

CHAPTER 1

THE MATRIX METALLOPROTEINASES AND THEIR INHIBITORS

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1. Introduction

It has been over 40 years since the identification by Gross and Lapiere (1962) (1) of a collagenolytic activity involved in tadpole tail resorption, which was subsequently revealed as collagenase-1, the prototype of the protease family called the Matrix Metalloproteinases (MMPs) or matrixins (2). With the completion of the human genome sequence, the human MMP family is now known to contain 24 members (3). The MMPs have been and remain the focus of much research effort, which was fuelled originally by the recognition that these enzymes played critical roles in tissue remodelling processes, including the involvement in amphibian metamorphosis that set the ball rolling. Over the last twenty years it has been recognised that diseases involving pathological tissue destruction are associated with aberrant production or activation of MMPs, or a lack of their natural tissue inhibitors, the TIMPs. These perceptions led the pharmaceutical industry to develop synthetic MMP inhibitors (MPIs) which entered clinical trials in the late 1990s as potential treatments for cancer and arthritis. That the MPIs did not prove to be wonder drugs was a disappointment, but the lesson learned was that the biological roles of MMPs are much more complex than originally envisaged. That in addition to extracellular matrix (ECM) degradation during tissue remodelling, MMPs regulate the pericellular milieu by

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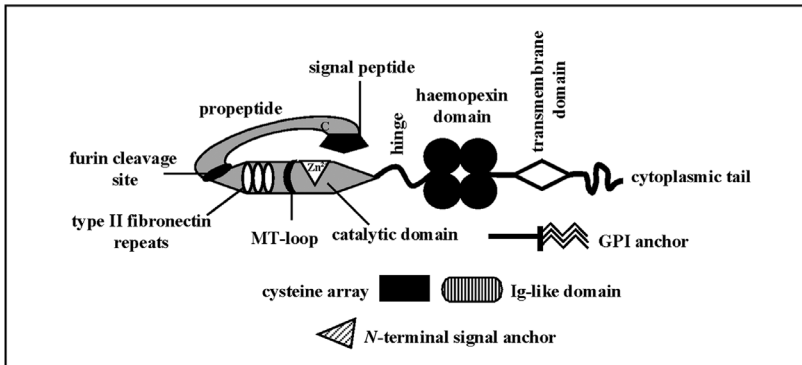
specific cleavage of ECM and cell-associated molecules to control the bioactivity of key cell signalling effectors.

This review sets out to summarise current knowledge of the composition of the MMP family, as well as their regulation and molecular activities. In particular we draw attention to new insights into the functions of the MMPs, many of which have originated from studies outside the CNS, but which may have relevance to an understanding of brain development and disease pathogenesis.

2. The MMP Family

The MMPs are a family of zinc- and calcium-dependent endopeptidases that form a subgroup of the metzincins, characterised by a conserved motif of three histidine residues binding a Zn^{2+} ion at the catalytic site followed by a methionine that introduces a turn into the molecule. The MMPs are the primary matrix degrading proteases, collectively able to degrade all protein components of the ECM (for a comprehensive list of MMPs and their substrates, see Egeblad and Werb (4); see also the excellent website of the Overall group at: http://www.clip.ubc.ca/mmp_timp_folder/mmp_substrates.shtml). All MMPs share a basic structural organisation comprising a signal peptide that targets them for secretion, a pro-peptide domain and an *N*-terminal catalytic domain. Most MMPs, with the exception of MMP-7, -23 and -26, also have a hinge region and *C*-terminal hemopexin-like domain. The MMPs can be arranged into different subgroups based on their structural features, as shown in Fig. 1.

MMPs are produced as inactive zymogens, with a cysteine residue within a PRCGV/NPD motif in the propeptide (with the possible exception of MMP-23) that binds to the Zn^{2+} in the active site cleft, thereby preventing activity (5). Activation requires disruption of the interaction between the cysteine and the Zn^{2+} and is referred to as the “cysteine switch”. This involves cleavage of the prodomain or disruption of its structure by thiol reactive reagents such as organomercurial compounds, reactive oxygen or denaturants. This can allow further cleavages to occur via inter- or intra-molecular proteolysis, thus generating the fully active enzyme (6). The *C*-terminal hemopexin-like domain has a β -propeller structure with pseudo-fourfold symmetry and is involved in both substrate binding and interaction with the tissue inhibitors of metalloproteinases (TIMPs) (7). The hinge region is a flexible linker peptide of variable length that links the *N*-terminal catalytic and *C*-terminal hemopexin domains and is important



• minimal domain: MMP-7, -26



• collagenases, stromelysins and other MMPs: MMP-1, -8, -13, -3, -10, -12, -19, -20, -27



• gelatinases: MMP-2, -9



• furin-activated MMPs: MMP-11, -21, -28



• membrane- type MMPs: MT1-MMP (MMP-14), MT2-MMP (MMP-15), MT3-MMP (MMP16), MT5-MMP (MMP-24)



• GPI-anchored MMPs: MT4-MMP (MMP-17), MT6-MMP (MMP25)



• type II transmembrane: MMP-23A, -23B



Fig. 1. Structural sub-grouping of the matrix metalloproteinases. The MMPs can be divided into several subgroups depending on their structure. All MMPs possess a signal peptide that targets the MMPs for secretion, a pro-peptide domain (containing a conserved Cys residue), and a catalytic domain. Most MMPs (with the exception of MMP-7 and MMP-26) contain a C-terminal haemopexin domain and a hinge region. Other MMP subgroups contain unique features such as a transmembrane domain, cytoplasmic tail and an MT-loop (MT1-, MT2, MT3, and MT5-MMP), a GPI anchor (MT4-MMP and MT6-MMP), a furin recognition site (MT-MMPs, MMP-11, -21, -23, -28), fibronectin type II repeats (MMP-2 and -9), and an N-terminal signal anchor, a cysteine array and an Ig-like domain (MMP-23).

for MMP function. Both gelatinases (MMP-2 and -9) have a fibronectin-like domain consisting of three tandem copies of a fibronectin type II-like module. This domain has been shown to bind denatured type IV and V collagens, elastin, and type-I collagen thereby contributing to substrate specificity (8).

A group of six cell membrane-associated MMPs has also been identified, known as the membrane-type MMPs (MT-MMPs). The MT-MMPs can be divided into two subgroups, the transmembrane type (MT-MMP-1, -2, -3, -5) and the glycosylphosphatidyl inositol (GPI)-anchored forms (MT-MMP-4 and -6) (9–13). Like most MMPs, the GPI-anchored type are sensitive to all members of the TIMP-family, but the transmembrane MT-MMPs are inhibited by TIMP-2, -3, -4, but are relatively insensitive to TIMP-1 inhibition (10, 14, 15). The cytoplasmic tail of the transmembrane MT-MMPs interacts with intracellular proteins that regulate the subcellular trafficking of the enzymes from the Golgi to the cell surface, and to specific membrane domains, for instance to protruding structures called ‘invadopodia’ in invasive cancer cells (4, 16).

3. Regulation of MMP Activity

The activity of MMPs can be regulated by various mechanisms — gene transcription, mRNA stability, translational control, cell compartmentalisation, zymogen activation via proteolysis, and inhibition by endogenous inhibitors.

MMP activation is often the result of a complex proteinase cascade. Certain MMPs, including MMP-1, MMP-3, MMP-7, MMP-8, MMP-9 and MMP-10, can be cleaved in their propeptide domain, at least *in vitro*, by serine proteinases such as the uPA–plasmin system and trypsin (17, 18). In turn, some of these activated MMPs can then go on to activate other proMMPs, e.g. MMP-3, which can activate proMMP-1 and proMMP-9 (19, 20). MT1-MMP can activate proMMP-13 (21) and this activated MMP-13 can then go on to activate MMP-9 (22). A subset of MMPs is activated primarily intracellularly by serine proteases of the pro-protein convertase class such as furin (23). This includes MMP-11, MMP-21, MMP-23 and MMP-28 (24, 25) as well as the MT-MMPs (13, 26–29).

ProMMP-2 has a unique cell-mediated activation mechanism, which is illustrated in Fig. 2. Activation of proMMP-2 by MT1-MMP has been the most extensively studied mechanism and is used as the basis for the

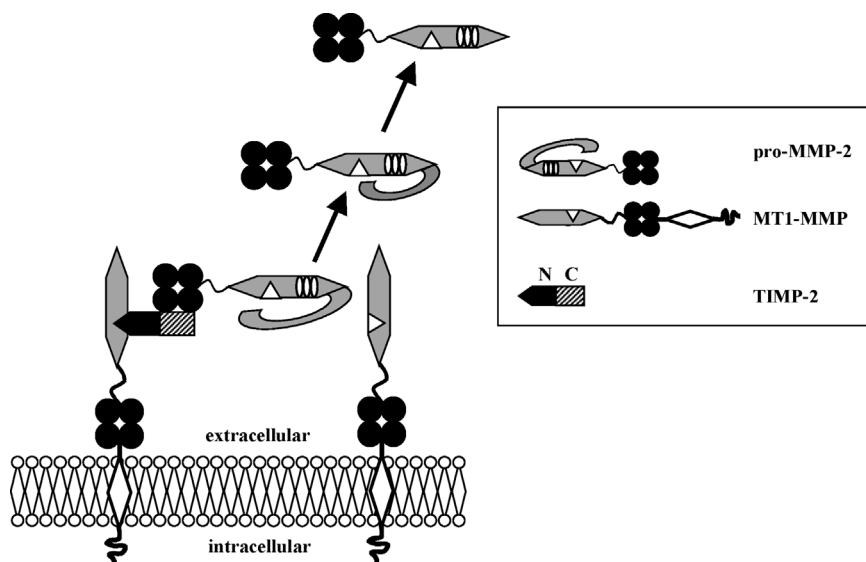


Fig. 2. Cell surface model of proMMP-2 activation. MT1-MMP on the cell surface acts as a receptor for TIMP-2, which binds via its *N*-terminal domain to the active site of the MT-MMP. This binary complex acts as a receptor for pro-MMP-2, the TIMP-2 *C*-terminal domain binding to the *C*-terminal domain of pro-MMP-2. A free MT1-MMP molecule in close proximity can then cleave the propeptide of pro-MMP-2 generating an intermediate species. Further proteolysis of the propeptide through an autocatalytic mechanism results in the generation of the fully active enzyme.

current model, in which MT1-MMP acts as a receptor for TIMP-2, which binds via its *N*-terminal domain to the active site of the MT-MMP. This binary complex can then act as a receptor for proMMP-2, the TIMP-2 *C*-terminal domain binding to the *C*-terminal domain of proMMP-2 (30). A free MT1-MMP molecule, positioned in close proximity via the interaction of the hemopexin domains of the two MT1-MMPs (31), can then cleave the propeptide of proMMP-2 at the N37-L38 bond, generating an intermediate MMP-2 species. Further autocatalytic proteolysis of the intermediate MMP-2 generates the fully active enzyme (32). Activation of proMMP-2 in this model requires TIMP-2 to be at a level that is adequate for the generation of the tri-molecular complex, but not high enough to saturate all the MT1-MMPs. High levels of TIMP-2 (as well as TIMP-3 and -4) will inhibit all MT1-MMP activity, preventing proMMP-2 activation (33). However, if no TIMP-2 is present, MT1-MMP undergoes autocatalytic processing to a 45 kDa inactive form (34). Certain synthetic MMP inhibitors have been

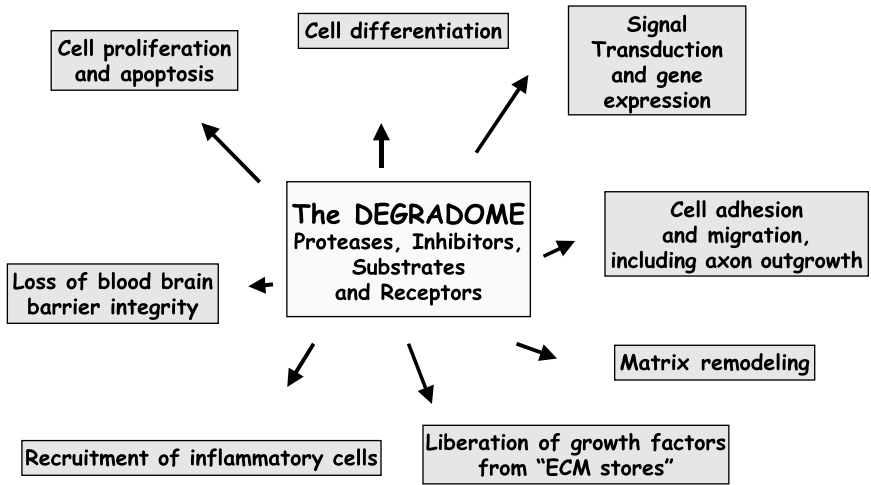


Fig. 3. The Biology of the CNS Degradome MMPs and other metalloproteases play a number of roles (which will interact and/or overlap with each other) in the CNS both in normal and pathological conditions.

shown to stabilize ‘active’ MT1-MMP on the cell surface, thereby potentiating proMMP-2 activation (35). MT2-MMP-mediated proMMP-2 activation does not require TIMP-2 and so appears to function via a different mechanism than that described for MT1-MMP (36).

The transmembrane MT-MMPs (MT1-, MT2-, MT3- and MT5-) are all able to activate proMMP-2 *in vitro* (37–40). However, MT4-MMP is without activity in this respect (26) and MT6-MMP is at best poorly active (10). The formation of homophilic complexes of MT1-MMP molecules at the plasma membrane is necessary for efficient activation of proMMP-2, as well as for MT1-MMP’s invasion promoting properties. Complex formation involves the interaction between the hemopexin domains and also between the cytoplasmic domains of the MT1-MMP molecules (31, 41).

4. Endogenous Inhibitors of Metalloproteinases

There are four vertebrate TIMPs which can influence the degradome (Fig. 3) (42). TIMP-1, -2, and -4 are all diffusible, secreted proteins while TIMP-3 is matrix-associated (15, 43). The TIMPs share a basic structural arrangement consisting of 12 conserved cysteine residues paired into 6 disulphide bonds forming 6 peptide loops and two knots. This common structure can be divided into 2 discrete domains, the N-terminal domain, primarily

responsible for its MMP inhibitory activity, and the C-terminal domain. The C-terminal domain is more variable in sequence than the N-terminal domain between the 4 TIMPs and may in part be responsible for the distinct properties of the 4 TIMPs (44).

While α_2 -macroglobulin is the principal endogenous MMP-inhibitor in the circulation, the TIMPs are the primary tissue inhibitors of MMPs and as such their primary role is to limit proteolysis during ECM remodeling (15). The TIMPs can inhibit most MMPs without major selectivity between them (with the exception that TIMP-1 is a very poor inhibitor of MT1-, MT2-, MT3-, MT5-MMP and MMP-19) (32). However, TIMPs differ in other properties such as tissue distribution, transcriptional regulation, and specific association with latent MMPs (i.e. TIMP-1/proMMP-9 and TIMP-2/proMMP-2). These differences suggest that they each have separate and specific physiological roles. TIMPs bind noncovalently the activated MMPs in a 1:1 stoichiometry, with inhibition constants in the subnanomolar range (45), forming an extended wedge structure with contacts in both the catalytic and hemopexin domains of the protease. The N-terminal cysteine of the mature TIMP molecule is essential for coordination with the active site Zn^{2+} in the MMP, resulting in inhibition. TIMPs can also be inactivated by a variety of proteinases such as neutrophil elastase and trypsin but are mainly regulated at the level of gene expression (46).

“Reversion-inducing cysteine-rich protein with kazal motifs” (RECK) is a recently discovered endogenous cell surface glycoprotein that inhibits the catalytic activities of MMP-9 (47), MMP-2 and MT1-MMP (48). RECK does not bear any structural resemblance to the TIMPs and apparently acts differently to the TIMPs, leading to the suppression of MMP-9 expression as well as inhibition of pro-MMP-2 activation. RECK expression has been detected in a wide range of tissues and loss of RECK expression has been linked to cancer progression (47). Other molecules that have been suggested as possible MMP inhibitory molecules include thrombospondins-1 (49) and -2, and proteins that resemble the N-terminal structure of the TIMPs, including netrin, secreted frizzled-related proteins, type I collagen C-proteinase enhancer protein (PCPE), and the serine proteinase tissue factor pathway inhibitor-2 (TFPI-2) [reviewed in Baker *et al.*, 2002] (42).

TIMPs also possess properties independent of their MMP inhibitory activity, including the ability of both TIMP-1 and TIMP-2 to promote growth in a variety of cell types (50). TIMP-1 proteins that have been engineered to lack anti-proteolytic activity retain growth-stimulating activity, indicating that the anti-proteolytic and growth factor activities of TIMP-1

are separable (51). Overexpression of TIMP-3 (but not TIMP-1 or TIMP-2) by adenoviral delivery induces apoptosis in smooth muscle cells and cancer cell lines (52–54). However, it is probable that these pro-apoptotic effects of TIMP-3 relate to the selective ability of this TIMP to inhibit another family of cell surface metalloproteinases called the adamalysins, or ADAMs (for a disintegrin and metalloproteinase). The ADAM metalloproteinases are involved both in cell adhesion and proteolysis, in particular, ADAM-17 acts as an “ectodomain sheddase” of various receptors and adhesion molecules as well as processing membrane-bound pro-TNF- α and pro-TGF- α liberating the active ligand (55). Several pro-apoptotic ligands and receptors are substrates of ADAM-17, thus its inhibition by TIMP-3 can potentiate the actions of apoptosis-inducing stimuli (56). The ADAMs and their close relatives, the ADAMTS metalloproteinases (a disintegrin and metalloproteinase with thrombospondin motifs) (57), are outside the scope of this current review, but they are likely to be as important as the MMPs in the development and disease of the CNS.

5. Novel Roles for MMPs

Whilst many ECM substrates have been identified for MMPs, interest has shifted to potential roles in the generation of matricryptic sites within ECM substrates, sometimes generating for example gradients of motogenic factors as well as liberating growth factors from the matrix or in some cases from the cell surface. Here we will also consider data obtained in other systems which may reveal ways in which novel roles for MMPs in the CNS could be explored (some of which are discussed in later chapters).

6. ECM Targets in the CNS

ECM components in the CNS include proteoglycans, laminins, tenascins and to a certain extent collagens, although these proteins are less abundant than in the PNS under normal conditions. Following injury to the CNS and subsequent formation of the glial scar, the production of several ECM molecules, including collagens, is up-regulated (58). The major recent focus for ECM study in the glial scar has, however been the proteoglycans, including brevican, versican and neurocan (59). These chondroitin sulphate PGs (CS-PGs) have been shown to be inhibitory for axon outgrowth *in vitro* and for the regeneration of severed axons in lesioned CNS *in vivo*. Degradation of CS side chains with bacterial chondroitinase ABC

relieves this inhibition (60). In the CNS, this type of role for MMPs has not been extensively explored but in the PNS degradation of CSPGs by MMP-2 enhances axon outgrowth (61). However MMP-9 has been strongly implicated in oligodendrocyte outgrowth (62) although the mechanism involved remains to be elucidated.

Nidogen has been shown to be critical to normal CNS function (63) and deletion of the nidogen-binding domain of the laminin gamma 1 chain results in disrupted cortical histogenesis (64). It is unknown however whether nidogen degradation plays a role in normal or pathological CNS development/repair although it has long been established that nidogen-1 is a substrate for many MMPs (65).

7. Generation of Matricryptic Sites in ECM Components

Although degradation of the ECM by MMPs has been extensively studied, the cell biological consequences of this process have not often been investigated in detail. However, some information has been obtained in systems other than the CNS which will be explored briefly here. One of the most abundant ECM components in the body, type I collagen, when degraded by collagenolytic MMPs into classic 3/4 and 1/4 fragments exposes multiple RGD sites thus enabling cells to adhere through for example alpha v beta 3 integrins. This can alter radically the ability of, for instance vascular smooth muscle cells, to migrate in response to motogens such as PDGF-BB (66). It would be of interest in the future to establish whether collagen degradation occurs in the permissive environment of the PNS but not in the glial scar of the CNS. A less abundant but extremely important ECM component, laminin-5, also contains matricryptic sites, which are revealed following cleavage with MT1-MMP resulting in enhanced tumour cell migration (67). Laminins are important ECM components for neurite outgrowth *in vitro* and axon outgrowth *in vivo* but as yet roles for MMPs in generation of bioactive matricryptic sites has not been demonstrated. However, Costa *et al* (68) have shown that in neuron-astrocyte co-cultures laminin plays a key role in neuronal migration and MMP-2 is concomitantly synthesised.

8. Growth Factor Liberation from ECM “Stores”

It is well-established in a number of systems that growth factors bound to the ECM can be liberated from these “stores” following degradation by MMPs. For example, MMP-9 degrades ECM to release VEGF and thus

influences ossification through modulation of angiogenesis in developing bone (69). In the CNS, however, whilst there are many reports of growth factor ECM association as yet roles for MMPs in the liberation of growth factors remains little investigated.

9. Chemokine Gradient Generation

Whilst chemokines have been implicated in the recruitment of leukocytes across the blood brain barrier (BBB) in inflammatory conditions within the CNS, the role for MMPs in the generation of a chemokine gradient has not been explored. MMP-7 has been shown to play a key role in the generation of soluble syndecan-1 from lung alveolar epithelial cells (70). The chemokine IL8 binds to syndecan-1 and MMP-7 was shown to be critical in the generation of an IL8 gradient important in recruiting leukocytes to the alveolar space. As yet it is not established whether MMPs could play such a role in the CNS but it is established that syndecans play a role in transendothelial migration of monocytes across the brain endothelium (71).

Another important way that MMPs may act to blunt the immune response is through the direct cleavage and inactivation of the CC class of chemokines. McQuibban *et al* (72) demonstrated that certain MMPs could cleave MCPs 1-4, showing specificity depending on the substrate and enzyme. Of great interest is the fact that once cleaved by MMPs, MCP2, -3 and -4 were able to block monocyte migration *in vitro* and inflammation *in vivo*.

10. Blood-Brain Barrier Substrates for MMPs

Whilst MMPs have been implicated in penetration of the BBB for some years, it is only recently that the nature of relevant substrates for MMPs has been explored. Wachtel *et al* (73) and Lohmann *et al* (74) have provided evidence that when loss of BBB integrity is induced by tyrosine phosphatase inhibition, the tight junction protein, occludin, is degraded by MMP(s) in an *in vitro* model of the BBB. The identity of the MMP(s) involved remains to be determined.

11. Conclusion

Metalloprotease research in the CNS is still in its infancy although MMPs have been a growing focus of research in this area over the past 10 years,

revealing roles in many aspects of CNS biology. Future studies will witness discovery of roles for ADAM and ADAMTS proteases whose expression and function in the nervous system is only beginning to emerge.

References

1. Gross, J., and Lapiere, C. M. (1962) *Proc Natl Acad Sci* 48, 1014–1022
2. Brinckerhoff, C. E., and Matrisian, L. M. (2002) *Nat Rev Mol Cell Biol* 3, 207–214
3. Puente, X. S., Sanchez, L. M., Overall, C. M., and Lopez-Otin, C. (2003) *Nat Rev Genet* 4, 544–558
4. Egeblad, M., and Werb, Z. (2002) *Nat Rev Cancer* 2, 161–174
5. Becker, J. W., Marcy, A. I., Rokosz, L. L., Axel, M. G., Burbaum, J. J., Fitzgerald, P. M., Cameron, P. M., Esser, C. K., Haggmann, W. K., and Hermes, J. D. (1995) *Protein Sci* 4, 1966–1976
6. Nagase, H., Ogata, Y., Suzuki, K., Enghild, J. J., and Salvesen, G. (1991) *Biochem Soc Trans* 19, 715–718
7. Gohlke, U., Gomis-Ruth, F. X., Crabbe, T., Murphy, G., Docherty, A. J., and Bode, W. (1996) *FEBS Lett* 378, 126–130
8. Steffensen, B., Wallon, U. M., and Overall, C. M. (1995) *J Biol Chem* 270, 11555–11566
9. Itoh, Y., Kajita, M., Kinoh, H., Mori, H., Okada, A., and Seiki, M. J. (1999) *Biol Chem* 274, 34260–34266
10. Kojima, S., Itoh, Y., Matsumoto, S., Masuho, Y., and Seiki, M. (2000) *FEBS Lett* 480, 142–146
11. Will, H., and Hinzmann, B. (1995) *Eur J Biochem* 231, 602–608
12. Takino, T., Sato, H., Shinagawa, A., and Seiki, M. (1995) *J Biol Chem* 270, 23013–23020
13. Pei, D. (1999) *J Biol Chem* 274, 8925–8932
14. Bigg, H. F., Morrison, C. J., Butler, G. S., Bogoyevitch, M. A., Wang, Z., Soloway, P. D., and Overall, C. M. (2001) *Cancer Res* 61, 3610–3618
15. Brew, K., Dinakarpanian, D., and Nagase, H. (2000) *Biochim Biophys Acta* 1477, 267–283
16. Nakahara, H., Howard, L., Thompson, E. W., Sato, H., Seiki, M., Yeh, Y., and Chen, W. T. (1997) *Proc Natl Acad Sci* 94, 7959–7964
17. Okada, Y., Gonoji, Y., Naka, K., Tomita, K., Nakanishi, I., Iwata, K., Yamashita, K., and Hayakawa, T. (1992) *J Biol Chem* 267, 21712–21719
18. Nagase, H., Suzuki, K., Enghild, J. J., and Salvesen, G. (1991) *Biomed Biochim Acta* 50, 749–754
19. Nagase, H., Suzuki, K., Morodomi, T., Enghild, J. J., and Salvesen, G. (1992) *Matrix Suppl* 1, 237–244
20. Ogata, Y., Enghild, J. J., and Nagase, H. (1992) *J Biol Chem* 267, 3581–3584
21. Knauper, V., Bailey, L., Worley, J. R., Soloway, P., Patterson, M. L., and Murphy, G. (2002) *FEBS Lett* 532, 127–130

22. Cowell, S., Knauper, V., Stewart, M. L., d'Ortho, M. P., Stanton, H., Hembry, R. M., Lopez-Otin, C., Reynolds, J. J., and Murphy, G. (1998) *Biochem J* 331, 453–458
23. Pei, D., and Weiss, S. J. (1995) *Nature* 375, 244–247
24. Ahokas, K., Lohi, J., Lohi, H., Elomaa, O., Karjalainen-Lindsberg, M. L., Kere, J., and Saarialho-Kere, U. *Gene* 301, 31–41
25. Illman, S. A., Keski-Oja, J., Parks, W. C., and Lohi, J. (2003) *Biochem J* 375, 191–197
26. English, W. R., Puente, X. S., Freije, J. M., Knauper, V., Amour, A., Merryweather, A., Lopez-Otin, C., and Murphy, G. (2000) *J Biol Chem* 275, 14046–14055
27. Yana, I., and Weiss, S. J. (2000) *Mol Biol Cell* 11, 2387–2401
28. Kang, T., Nagase, H., and Pei, D. (2002) *Cancer Res* 62, 675–681
29. Pei, D. (1999) *Cell Res* 9, 291–303
30. Butler, G. S., Butler, M. J., Atkinson, S. J., Will, H., Tamura, T., van Westrum, S. S., Crabbe, T., Clements, J., d'Ortho, M. P., and Murphy, G. (1998) *J Biol Chem* 273, 871–880
31. Itoh, Y., Takamura, A., Ito, N., Maru, Y., Sato, H., Suenaga, N., Aoki, T., and Seiki, M. (2001) *EMBO J* 20, 4782–4793
32. Will, H., Atkinson, S. J., Butler, G. S., Smith, B., and Murphy, G. (1996) *J Biol Chem* 271, 17119–17123
33. Hernandez-Barrantes, S., Shimura, Y., Soloway, P. D., Sang, Q. A., and Fridman, R. (2001) *Biochem Biophys Res Commun* 281, 126–130
34. Hernandez-Barrantes, S., Toth, M., Bernardo, M. M., Yurkova, M., Gervasi, D. C., Raz, Y., Sang, Q. A., and Fridman, R. (2000) *J Biol Chem* 275, 12080–12089
35. Toth, M., Bernardo, M. M., Gervasi, D. C., Soloway, P. D., Wang, Z., Bigg, H. F., Overall, C. M., DeClerck, Y. A., Tschesche, H., Cher, M. L., Brown, S., Mobashery, S., and Fridman, R. (2000) *J Biol Chem* 275, 41415–41423
36. Morrison, C. J., Butler, G. S., Bigg, H. F., Roberts, C. R., Soloway, P. D., and Overall, C. M. (2001) *J Biol Chem* 276, 47402–47410
37. Imai, K., Ohuchi, E., Aoki, T., Nomura, H., Fujii, Y., Sato, H., Seiki, M., and Okada, Y. (1996) *Cancer Res* 56, 2707–2710
38. Kinoshita, T., Sato, H., Takino, T., Itoh, M., Akizawa, T., and Seiki, M. (1996) *Cancer Res* 56, 2535–2538
39. Butler, G. S., Will, H., Atkinson, S. J., and Murphy, G. (1997) *Eur J Biochem* 244, 653–657
40. Shofuda, K., Yasumitsu, H., Nishihashi, A., Miki, K., and Miyazaki, K. (1997) *J Biol Chem* 272, 9749–9754
41. Lehti, K., Valtanen, H., Wickstrom, S., Lohi, J., and Keski-Oja, J. (2000) *J Biol Chem* 275, 15006–15013
42. Baker, A. H., Edwards, D. R., and Murphy, G. (2002) *J Cell Sci* 115, 3719–3727
43. Leco, K. J., Khokha, R., Pavloff, N., Hawkes, S. P., and Edwards, D. R. (1994) *J Biol Chem* 269, 9352–9360

44. Gomis-Ruth, F. X., Maskos, K., Betz, M., Bergner, A., Huber, R., Suzuki, K., Yoshida, N., Nagase, H., Brew, K., Bourenkov, G. P., Bartunik, H., and Bode, W. (1997) *Nature* 389, 77–81
45. Bode, W., Fernandez-Catalan, C., Grams, F., Gomis-Ruth, F. X., Nagase, H., Tschesche, H., and Maskos, K. (1999) *Acad Sci* 878, 73–91
46. Okada, Y., Watanabe, S., Nakanishi, I., Kishi, J., Hayakawa, T., Watorek, W., Travis, J., and Nagase, H. (1988) *FEBS Lett* 229, 157–160
47. Takahashi, C., Sheng, Z., Horan, T. P., Kitayama, H., Maki, M., Hitomi, K., Kitaura, Y., Takai, S., Sasahara, R. M., Horimoto, A., Ikawa, Y., Ratzkin, B. J., Arakawa, T., and Noda, M. (1998) *Proc Natl Acad Sci* 95, 13221–13226
48. Oh, J., Takahashi, R., Kondo, S., Mizoguchi, A., Adachi, E., Sasahara, R. M., Nishimura, S., Imamura, Y., Kitayama, H., Alexander, D. B., Ide, C., Horan, T. P., Arakawa, T., Yoshida, H., Nishikawa, S., Itoh, Y., Seiki, M., Itohara, S., Takahashi, C., and Noda, M. (2001) *Cell* 107, 789–800
49. Rodriguez-Manzaneeque, J. C., Lane, T. F., Ortega, M. A., Hynes, R. O., Lawler, J., and Iruela-Arispe, M. L. (2001) *Proc Natl Acad Sci* 98, 12485–12490
50. Jiang, Y., Goldberg, I. D., and Shi, Y. E. (2002) *Oncogene* 21, 2245–2252
51. Chesler, L., Golde, D. W., Bersch, N., and Johnson, M. D. (1995) *Blood* 86, 4506–4515
52. Baker, A. H., Zaltsman, A. B., George, S. J., and Newby, A. C. (1998) *J Clin Invest* 101, 1478–1487
53. Smith, M. R., Kung, H., Durum, S. K., Colburn, N. H., and Sun, Y. (1997) *Cytokine* 9, 770–780
54. Ahonen, M., Poukkula, M., Baker, A. H., Kashiwagi, M., Nagase, H., Eriksson, J. E., and Kahari, V. M. (2003) *Oncogene* 22, 2121–2134
55. Itai, T., Tanaka, M., and Nagata, S. (2001) *Eur J Biochem* 268, 2074–2082
56. Edwards, D. R. (2004) *J Pathol* 202, 391–394
57. Apte, S. S. (2004) *Int J Biochem Cell Biol* 36, 981–985
58. Hermanns, S., Reiprich, P., and Muller, H. W. (2001) *J Neurosci Methods* 110, 141–146
59. Rhodes, K. E., and Fawcett, J. W. (2004) *J Anat* 204, 33–48
60. Bradbury, E. J., Moon, L. D., Popat, R. J., King, V. R., Bennett, G. S., Patel, P. N., Fawcett, J. W., and McMahon, S. B. (2002) *Nature* 416, 636–640
61. Ferguson, T. A., and Muir, D. (2000) *Mol Cell Neurosci* 16, 157–167
62. Ohu, L. Y., Larsen, P. H., Krekoski, C. A., Edwards, D. R., Donovan, F., Werb, Z., and Yong, V. W. (1999) *J Neurosci* 19, 8464–8475
63. Dong, L., Chen, Y., Lewis, M., Hsieh, J. C., Reing, J., Chaillet, J. R., Howell, C. Y., Melhem, M., Inoue, S., Kuszak, J. R., DeGeest, K., and Chung, A. E. (2002) *Lab Invest* 82, 1617–1630
64. Halfter, W., Dong, S., Yip, Y. P., Willem, M., and Mayer, U. (2002) *J Neurosci* 22, 6029–6040
65. Mayer, U., Mann, K., Timpl, R., and Murphy, G. (1993) *Eur J Biochem* 217, 877–884

66. Stringa, E., Knauper, V., Murphy, G., and Gavrilovic, J. (2000) *J Cell Sci* 113, 2055–2064
67. Gilles, C., Polette, M., Coraux, C., Tournier, J. M., Meneguzzi, G., Munaut, C., Volders, L., Rousselle, P., Birembaut, P., and Foidart, J. M. (2001) *J Cell Sci* 114, 2967–2976
68. Costa, S., Planchenault, T., Charriere-Bertrand, C., Mouchel, Y., Fages, C., Juliano, S., Lefrancois, T., Barlovatz-Meimon, G., and Tardy, M. (2002) *Glia* 37, 105–113
69. Vu, T. H., Shipley, J. M., Bergers, G., Berger, J. E., Helms, J. A., Hanahan, D., Shapiro, S. D., Senior, R. M., and Werb, Z. (1998) *Cell* 93, 411–422
70. Li, Q., Park, P. W., Wilson, C. L., and Parks, W. C. (2002) *Cell* 111, 635–646
71. Floris, S., van den, B. J., van der Pol, S. M., Dijkstra, C. D., and De Vries, H. E. (2003) *J Neuropathol Exp Neurol* 62, 780–790
72. McQuibban, G. A., Gong, J. H., Wong, J. P., Wallace, J. L., Clark-Lewis, I., and Overall, C. M. (2002) *Blood* 100, 1160–1167
73. Wachtel, M., Frei, K., Ehler, E., Fontana, A., Winterhalter, K., and Gloor, S. M. (1999) *J Cell Sci* 112, 4347–4356
74. Lohmann, C., Krischke, M., Wegener, J., and Galla, H. J. (2004) *Brain Res* 995, 184–196