyte growth factor (HGF) stimulates MMP-13 transcription in human osteoarthritic chondrocytes.[27] This effect was mediated by the SAPK/JNK pathway, although not involving p38 MAPK, which is frequently involved in MMP-13 upregulation in response to several other factors.

The MMP-13 gene is down-regulated by wild-type p53, although this effect does not occur with six different p53 mutants.[28] This dominant effect of the mutants appears to be both promoter- and mutant-specific and provides a mechanism that could contribute to the dysregulation of MMP-13 observed in cancer.

C. Cytokines

The major cytokines to induce MMP-13 expression include interleukin (IL)-1 and -6, and tumor necrosis factor alpha (TNFα). IL-1 induced a marked increase in MMP-13 mRNA as detected by Northern blot analysis in mouse calvarial bone cultures, while the effects of IL-6 are more moderate.[29] In primary human chondrocyte cultures, MMP-13 mRNA is inducible by IL-1β, TNFα, and only slightly by PDGF and epidermal growth factor (EGF).[30] IL-1 appears to act through the p38MAPK and JNK pathways.[31] Interestingly, MMP-13 was not expressed by isolated synovial fibroblasts in this study, emphasising the importance of tumor stimulation of stromal cells for MMP-13 induction.

Peroxisome proliferator-activated receptor (PPAR)-γ ligands reduce IL-1β-induced transcription of MMP-13. Furthermore, one of these ligands, 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2), inhibited TNFα and IL-17 induced MMP-13 production, probably by interfering with AP-1 and NF-κB activation.[32] This ligand also attenuated PPARγ-dependent activation of the MMP-13 promoter and MMP-13 mRNA in chondrocytes.

In breast cancer, MMP-13 has been found to be expressed by stromal cells immediately adjacent to tumour cells. Uria et al.[33] demonstrated by co-culture experiments of stromal cells and breast cancer cells that fibroblastic MMP-13 expression was induced by breast cancer cells and that IL-1α and IL-1β were most likely to be the signals involved. IL-1 induction of MMP-13 requires nuclear factor (NF)-κB nuclear translocation, p38 MAPK activity, and c-Jun N-terminal kinase (JNK) activity in SW-1353 chondrosarcoma cells.[31] These findings suggest NF-κB and AP-1 transcription factors are required for the induction of MMP-13. Moreover, TNFα stimulation of two human epidermal keratinocyte cell lines also led to extracellular signal related kinase (ERK), JNK, and p38 MAPK activation.[25] Specific inhibition of p38 prevents

the enhancement of MMP-13 expression caused by either TNFα or TGFβ in these cells, indicating its central importance.

Integrins are capable of inducing MMPs in response to extracellular conditions. Indeed, $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins use MAPKs to translate a three-dimensional collagen cellular signal into MMP-13 induction.[34] p38 MAPK is required to be active, whereas activation of the ERK1,2 pathway inhibits MMP-13 induction; hence, the correct balance of MAPKs is important for MMP-13 expression *in vivo*.

The induction of MMP-13 expression by oncostatin M requires phosphorylation of JAK3, a member of the Janus kinase/STAT pathway.[35] When this is specifically inhibited, STAT1 tyrosine phosphorylation, DNA binding activity of STAT1, and MMP-13 RNA expression were all blocked in chondrocytes.

D. Protein Structure of MMP-13

MMP-13 has all the domains characteristic of an MMP (Figure 2), sharing more than 50% sequence identity with the other collagenases, as well as several residues specific to the collagenase subfamily (tyr 214, asp 235, gly 237). The crystal structure of the C-terminal haemopexin-like domain (CTD) has been solved by molecular replacement.[36] The structure of this domain has a disk-like shape. The amino acid chain is folded into a β-propeller structure, with four propeller blades arranged around a funnel-like tunnel. This tunnel holds two calcium ions and two chloride ions. Interestingly, the MMP-13 CTD has an even distribution of protein mass across its "disk", despite of an increase in volume on moving away from the center. This is explained by a generally increasing mass of amino acid residue upon moving toward the periphery. When considering the detailed structure, surface contoring and charge distribution, the CTD of MMP-13 shares more similarity with the MMP-1 (collagenase) CTD as opposed to MMP-2 (gelatinase).[36]

In both MMP-1 and MMP-13, a fully stretched linker domain acts as a spacer to allow the CTD to be neatly positioned above the catalytic domain and thereby assist in triple helicase activity.[37,38] After binding a triple helical substrate, the CTD folds over the catalytic site, trapping the substrate at the active site. This interaction is probably associated with unwinding of the triple helix, allowing single strands to occupy the catalytic site and subsequently be hydrolyzed.[36]

X-ray crystal structure analysis[39] and multidimensional heteronuclear magnetic resonance[40] have both been used to determine the structure of the MMP-13 catalytic domain. Both techniques indicate a five-stranded β-sheet with a mixed parallel and antiparallel arrangement, and three α-helices as have been found in other MMP structures.[39,40] A specific structure called the S1' subsite is critical for determining MMP-inhibitor selectivity, usually defined by the residue at position 218 and the form of the loop at the back of the pocket.[39] The structure and sequential variability of the S1' loop contributes to the overall size and shape of the S1' pocket in different MMPs.

In MMP-13, the S1' pocket is a long, open channel that is defined by a leucine at 218 and by residues 245 to 253 that form the back of the pocket. As such, this pocket is similar to that of MMP-8 (neutrophil collagenase), whereas MMP-1 (interstitial collagenase) has a small, closed pocket.[39] These pockets do not appear to heavily influence collagenase activity, as all three are capable of degrading fibrillar collagens, but do affect synthetic inhibitor selectivity.

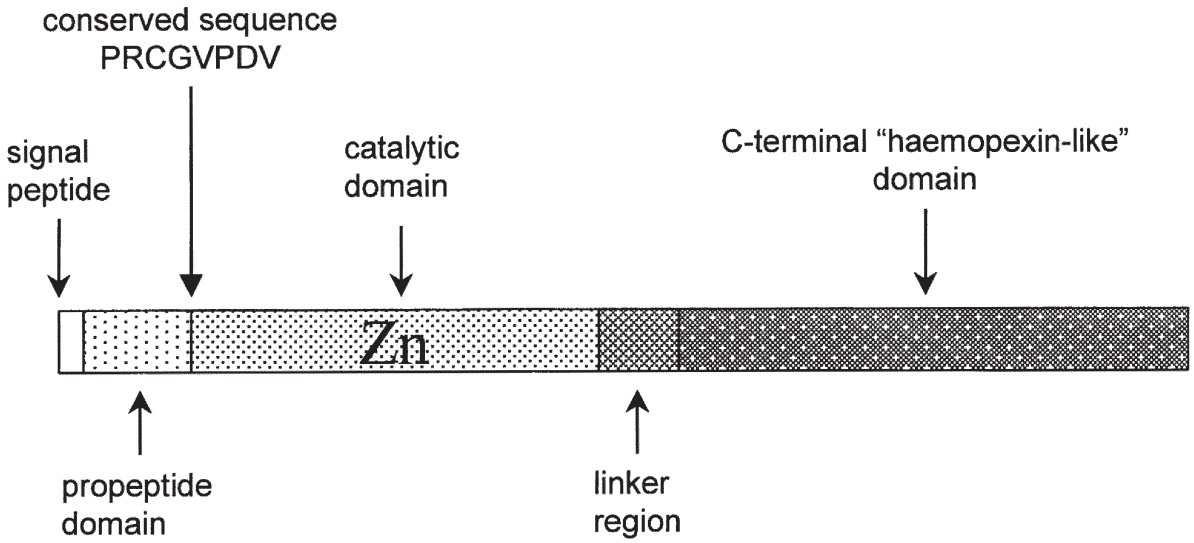

FIGURE 2. The domain structure of MMP-13. MMP-13 is synthesized as an inactive precursor and is activated by cleavage of the N-terminal propeptide.

E. Activation and Inhibition of MMP-13

MMP-13 has a central position in the MMP activation cascade (Figure 3). MMP-13 is produced as an inactive proenzyme and activation by cleavage of the N-terminal propeptide can be carried out by a various compounds. Activation can be autoproteolytic, can be catalyzed by several MMPs, and occurs by a three-step process when catalyzed by aminophenylmercuric acetate (APMA, used to activate proMMP-13 *in vitro*).[41] MMP-13 is inhibited by tissue inhibitors of matrix metalloproteinases (TIMPs) in a 1:1 stoichiometric fashion. Kinetic analysis shows that TIMP-3 interacts with MMP-3 1.1 times faster than TIMP-1 and 5.5 times faster than TIMP-2.[41]

Activation of MMP-13 by stromelysin-1 is concentration dependent.[41] An intermediate is formed with a molecular mass of 50 kDa by cleavage of Gly^{57}-Leu^{58}, which is then converted to the fully active 48-kDa form, by cleavage at the characteristic Glu^{84}-Tyr^{85} bond.[41] MMP-2 alone directly hydrolyzes the 60-kDa proenzyme at the Glu^{84}-Tyr^{85} bond in a 23:1 molar ratio.[42] Using the recombinant catalytic domain of membrane-type matrix metalloproteinase MT1-MMP, activation occurs in a 9:1 molar ratio. The introduction of TIMP-1 to this system leads to the production of a 56-kDa intermediate, via cleavage at the Gly^{35}-Ile^{36} bond. The activation by MT1-MMP is potentiated by the presence of MMP-2, resulting in a faster reaction rate. This interaction requires an intact MMP-2 active site, as a mutant with a $Glu^{375} \rightarrow Ala$ substitution had no effect. MT1-MMP generates active MMP-2 during the proMMP-13 activation reaction, which then significantly contributes to MMP-13 activation. Furthermore, concavalin-A stimulated fibroblast monolayers, which express MT1-MMP, activate

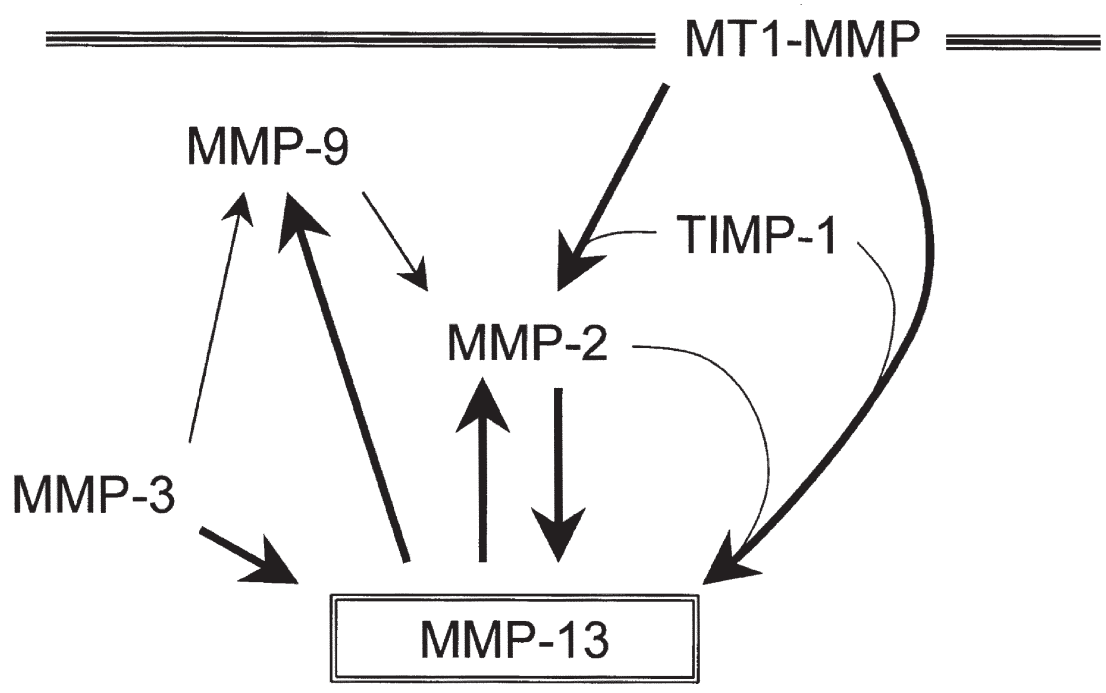

FIGURE 3. The central role of MMP-13 in the MMP activation cascade. MMP-13 is activated by MT1-MMP, MMP-2, and MMP-3; MMP-2 and -9 are activated by MMP-13.

MMP-13. This is potentiated by the presence of MMP-2.[42] Similarly, conA-treated chondrosarcoma cell (SW1353) membranes, which is increased MT1-MMP display levels, had a greater ability to activate MMP-13.[43]

In a human chondrosarcoma cell line (SW1353), proMMP-2 is constitutively produced.[43] However, active MMP-9 is seen only when active MMP-13 and MMP-2 are present. This is consistent with another study in which active MMP-13 activated proMMP-9.[44] In SW1353 cultures, there are independent activation mechanisms for MMP-1 and MMP-13 in these cells, and activation of MMP-1 is independent of MMP-2, –9, and –13.[43] It is also unlikely that MMP-3 has any role in MMP-13 activation in this system, which suggests that there are multiple, potentially independent collagenase activation pathways. MMP-13 can also be activated by plasmin, being cleaved at the Lys^{38}-Glu^{39} and Arg^{76}-Cys^{77} bonds in the propeptide domain.[42]

A specific MMP-13 receptor has been characterized: the 170-kDa protein was initially identified in a rat osteosarcoma cell line (UMR 106-01),[45] but subsequently has been found in other cell types, including osteoblasts and fibroblasts.[46] The binding of MMP-13 to the receptor is a calcium-dependent process, which is followed by internalization of MMP-13 and its intracellular degradation. The internalization step requires the cooperation of the low-density lipoprotein receptor-related protein (LRP). Parathyroid hormone influences the rate of degradation of MMP-13. This represents another level of regulation of MMP activity.

F. MMP-13 Substrates

When first characterized in 1994, MMP-13 displayed structural features consistent with expected activity against fibrillar collagens.[4] As described above, the three residues conserved among collagenases, Tyr-214, Asp-235, and Gly-237, are all conserved in MMP-13. It was also demonstrated that MMP-13 was active against type 1 collagen and a synthetic peptide, commonly used for investigating collagenase activity. A range of studies has shown that MMP-13 is active *in vitro* against a variety of natural and synthetic substrates.

MMP-13 cleaves the interstitial collagens (I, II, and III) into typical C-terminal and N-terminal polypeptide fragments, also seen for other MMPs.[41] MMP-13 is particularly potent against type II collagen, cleaving it five times faster than type I collagen and six times faster than type III collagen. Type II collagen is the major collagen constituent in cartilage, and this activity is consistent with the fact that MMP-13 is physiologically restricted to expression during bone remodelling. Furthermore, the type II collagenase activity of MMP-13 is more potent than that of the other collagenases, suggesting specific roles in the degradation of different types of collagen.[41] For comparison, MMP-1 preferentially hydrolyzes type III collagen, whereas MMP-8 is more active against type I collagen. MMP-13 activity against type II collagen was at least 10 times greater than that of MMP-1.[47] Furthermore, MMP-13 cleaves type II collagen at the same bond as MMP-1 (Gly^{906}-Leu^{907}) and then further cleave one of the resulting fragments at two other bonds (Gly^{909}-Gln^{910}; Gly^{912}-Ile^{913}).

Studies using a C-terminal deletion mutant of MMP-13 were used to investigate the function of the C-terminal domain (CTD). The mutant ($\Delta_{249\text{-}451}$)MMP-13 did not have triple helicase activity against type I or II collagen, indicating the importance of the CTD. However, the mutant did cleave the β1,2(I) chains of type I collagen, generating smaller such chains, indicating that the catalytic domain independently has effi-

cient telopeptidase activity that does not require substrate binding.[48] This is unique among the collagenases. Furthermore, the use of triple helical peptides (THP) has also provided evidence that MMP-13 has some N-terminal exopeptidase or endopeptidase activity, whereas MMP-1 has none.[49]

Both mutant and wild-type MMP-13 degraded type IV collagen, in both the α2 (IV) and α1(IV) chains. It is interesting to note that MMP-13 degraded type IV collagen at a lower temperature than gelatinase A, suggesting another important role physiologically.[48] Similarly, wild-type and mutant MMP-13 had comparable activity against type IX, X, and XIV collagens, while collagen type XI was completely resistant to both.

MMP-13 also shows the highest activity against gelatin among the collagenases, being 44 times more efficient than MMP-1 and 3 to 8 times more efficient than MMP-8.[41] This suggests that MMP-13 not only acts against intact fibrillar collagens, but also assists in the further cleavage of breakdown products.

Aggrecan and perlecan are two further ECM components susceptible to degradation by MMP-13. Cartilage aggrecan is a large cartilage proteoglycan. MMP-13 cleaves aggrecan in the interglobular domain (IGD) at the same site identified for other MMPs (…PEN_{341}-FFG…), such as stromelysins, collagenase, gelatinases, and matrilysin, as well as at a novel site not previously observed for other proteinases (ÖVKP_{384}-VFEÖ), although *in vitro* a high concentration was required to reveal the existence of this cleavage site.[50]

Perlecan is a heparin sulfate proteoglycan. The heparin sulfate sequence is capable of binding bFGF for storage, whereupon MMP-13 action allows its release and promotion of angiogenesis.[51] Rat MMP-13 was used in these experiments and resulted in cleavage of the protein core. It was also noted that perhaps the bFGF was being cleaved by the rat collagenase during release.

A recent study using a phage-display peptide library detected a potential MMP-13 cleavage site in TGFβ3.[52] MMP-13 has been identified recently in matrix vesicles (MV) from growth plate cartilage, structures that have a key role in initiating the mineralization of cartilage. MMP-13 associated with these matrix vesicles can at least partially activate latent TGFβ within the MV.[53] Furthermore, a link between TGFβ activation and the gelatinases has already been established.[54] Biglycan, a connective tissue proteoglycan, was identified as another potential substrate.[52] The most significant finding, however, was that of a highly sensitive and specific substrate for MMP-13. This substrate, CP (2,4-dinitrophenyl-GPLGMRSGL-NH_2), may be used to facilitate the future development of synthetic MMP-13 inhibitors of greater specificity.

Tenascin C is an ECM component consisting of two isoforms produced by alternative splicing. The large isoform is highly susceptible to proteolytic cleavage by MMP-13, while the small isoform is not.[48] This suggests that the MMP-13-sensitive sites are situated in fibronectin type III repeats that are alternatively spliced between the two isoforms. Fibronectin itself is also cleaved by MMP-13.

Both fibrillin and fibrillin-rich microfibrils (further ECM components) are broken down by MMP-13.[55] The cleavage site of fibrillin-1 and -2 may be located in the proline-rich region, whereas there may be additional degradation of the CTD of fibrillin-1. Furthermore, MMP-13 affected microfibril organization and integrity, leading to loss of of normal microfibril function.

MMP-13 is also active against two serpins: α2-antichymotrypsin and plasminogen activator inhibitor (PAI)-2.[41] α2-antichymotrypsin is degraded at the same

site (Ala^{362}-Leu^{363}) by MMP-13 as by MMPs 1 and 3.[56] The activity against PAI-2 is interesting in light of recent research that has demonstrated MMP-13 activity against fibrinogen and Hageman factor (factor XII).[57] *In vitro* MMP-13 catalyzed fast degradation of the α chain of fibrinogen (within 1 min), while longer incubation was necessary for the degradation of β and γ chains. Furthermore, treatment of fibrinogen with MMP-13 prevents self-assembly of large protofibrils and fibers. In this way, MMP-13 reduced thrombin-induced fibrinogen clotting activity by 50% after only 8 min. The cleavage of factor XII again occurred in a type II fibronectin-like domain, as well as in an EGF-like domain. There was also a cleavage at Gly^{376}-Leu^{377}. This bond was located within the catalytic region, four residues downstream of the kallikrein cleavage site, such that MMP-13-treated latent factor XII cannot be activated by kallilkrein.

G. MMP-13 Expression

The genetic and regulatory features of MMP-13 are typical of the stringent control mechanisms associated with the MMPs. In light of the potency of action and wide range of substrates for MMP-13, it is not surprising that physiological expression is limited to situations of rapid extracellular matrix remodeling.

H. Developmental Expression of MMP-13

The earliest stage MMP-13 may be found at developmentally is the body stalk at 4 weeks gestation. Thereafter, MMP-13 is restricted to skeletal tissues involving rapid turnover.[58] *In situ* hybridization studies have demonstrated MMP-13 mRNA in chondrocytes, osteoblasts, and periosteal cells, both in the periostea of ribs and vertebrae, at 15 weeks of fetal bone development.[58]

MMP-13 is present in chondrocytes and osteoblastic cells associated with periosteal blood vessels in mineralizing primary ossification centers in the shaft of long bones and feet from 10 weeks throughout gestation.[59] It was also detected in osteoblasts and fibroblasts of calvarial bone at 16 weeks[58] (Johansson et al., 1997a). It is interesting to note that MMP-13 is expressed during both intramembranous ossification (skull) and endochondral ossification (long bones) during gestation. Furthermore, all organs were investigated at 20 weeks gestation, with MMP-13 expression being confined to developing skeletal tissue.[59]

I. MMP-13 Expression in Cancer

1. Breast Carcinoma

Human MMP-13 was first cloned from a breast cancer cDNA library, and initial immunohistochemical studies indicated strong immunoreactivity in the cytoplasm of breast carcinoma cells, with some cases showing slight staining in surrounding stromal cells.[4] Subsequent *in situ* hybridization studies showed the presence of MMP-13 mRNA breast cancer in two other studies. The first demonstrated MMP-13 positivity in cancer cells in 3 of 11 cases (27.3%),[60] while a second study detected positivity in fibroblasts surrounding the tumor cells in 3 of 10 cases (30%).[33] These apparently contradictory findings may be explained by differences in tumor biology (histological subtype, grade) and the small sample size. Also, it may be that stromal MMP-13 expression occurs earlier in tumor progression

before a switch to epithelial cell expression in later stages, as occurs for other MMPs.[61]

There is convincing *in vitro* evidence that breast carcinoma cells secrete diffusible factors, including IL-1α and IL-1β, which induce surrounding stromal fibroblasts to express MMP-13.[33,62]

2. Head and Neck Squamous Carcinoma

MMP-13 expression was related to tumor aggressiveness in a study of squamous cell carcinomas of the head and neck (n = 35), where 85.7% of the cases by immunohistochemistry were MMP-13 positive.[63] Similarly, by *in situ* hybridization, Johansson and co-workers showed MMP-13 mRNA in 88.2% of a series (n = 17) of head and neck squamous carcinomas. Expression was largely in tumor cells at the invading front, but three cases displayed MMP-13 exclusively in stromal fibroblasts.[64] The study by Cazorla et al.[65] showed MMP-13 mRNA in 20 of 35 (57.1%) laryngeal squamous carcinomas by Northern blotting: by immunohistochemistry, MMP-13 expression in this series was mainly confined to tumor cells, with occasional positive stromal cells.[65]

3. Female Reproductive Tract Malignancies

Johansson and colleagues showed the presence of MMP-13 mRNA (mainly confined to tumor cells) in 75% of cell lines established from invasive squamous carcinomas of the vulva n = 12).[66] With regard to squamous carcinoma of the uterine cervix, MMP-13 mRNA was detected in two-thirds of cases (n = 6): An association between HPV types 16 or 68 and transcription of MMP-13 was noted.

4. Melanoma and Non-Melanoma Skin Cancer

MMP-13 has been demonstrated in 12 of 23 (52.2%) malignant melanomas, being more common in tumors showing a greater extent of local invasion.[67] Basal cell carcinoma (BCC) of the skin shows a more unusual expression pattern for MMP-13, with weak staining in stromal and tumor epithelium but intense staining in normal epithelium adjacent to the tumor.[68] Airola et al.[69] compared MMP-13 expression in malignant, premalignant (solar keratosis), and benign proliferative epithelial skin lesions, using *in situ* hybridization. The majority of premalignant and benign lesions failed to show expression of MMP-13 mRNA, while squamous carcinomas displayed MMP-13 expression at the invading edge of the tumors. Interestingly, MMP-13 co-localized with laminin-5 immunostaining, which has a suggested role in tumor cell migration.

5. Cartilaginous Tumors

The tumor type most commonly expressing MMP-13 in the literature to date is chondrosarcoma. One-quarter of a series (n = 8) of benign collagen-forming neoplasms expressed MMP-13, whereas all malignant collagen-forming tumors showed positive staining for MMP-13 by immunohistochemistry.[24] This finding is consistent with a physiological role for MMP-13 in the musculoskeletal system.

III. CONCLUSIONS

MMP-13 has central roles in modulating extracellular matrix degradation through

its direct matrix degrading capability as well as having a key involvement in the activation of other MMPs. This review has outlined the regulatory mechanisms, structure, and function of this important MMP. The physiological expression of MMP-13 is even more restricted than some of the other collagenases, (e.g., MMP-1), being limited to skeletal tissues undergoing remodeling. MMP-13 has been detected in a variety of neoplastic cells, in particular, breast carcinoma cells and chondrosarcoma cells, and in stromal cells within several different types of malignancy. Some of the mechanisms that control the normal regulation of MMP-13 have been elucidated. However, the mechanisms underlying the dysregulation of MMP-13 in cancer have still to be fully characterized. It is also becoming apparent that the MMPs have broad biological roles of which the best characterized is extracellular matrix degradation, but other roles in cell growth and regulation are now being identified, and further elucidation of the biological roles of MMP-13 can be expected.[3,70]

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