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Molecules in focus

MMP-1: the elder of the family

Annie Pardo*, Moisés Selman

Facultad de Ciencias, Universidad Nacional Autónoma de México and Instituto Nacional de Enfermedades Respiratorias, Apartado Postal 21-630, Coyoacan, México, DF, CP 04000, México

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Abstract

The matrix metalloproteinases (MMPs) are a family of zinc-containing endopeptidases that play a key role in both physiological and pathological tissue remodeling. Human fibroblast collagenase (MMP-1) was the first vertebrate collagenase purified as a protein and cloned as a cDNA, and is considered the prototype for all the interstitial collagenases. It is synthesized as a zymogen where N-terminal residues are removed by proteolysis and shares with other MMPs a catalytic domain and a carboxy terminal domain with sequence similarity to hemopexin. Importantly, MMP-1 should be considered a multifunctional molecule since it participates not only in the turnover of collagen fibrils in the extracellular space but also in the cleavage of a number of non-matrix substrates and cell surface molecules suggesting a role in the regulation of cellular behaviour. Furthermore, an extensive body of evidence indicates that MMP-1 plays an important role in diverse physiologic processes such as development, tissue morphogenesis, and wound repair. Likewise, it seems to be implicated in a variety of human diseases including cancer, rheumatoid arthritis, pulmonary emphysema and fibrotic disorders, suggesting that its inhibition or stimulation may open therapeutic avenues.

Keywords: Fibrosis; Collagenase; Matrix metalloproteinases; Cancer

1. Introduction

Interstitial collagens, types I, II, and III are the most abundant proteins in animals. In particular, types I and II collagens are highly resistant to general proteolysis and require specific proteases for their degradation. In 1962 Gross and Lapiere described the observation that in metamorphosing tadpole the resorbing tail released an enzymatic activity that had the ability to degrade the collagen triple helix at neutral pH. This report involving interstitial collagenase opened a new field strongly related to biomedical research that currently comprise a family of 23 human matrix metalloproteinases/matrixins (MMPs) genes. Human fibroblast collagenase was the first vertebrate collagenase both purified to homogeneity as a protein, and cloned as a cDNA (Bauer, Eisen, & Jeffrey, 1970; Goldberg et al., 1986). This enzyme has been designated as matrix metalloproteinase-1 (MMP-1) and has served as the prototype for all the interstitial collagenases.

MMP-1 is also known as collagenase-1, fibroblast collagenase and interstitial collagenase which is the official EC name (EC number 3.4.24.7). As many other

* Corresponding author. Fax: +52 5 622 4910.
E-mail address: aps@hp.fciencias.unam.mx (A. Pardo).

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MMPs it is undetectable in normal resting tissues, although in vitro is produced by a wide variety of normal cells as fibroblasts, macrophages, endothelial and epithelial cells. MMP-1 is mainly expressed during physiological and pathological tissue remodeling in vivo suggesting a broad-based role in biology (Woessner & Nagase, 2000).

2. Structure

Starting from the N terminus the following features of domain organization are observed (Woessner & Nagase, 2000).

The pre-domain specifies a signal rich in hydrophobic amino acids that destines the synthesized polypeptide to the endoplasmic reticulum where it is removed during the transportation of the molecule from the cell to the outside.

The propeptide domain indicates a sequence that is responsible of keeping the proenzyme inactive. It presents a cysteine residue 73 that is located in a conserved sequence PRCGVPD opposite to a zinc atom at the active site and coordinated to it through an –SH group. The enzymatic activity of the proenzyme is turned on by the displacement of this cysteine residue ("cysteine switch") and occurs by proteolytic cleavage, or by chemical disruption as in the case of oxidation or treatment with mercurial compounds (Springman, Angleton, Birkedal-Hansen, & Van Wart, 1990).

The catalytic domain contains a catalytic zinc, bound in the sequence HELGHXXGXXH by the three His residues. The crystal structure revealed that the catalytic domain has a five-stranded β sheet, three α helices, two zinc ions, one structural and one catalytic, and one calcium (Fig. 1) (Li et al., 1995). A cleft in the active site with a well defined pocket contributes to the enzyme specificity since variations in the residues of this pocket differentiate one MMP from another. According to the proposed catalytic mechanism the Glu residue at the active centre HEXXH would act as a general base catalyst (Spurlino et al., 1994).

The catalytic domain is connected by a linker region to a carboxy terminal domain, with homology to hemopexin a serum glycoprotein involved in haem transport. This hemopexin domain has about 200 residues and contains four repeats with a Cys residue at either end. It presents a four bladed β-propeller-like

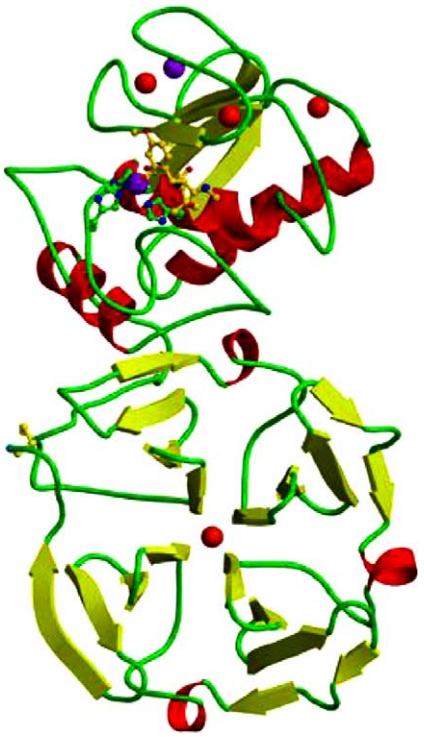

Fig. 1. Porcine MMP-1. The catalytic domain (residues 100–260) is at the top. The hemopexin domain (residues 276–466) is at the bottom. Calcium atoms are coloured red. Zinc atoms are coloured purple (magenta). A specific metalloproteinase inhibitor *N*-[3-*N*′-hydroxycarboxamido)-2-(2-methylpropyl)-propananoyl]-*O*-methyl-L-tyrosine-*N*-methylamide bound at the active site is displayed using a ball and stick representation with yellow carbon atoms. His218, His222, and His228 which coordinate the Zn at the active site are draw with green carbon atoms. (Figure donated by Peter Brick, Blackett Laboratory, Department of Biological Sciences, Imperial College, London UK).

structure arranged symmetrically around a central axis as observed in Fig. 1 (Li et al., 1995). It helps to confer enzyme substrate-binding specificity for collagen, as demonstrated when hybrid enzymes of MMP-1 and MMP-3 representing an exchange of the C-terminal domains, were neither able to degrade collagen (Murphy et al., 1992).

3. Synthesis and regulation

MMP-1 is synthesized as a single polypeptide and is secreted as a proenzyme. It comprises a major

unglycosylated form of ∼57 kDa, and a minor glycosylated species of 61 kDa.

The structure of the human gene has 10 exons and is located on the human chromosome 11q22.2–22.3. This gene is tightly linked to a cluster of other eight MMPs genes that include MMP-3, MMP-7, MMP-8, MMP-10, MMP-12, MMP-13, MMP-20, MMP-27 and two pseudogenes (Puente, Sánchez, Overall, & López-Otín, 2003). Interestingly, the mouse ortholog (McolA) for human MMP-1 shows only 58% amino-acid identity and is distantly related suggesting a rapid evolution in one or both lineages (Balbin et al., 2001).

4. Regulation

In normal adult tissues, the levels of MMP-1 are usually low. By contrast, its expression is elevated when the system faces a disturbance, such as wound healing, repair, or remodelling processes as occur in several pathological conditions (Brinckerhoff & Matrisian, 2002).

The expression of collagenase-1 is transcriptionally regulated by growth factors, hormones, and cytokines, and the proteolytic activity is accurately controlled through activators and inhibitors such as α-macroglobulins, and tissue inhibitors of metalloproteinases (TIMPs).

4.1. Transcriptional regulation

Several cytokines and growth factors induce the transcription of MMP-1. Its promoter contains a TATA box at approximately −30 bp, and an AP-1 site at approximately −70 bp. AP-1 binds Fos–Jun heterodimers or Jun–Jun homodimers. An additional AP-1 site is found at −186 bp which has a modest role in basal transcription but is responsible for the increased transcription in response to phorbol esters (Vincenti & Brinckerhoff, 2002). AP-1 site cooperate with a variety of cis-acting sequences, and particularly, the Ap-1 site at −1602 bp is of interest because it cooperates with an Ets site created by a single nucleotide polymorphism at −1607 bp. This polymorphism is represented by the insertion of an extra guanine base which creates a binding site for the Ets transcription factor to enhance the expression of MMP-1. Interestingly, patients with some types of cancer exhibit a higher frequency of the two guanine allele compared with unaffected individuals (Nishioka, Sagae, Nishikawa, Ishioka, & Kudo, 2003).

Cytokine inducers of MMP-1 include epidermal growth factor, fibroblast growth factor (FGF)-1, -2, -7, and -9, hepatocyte growth factor, granulocyte-macrophage colony-stimulating factor, interferon beta and gamma, platelet-derived growth factor, transforming growth factor (TGF) alpha, and interleukin (IL)-1, -4, -5, -6, -8, and -10 (Woessner & Nagase, 2000). On the other hand, TGFβ and the vitamin A derivatives all-trans retinoic acid and the synthetic retinoids suppress MMP-1 transcription. TGFβ mediates its effect through a TGFβ inhibitory element (TIE). Mutation in this element results in a significant increase of both basal and tissue polypeptide antigen (TPA) induced MMP-1 gene transcription in fibroblasts suggesting that TIE may have a role as an MMP-1 constitutive repressor in these cells (White, Mitchell, & Brinckerhoff, 2000).

4.2. Procollagenase activation

The activation of the pro-enzyme occurs when the linkage of the unpaired cysteine, in the pro-domain PRCGXPD sequence, forming a bridge with the catalytic zinc is chemically disrupted as for example, by amino phenyl mercuric acid (APMA). Of biological relevance is the activation of pro-MMP-1 by pro-domain proteolytic cleavage that seems to take place in a stepwise manner requiring at least two proteinases for full activation, the interaction of the plasminogen activator urokinase and stromelysin-1 (Suzuki, Enghild, Morodomi, Salvesen, & Nagase, 1990).

4.3. Local inhibition of MMP-1

Extracellular activation of the zymogen can be followed either by binding to substrate or by the interaction with inhibitor. In general, the activity of the matrix metalloproteinases is inhibited by a family of four endogenous inhibitors known as ‘tissue inhibitors of metalloproteinases’ (TIMPs). TIMP-1 acts by forming a 1:1 complex with the activated catalytic zinc in the MMP-1. MMP–TIMP interaction in the crystal structure of the stromelysin-1 catalytic domain–TIMP-1 complex showed that most of the intermolecular contacts between these two proteins are restricted to the first five amino-terminal

residues of TIMP-1, which binds to the active site in a substrate-like manner. A very similar "substrate-like" interaction has been found in two crystal forms of MMP-1 (Woessner & Nagase, 2000).

5. Biological function

5.1. Substrate specificity

Only four members of the MMPs including MMP-1, MMP-8, MMP-13 and MMP-14 (MT1-MMP) can degrade fibrillar collagens in their triple-helical domain which leave the molecules thermally unstable so that they unwind to form gelatin after which they can be degraded by other members of the MMP family. These collagenases cleave fibrillar collagens at sites with similar amino acid sequences; in the α1(I) chain the cleavage occurs between Gly775/Ile776, and in the α2(I) chain in a corresponding Gly/Leu, three quarters of the distance from the NH2-terminus.

Additionally, other matrix molecules are substrates for MMP-1, including aggrecan, versican, perlecan, casein, nidogen, serpins, and tenascin-C (McCawley & Matrisian, 2001). Therefore, MMP-1 should play a pivotal role in the physiologic remodelling of extracellular matrix. Importantly, MMP-1 may regulate the function of biologically active molecules by releasing them from ECM stores; for example, through degradation of perlecan, MMP-1 can release bound FGF (Whitelock, Murdoch, Iozzo, & Underwood, 1996).

In the last years it has become evident that MMP-1 is also able to cleave cell surface molecules and other non-matrix substrates. Some of them comprise antichymotrypsin, antitrypsin, insulin-growth factor binding protein (IGFBP)-3, IGFBP-5, IL-Iβ, L-selectin, ovostatin, tumour necrosis factor-α, and stromal cell-derived factor-1 (McCawley & Matrisian, 2001). This wide variety of substrates assigns MMP-1 as a multifunctional molecule. For example, MMP-1 may participate in the regulation of insulin-like growth factor (IGF) for its ability to degrade IGFBPs which bind IGF with high affinity. Likewise, MMP-1 appears to play a pivotal role in epithelial morphogenesis which depends on specific movement of epithelial cells. Thus, keratinocyte migration on collagen-1 requires specific cleavage of the collagen molecule by collagenase (Pilcher et al., 1997).

The creation of knock-out mice that are null for specific genes has been useful in attributing functions to a number of MMP family members. Nevertheless, this approach has been not possible in the case of MMP-1 as the human enzyme does not have a clear-cut ortholog in the adult mouse. However, the transgenic mouse expressing the enzyme has provided some interesting results. Lung-specific expression of human MMP-1 by alveolar epithelial cells has demonstrated that up-regulation of this enzyme provokes emphysematous changes similar to those seen in smoking-induced emphysema in humans, supporting a putative role in the development of this disease (D'Armiento, Dalal, Okada, Berg, & Chada, 1992).

6. Medical applications

An extensive body of literature supports an association of MMPs with a number of human diseases including cancer, rheumatoid arthritis and other autoimmune disorders, cardiovascular and fibrotic diseases, suggesting that their inhibition or stimulation may have a therapeutic role.

6.1. Therapeutic opportunities for MMP-1 downregulation

In arthritic disease, it has been demonstrated a correlation between the increase in MMP-1 and collagen degradation suggesting that cleavage of cartilage collagen is directly related to the activity of this enzyme (Vincenti & Brinckerhoff, 2002). Cartilage destruction correlates with the concentration of MMP-1 and can be blocked by broad-range MMP inhibitors in a dose-dependent manner (Neidhart et al., 2003).

The therapeutic exploration of MMP-1 inhibition has been not only limited to diseases associated with excessive extracellular matrix destruction. Because MMP-1 is able to degrade IGFBPs, the effect of its inhibition was recently investigated in cardiac hypertrophy induced by beta-adrenergic stimulation (Miura et al., 2003). Reduction of MMP-1 and MMP-2 activities by the MMP inhibitor SI-27 caused a regression in the myocyte hypertrophy and the suppression of IGFBP-3 degradation.

MMP-1 is up-regulated in a wide variety of advanced cancers, and in nearly all instances, a significant

negative correlation between its expression and survival has been found (Brinckerhoff, Rutter, & Benbow, 2000). In this context, this and other MMPs have been selected as targets for therapeutic strategies aiming to block enzyme activity or synthesis at all stages of tumour invasion and metastasis. However, these compounds act on many genes and have resulted in undesirable side-effects and toxicities. A main factor contributing for failure is the specificity for the targeted MMP, and the lack of assays to verify inhibition of target enzymes in vivo (Coussens, Fingleton, & Matrisian, 2002).

6.2. Therapeutic opportunities for MMP-1 upregulation

The opposite process to the destructive degradation observed in arthritis occurs in fibrotic disorders that are characterized by exaggerated extracellular matrix accumulation. Here, at least theoretically, fibrillar collagen deposition in the scarring tissue might be partially related to a decreased collagenolytic activity. Some studies in experimental fibrosis support this notion. The development of liver and lung fibrosis seems to be associated with a reduction of rat MMP-13 collagenase activity (Iredale et al., 1998; Ruiz et al., 2003). These findings open new therapeutic avenues for fibrotic disorders, including gene therapy. In this context, it has been shown that gene delivery of human MMP-1, with transient over-expression of the enzyme in the liver attenuated established experimental hepatic fibrosis (Iimuro et al., 2003).

However, analysis of the expression of MMP-1 in fibrotic disorders has given contradictory results, and the possibility of inhibition should be considered carefully. In human lung fibrosis, studies on gene and protein expression have unexpectedly shown a strong up-regulation of MMP-1 (Zuo et al., 2002; Selman et al., 2000). The paradox is that excessive accumulation of collagens is associated with over-expression of the cleaving enzyme not an absence as might have expected. Although there is no a clear explanation for this paradox, the location of the enzyme in the fibrotic lung may partially explain it. Thus, although MMP-1 was highly expressed in fibrotic lung tissue, the localization of the enzyme was noticed mainly in reactive alveolar epithelial cells (Fig. 2) (Selman et al., 2000). Furthermore, the so called *fibroblast type collagenase* was virtually absent in fibroblasts from the interstitial fibrotic areas where collagens are being deposited. The role of epithelial MMP-1 is presently unknown but it might be related with cell migration as occurs with keratinocytes (Pilcher et al., 1997).

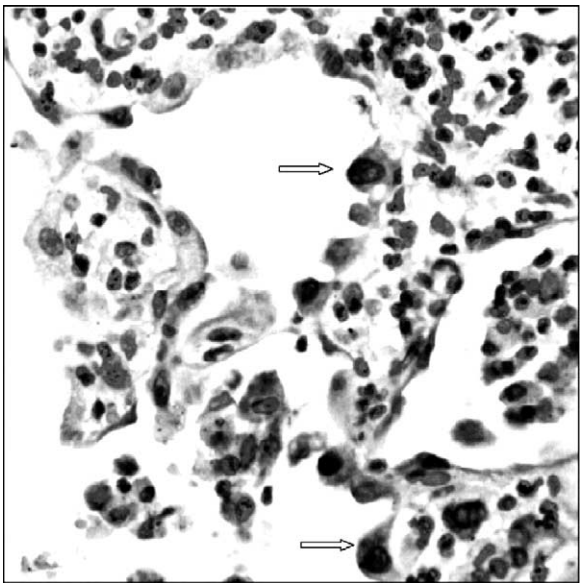

Fig. 2. Immunolocalization of MMP-1 in idiopathic pulmonary fibrosis. Arrows indicate alveolar epithelial cells with strong positive cytoplasmic staining.

It is important to emphasize that the field of therapeutic inhibition or stimulation of MMP-1 is just opening and it will be likely associated with several problems in the clinical practice. MMP-1 is a pleiotropic molecule that has multiple substrate activities and is expressed in several cell types. Difficulties associated with enzyme specificity, drug stability, delivery and clearance, as well as achievement of clinically effective concentrations avoiding undesirable side-effects remain to be resolved.

References

Balbin, M., Fueyo, A., Knauper, V., Lopez, J. M., Alvarez, J., Sanchez, L. M., et al. (2001). Identification and enzymatic characterization of two diverging murine counterparts of human interstitial collagenase (MMP-1) expressed at sites of embryo implantation. *Journal of Biological Chemistry*, *276*, 10253–10262.

Bauer, E. A., Eisen, A. Z., & Jeffrey, J. J. (1970). Immunologic relationship of a purified human skin collagenase to other human

and animal collagenases. *Biochimica et Biophysica Acta*, *206*, 152–160.

Brinckerhoff, C. E., & Matrisian, L. M. (2002). Matrix metalloproteinases: a tail of a frog that became a prince. *Nature Review Molecular Cell Biology*, *3*, 207–214.

Brinckerhoff, C. E., Rutter, J. L., & Benbow, U. (2000). Interstitial collagenases as markers of tumor progression. *Clinical Cancer Research*, *6*, 4823–4830.

Coussens, L. M., Fingleton, B., & Matrisian, L. M. (2002). Matrix metalloproteinase inhibitors and cancer: trials and tribulations. *Science*, *295*, 2387–2392.

D'Armiento, J., Dalal, S. S., Okada, Y., Berg, R. A., & Chada, K. (1992). Collagenase expression in the lungs of transgenic mice causes pulmonary emphysema. *Cell*, *71*, 955–961.

Goldberg, G. I., Wilhelm, S. M., Kronberger, A., Bauer, E. A., Grant, G. A., & Eisen, A. Z. (1986). Human fibroblast collagenase. Complete primary structure and homology to an oncogene transformation-induced rat protein. *Journal of Biological Chemistry*, *261*, 6600–6605.

Gross, J., & Lapiere, C. M. (1962). Collagenolytic activity in amphibian tissues; a tissue culture assay. *Proceedings of the National Academy of Sciences of the United States of America*, *48*, 1014–1022.

Hirata, H., Naito, K., Yoshihiro, S., Matsuyama, H., Suehiro, Y., & Hinoda, Y. (2003). A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter is associated with conventional renal cell carcinoma. *International Journal of Cancer*, *106*, 372–374.

Iimuro, Y., Nishio, T., Morimoto, T., Nitta, T., Stefanovic, B., Choi, S. K., et al. (2003). Delivery of matrix metalloproteinase-1 attenuates established liver fibrosis in the rat. *Gastroenterology*, *124*, 445–458.

Iredale, J. P., Benyon, R. C., Pickering, J., McCullen, M., Northrop, M., Pawley, S., et al. (1998). Mechanisms of spontaneous resolution of rat liver fibrosis. Hepatic stellate cell apoptosis and reduced hepatic expression of metalloproteinase inhibitors. *Journal of Clinical Investigation*, *102*, 538–549.

Li, J., Brick, P., O'Hare, M. C., Skarzynski, T., Lloyd, L. F., Curry, V. A., et al. (1995). Structure of full-length porcine collagenase reveals a C-terminal domain containing a calcium linked, four-bladed b-propeller. *Structure*, *3*, 541–549.

McCawley, L. J., & Matrisian, L. M. (2001). Matrix metalloproteinases: they're not just for matrix anymore. *Current Opinion in Cell Biology*, *3*, 534–540.

Miura, S., Ohno, I., Suzuki, J., Suzuki, K., Okada, S., Okuyama, A., et al. (2003). Inhibition of matrix metalloproteinases prevents cardiac hypertrophy induced by beta-adrenergic stimulation in rats. *Journal of Cardiovascular Pharmacology*, *42*, 174–181.

Murphy, G., Allan, J. A., Willenbrock, F., Cockett, M. I., O'Connell, J. P., & Docherty, A. J. (1992). The role of the C-terminal domain in collagenase and stromelysin specificity. *Journal of Biological Chemistry*, *267*, 9612–9618.

Neidhart, M., Seemayer, C. A., Hummel, K. M., Michel, B. A., Gay, R. E., & Gay, S. (2003). Functional characterization of adherent synovial fluid cells in rheumatoid arthritis: destructive potential in vitro and in vivo. *Arthritis and Rheumatism*, *48*, 1873–1880.

Nishioka, Y., Sagae, S., Nishikawa, A., Ishioka, S., & Kudo, R. (2003). A relationship between matrix metalloproteinase-1 (MMP-1) promoter polymorphism and cervical cancer progression. *Cancer Letters*, *200*, 49–55.

Pilcher, B. K., Dumin, J. A., Sudbeck, B. D., Krane, S. M., Welgus, H. G., & Parks, W. C. (1997). The activity of collagenase-1 is required for keratinocyte migration on a type I collagen matrix. *Journal of Cell Biology*, *137*, 1445–1457.

Puente, X. P., Sánchez, L. M., Overall, C. M., & López-Otín, C. (2003). Human and mouse proteases: a comparative genomic approach. *Nature Review Genetics*, *4*, 544–549.

Ruiz, V., Ordonez, R. M., Berumen, J., Ramirez, R., Uhal, B., Becerril, C., et al. (2003). Unbalanced collagenase/TIMP-1 expression and epithelial apoptosis in experimental lung fibrosis. *American Journal of Physiology Lung Cell Molecular Physiology*, *285*, L1026–L1036.

Selman, M., Ruiz, V., Cabrera, S., Segura, L., Ramirez, R., Barrios, R., et al. (2000). TIMP-1, -2, -3, and -4 in idiopathic pulmonary fibrosis. A prevailing nondegradative lung microenvironment? *American Journal of Physiology Lung Cell Moecular Physiology*, *279*, L562–L574.

Springman, E. B., Angleton, E. L., Birkedal-Hansen, H., & Van Wart, H. E. (1990). Multiple modes of activation of latent human fibroblast collagenase: evidence for the role of Cys 73 active site zinc complex in latency and a cysteine switch mechanism for activation. *Proceedings of the National Academy of Sciences of the United States of America*, *87*, 364–368.

Spurlino, J. C., Smallwood, A. M., Carlton, D. D., Banks, T. M., Vavra, K. J., Johnson, J. S., et al. (1994). 1.56 A structure of mature truncated human fibroblast collagenase. *Proteins*, *19*, 98–109.

Suzuki, K., Enghild, J. J., Morodomi, T., Salvesen, G., & Nagase, H. (1990). Mechanisms of activation of tissue procollagenase by matrix metalloproteinase 3 (stromelysin). *Biochemistry*, *29*, 10261–10270.

Vincenti, M. P., & Brinckerhoff, C. E. (2002). Transcriptional regulation of collagenase (MMP-1, MMP-13) genes in arthritis: integration of complex signaling pathways for the recruitment of gene-specific transcription factors. *Arthritis Research*, *4*, 157–164.

White, L. A., Mitchell, T. I., & Brinckerhoff, C. E. (2000). Transforming growth factor beta inhibitory element in the rabbit matrix metalloproteinase-1 (collagenase-1) gene functions as a repressor of constitutive transcription. *Biochimica et Biophysica Acta*, *1490*, 259–268.

Whitelock, J. M., Murdoch, A. D., Iozzo, R. V., & Underwood, P. A. (1996). The degradation of human endothelial cell-derived perlecan and release of bound basic fibroblast growth factor by stromelysin, collagenase, plasmin, and heparanases. *Journal of Biological Chemistry*, *271*, 10079–10086.

Woessner, J. F., & Nagase, H. (2000). *Matrix metalloproteinases and TIMPs*. New York: Oxford University Press.

Zuo, F., Kaminski, N., Eugui, E., Allard, J., Yakhini, Z., Ben-Dor, A., et al. (2002). Gene expression analysis reveals matrilysin as a key regulator of pulmonary fibrosis in mice and humans. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 6292–6297.