

METALLOPROTEINASES IN BIOLOGY AND PATHOLOGY OF THE NERVOUS SYSTEM

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Matrix metalloproteinases (MMPs) have been implicated in several diseases of the nervous system. Here we review the evidence that supports this idea and discuss the possible mechanisms of MMP action. We then consider some of the beneficial functions of MMPs during neural development and speculate on their roles in repair after brain injury. We also introduce a family of proteins known as ADAMs (a disintegrin and metalloproteinase), as some of the properties previously ascribed to MMPs are possibly the result of ADAM activity.

DISINTEGRINS

Peptides found in the venoms of various snakes that inhibit the function of integrins of the $\beta 1$ and $\beta 3$ classes.

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Extracellular proteases are crucial regulators of cell function. The family of matrix metalloproteinases (MMPs) has classically been described in the context of extracellular matrix (ECM) remodelling, which occurs throughout life in diverse processes that range from tissue morphogenesis to wound healing. Recent evidence has implicated MMPs in the regulation of other functions, including survival, angiogenesis, inflammation and signalling. There are at least 25 members of the MMP family and, collectively, these proteases can degrade all constituents of the ECM. As a result of their potent proteolytic activity, abnormal MMP function can also lead to pathological conditions. The most widely studied disease that involves MMPs is cancer metastasis. In this case, the tumour cell is thought to use MMPs to overcome multiple structural barriers and establish a new focus of growth at a distant site from the primary tumour mass. In the nervous system, MMPs have also been associated with pathogenesis, particularly in **multiple sclerosis** (MS) and malignant gliomas¹. A growing literature has linked MMPs to stroke, to **Alzheimer's disease** and to viral infections of the central nervous system (CNS). The goal of this review is to acquaint the reader with the biology of MMPs, particularly the functions of MMPs that are not associated with matrix turnover. We will highlight the roles of MMPs in pathology of the nervous system, and emphasize the fact that MMPs can also have physiological functions during CNS repair and

ontogeny. Last, we will discuss the function of another group of metalloproteinases — ADAMs (a **DISINTEGRIN** and metalloproteinase) — in CNS pathophysiology, as these proteins might be responsible for many of the activities previously ascribed to MMPs.

Metalloproteinases — the cast MMPs are part of a larger family of structurally related zinc-dependent metalloproteinases called metzincins. Other subfamilies of the metzincins are ADAMs, bacterial serralysins and the astacins. Metzincins use three histidine (H) residues to bind the zinc ion at their active site. These residues occur in the conserved sequence motif HExxHxxGxxHZ, where Z is a family-specific residue — serine in most MMPs, aspartate in ADAMs, proline in serralysins and glutamate in astacins. In addition, there is a distinct β -turn at the active site, which is delineated by a methionine residue ('met-turn') and seems to be essential for activity. There is about 20% similarity between metzincin subfamilies², but identity at the catalytic domain is much higher.

On the basis of substrate preference and protein-domain considerations, MMP family members (TABLE 1) have been categorized into subgroups that include gelatinases, stromelysins, collagenases, membrane-type (MT)-MMPs and 'other MMPs'. However, there is much overlap in substrate specificity between subgroups. Structurally, MMPs are divided into three

HEMOPEXIN
Serum glycoprotein that binds haem and transports it to the liver for breakdown and iron recovery.

domains (FIG. 1): an amino-terminal propeptide region, an amino-terminal catalytic domain (which contains the zinc-binding motif) and a carboxy-terminal domain, which has a high level of similarity to members of the HEMOPEXIN family and is involved in ECM substrate binding for many MMPs. The carboxy-terminal hemopexin-like domain is a four-bladed β -propeller structure that is present in all MMPs except MMP7 and MMP26 (matrilysin and matrilysin-2). A hinge region, which is short in collagenases and long in other MMPs, connects the carboxy- and amino-terminal domains. A short signal sequence ('pre-domain') lies at the amino-terminal end of the protein, before the propeptide region, and is clipped off as newly synthesized MMPs travel to the cell surface. Last, unlike other MMP groups, the six MT-MMPs are membrane proteins. MT4-MMP (MMP17) and MT6-MMP (MMP25) are glycosyl-phosphatidylinositol (GPI)-anchored MMPs (REF. 3), whereas the other MT-MMPs are transmembrane proteins.

The ADAM family of metalloproteinases is also referred to as MDC (metalloproteinase, disintegrin, cysteine-rich) proteins. Biological roles for ADAM members⁴ include specialized functions in cell adhe-

sion, sperm-egg fusion (for example, ADAM1 and -2), myoblast fusion (ADAM12) and the ectodomain shedding of cell-surface proteins (for example, ADAM9, -10 and -17). Most ADAMs have a prodomain, a metalloprotease region, a disintegrin domain for adhesion, a cysteine-rich region, epidermal-growth-factor (EGF) repeats, a transmembrane module and a cytoplasmic tail (FIG. 1). So, ADAMs are unique among cell-surface proteins in having both adhesive and proteolytic activities. The cysteine-rich region and EGF repeats are thought to mediate cell fusion or the interaction of ADAMs with other molecules such as chaperone proteins. The cytoplasmic tails of ADAM9 and -12 interact with protein kinase C and with src, respectively, implicating some ADAM family members as signalling molecules^{5,6}. Although most ADAMs are transmembrane proteins, some members (for example, ADAM11, -12, -17 and -28) also have alternatively spliced forms that diverge before the transmembrane module to generate a soluble, secreted protein. It is important to note that only about half of the ADAM family members have a metalloproteinase domain that contains the catalytic site consensus sequence, indicating that, in ADAMs

Table 1 | Matrix metalloproteinase family members

Member	Name	M latent/ active (kDa)	Furin activation site	Collagen substrates	Pro-MMP substrates	Other matrix substrates*
MMP1	Collagenase 1	55/45		I, II, III, VII, VIII, X	2, 9	Agg, Gel, PG
MMP2	Gelatinase A	72/66		I, III, IV, V, VII, X, XI, XIV	1, 9, 13	Agg, EL, FN, Gel, LN, PG, VN
MMP3	Stromelysin 1	57/45		III, IV, IX, X, XI	1, 7, 8, 9, 13	Agg, EL, FN, Gel, LN, PG, VN
MMP7	Matrilysin	28/19		IV, X	1, 2, 9	Agg, Casein, EL, FN, Gel, LN, PG, VN
MMP8	Collagenase 2	75/58		I, II, III, V, VII, VIII, X		Agg, EL, FN, Gel, LN,
MMP9	Gelatinase B	92/86		IV, V, VII, X, XIV		Agg, EL, FN, Gel, PG, VN
MMP10	Stromelysin 2	57/44		III, IV, V, IX, X	1, 8	Agg, EL, FN, Gel, LN, PG
MMP11	Stromelysin 3	51/44	Yes			
MMP12	Metalloelastase	54/45/22 [†]		IV		Casein, EL, FN, Gel, LN, PG, VN
MMP13	Collagenase 3	60/48		I, II, III, IV, VII, IX, X, XIV	9	Agg, FN, Gel
MMP14	MT1-MMP	66/56	Yes	I, II, III	2, 13	Agg, EL, FN, Gel, LN,
MMP15	MT2-MMP	72/60	Yes		2	Agg, FN, Gel, LN
MMP16	MT3-MMP	64/52	Yes	III	2	Gel, FN
MMP17	MT4-MMP	57/53	Yes			Fibrinogen/fibrin
MMP18	Collagenase 4	70/53		I		
MMP19	RAS I 1	54/45		IV		Gel, FN, LN
MMP20	Enamelysin	54/22				Amelogenin
MMP21	Xenopus MMP	70/53	Yes			
MMP22	Chick embryo MMP	51/42				Casein, Gel
MMP23			Yes			
MMP24	MT5-MMP		Yes		2	Gel
MMP25	MT6-MMP		Yes	IV		Gel, FN
MMP26	Matrilysin 2/endometase	28/19				Gel
MMP27	Human MMP22 [§]					
MMP28	Epilysin	56/45	Yes			Casein

MMP4, MMP5 and MMP6 were found to be identical to other MMPs, and are not listed according to this nomenclature. *The list of substrates, collated from REFS 15,56,100, is by no means exhaustive and emphasizes those from the extracellular matrix. [†]The activation of MMP12 involves the removal of the propeptide region to produce an intermediate active form (45 kDa), followed by atypical carboxy-terminal processing to produce the fully active enzyme (22 kDa). [§]MMP27 is the human orthologue of chick embryo MMP22. M, molecular mass; MMP, matrix metalloproteinase; Agg, aggrecan; Gel, gelatin; PG, proteoglycan link protein; EL, elastin; FN, fibronectin; LN, laminin; VN, vitronectin; MT, membrane type; RAS I 1, rheumatoid arthritis synovial inflammation 1.

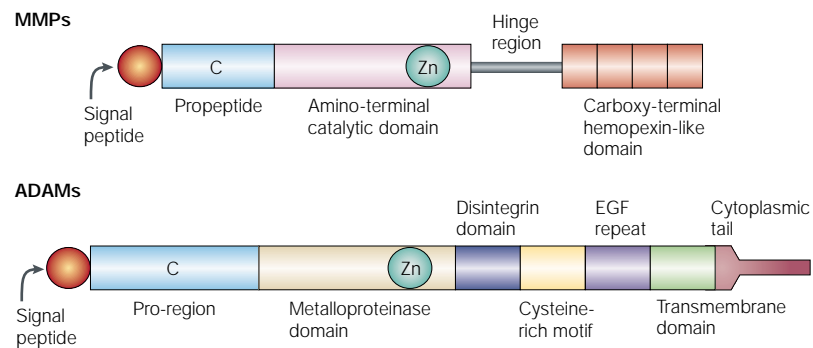


Figure 1 | Domain structure of MMPs and ADAMs. The generic structure of matrix metalloproteinases (MMPs) shown here describes most MMP family members. Note that gelatinases (MMP2 and -9) have a unique fibronectin type II-like domain inserted into the catalytic site, whereas MT-MMPs have a transmembrane domain at the carboxy terminus. Matrilysin (MMP7) lacks the hinge and the carboxyl terminus. The hemopexin-like module of MMPs contains four repeat units; the first and fourth are connected by a disulphide bridge. The 'C' at the propeptide region denotes the cysteine residue that ligates the zinc in the catalytic domain to keep the enzyme inactive. For ADAMs (a disintegrin and metalloproteinase), the core structure of most members is also depicted. Most ADAMs are integral membrane proteins owing to the presence of the transmembrane domain. However, alternative splicing generates a secreted form of some ADAMs. It should be noted that some ADAMs (ADAM2, -7, -11, -14, -18, -22 and -29) lack the intact zinc-binding site and, furthermore, that the metalloproteinase domain is not retained in several mature proteins of this family (for example, ADAM1 and -2). These proteins are therefore not considered true degradative enzymes. Last, the ADAMTS proteins are ADAMs that contain one or more thrombospondin type I motifs at the carboxyl terminus. They are also distinguished from ADAMs by the lack of epidermal-growth-factor (EGF)-like, transmembrane and cytoplasmic domains.

importance in the CNS: ADAM10 (kuzbanian) and ADAM17, also known as TACE (tumour-necrosis-factor- α -converting enzyme).

Regulation of metalloproteinase activity

The activity of metalloproteinases is tightly regulated, as these molecules are potent proteolytic enzymes that are capable of widespread destruction. Their first regulatory step is at the level of transcription, as most MMPs are not constitutively expressed but are transcribed after cell activation. Transcription of many MMPs is promoted by inflammatory cytokines, growth factors, CHEMOKINES, oncogenes and cell–cell or cell–matrix interactions¹².

Post-translational modifications provide a second level of MMP regulation. Many MMPs are expressed as inactive ZYMOGENS in which the cysteine residue at the propeptide region binds the zinc ion present at the catalytic site. Activating factors include the plasminogen-plasmin cascade, as well as other MMPs (TABLE 1) that disrupt the interaction between cysteine and zinc (the so-called 'cysteine switch' mechanism) and then remove the propeptide region for full activation¹³. Non-proteolytic compounds such as sulphhydryl-reactive agents (4-aminophenylmercuric acetate) and denaturants (urea) can also activate zymogens. A subset of MMPs contains a cleavage site for FURIN-like prohormone convertases between the propeptide and catalytic domains; this subset includes the MT-MMPs, which are activated during secretion and appear on the cell surface in the active form.

A third means to control MMP activity is by the interaction of active MMPs with tissue inhibitors of metalloproteinases (TIMPs; see REFS 14,15 for comprehensive reviews). Four TIMPs are now known and they cause inactivation by binding to the catalytic site of MMPs. Interestingly, TIMPs are also required for the activation of some MMPs. In this regard, a complex formed by TIMP2 and the carboxyl terminus of pro-MMP2 has been found to bind MT1-MMP (MMP14) on the cell surface. An adjacent MT1-MMP molecule then removes the propeptide region of MMP2 (REF. 16). The pro-MMP-TIMP-MT-MMP trimolecular complex highlights another feature of MMP regulation: the active proteinase is found focally in the pericellular region, rather than being diffusely distributed. In addition, other means also exist to localize MMP activity to the pericellular region. For example, activated MMP2 can bind the α v β 3 integrin, whereas active MMP9 can interact with the HYALURONAN receptor CD44 on the cell membrane¹⁷.

In the case of ADAMs, less is known about the regulation of their activity. Several ADAMs are kept in the inactive state through the interaction of a cysteine residue at the propeptide domain with zinc in the metalloproteinase module. So, as with MMPs, these ADAMs might be activated by the cysteine switch mechanism that disrupts the cysteine–zinc interaction to expose the catalytic site. ADAMs might also be regulated at the transcriptional level. For example, interleukin-1 (IL-1) can induce transcription of some ADAM members. Little is known of the physiological

that lack metalloproteinase activity, this domain might fill another purpose, such as allowing protein–protein interactions. The expression of six ADAMs (ADAM9, -10, -12, -15, 17 and -19) is widespread. By contrast, some ADAMs that are predicted to have metalloproteinase activity are expressed in a testis-specific fashion, and ADAM8 and -28 are predominantly haematopoietic. Recently, new members of the ADAM family have been identified⁷. They have THROMBOSPONDIN motifs and are therefore known as ADAMTSs. All ADAMTSs are secreted proteins. At present, at least 30 ADAMs and 10 ADAMTSs have been described.

The literature on the role of ADAMs in the CNS is still sparse. For example, an early description of ADAM10 in the CNS implicated this protein in oligodendrocyte and myelin biology⁸, although the molecule involved was not recognized as an ADAM until later. But despite the limited evidence, ADAMs might be important proteins in the nervous system. At least 17 ADAMs are found in the CNS (REF. 9) and, in fact, some of these are expressed predominantly in the brain (for example, ADAM22 and -23). Furthermore, many pharmacological studies on the role of MMPs have failed to discriminate between MMP or ADAM functions. In particular, many of the inhibitors based on the functional group hydroxamate, which chelate the zinc ion from the active site of MMPs, also have activity against ADAMs (REFS 10,11). Last, the thrombospondin motif of ADAMTSs confers degradative activity on several proteoglycans that are enriched in the CNS. Here, we will centre our discussion on two ADAMs, as several lines of evidence indicate their

THROMBOSPONDIN
Homotrimeric glycoprotein found in platelets, and in the extracellular matrix of endothelial cells and fibroblasts. It is involved in platelet aggregation.

CHEMOKINES
Small, secreted proteins that stimulate motile behaviour of leukocytes.

ZYMOGEN
Inactive precursor of a proteolytic enzyme that is synthesized and secreted in this form, and then activated by proteolytic cleavage.

FURIN
Endopeptidase with specificity for the consensus sequence Arg-X-Lys/Arg-Arg.

HYALURONAN
Also known as hyaluronate. Polysaccharide of the glycosaminoglycans class highly abundant in the vitreous humour, the synovial fluid of joints and the umbilical cord.

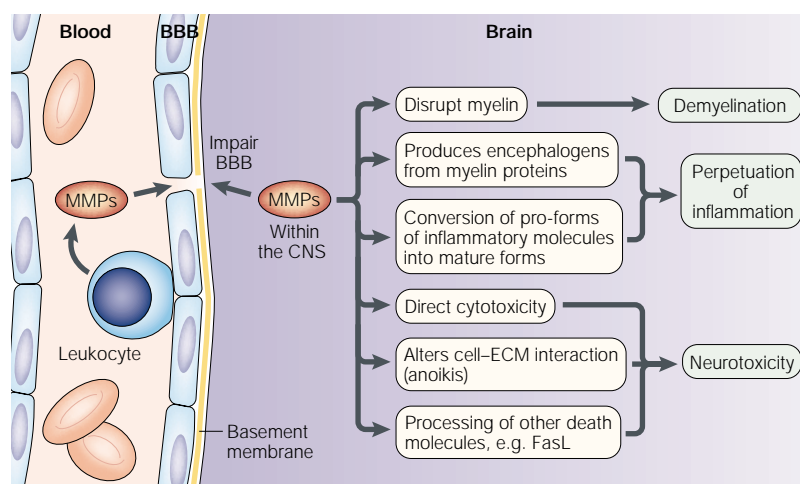


Figure 2 | Mechanisms by which MMPs contribute to neuropathology. Leukocytes express matrix metalloproteinases (MMPs) to facilitate their entry into the central nervous system (CNS). This process disrupts the basement membrane that surrounds the vasculature and results in blood–brain barrier (BBB) impairment. In the central nervous system, high MMP content and its indiscriminate localization result in perpetuation of an inflammatory response, which contributes to demyelination and neuronal or oligodendrocyte death. ECM, extracellular matrix.

inhibitors of ADAMs. The crystal structure of the protease domain of human ADAM17 (TACE) shows that, although the active-site cleft is similar to what is found in MMPs, its secondary structure differs. These features might account for the finding that, whereas **TIMP3** inhibits TACE, **TIMP1** does not. Also, ADAM10 is inhibited by TIMP1 and -3, and by hydroxamates, but not by TIMP2 and -4 (REF. 18). So, TIMP3 might be a more general inhibitor of several ADAM family members, including the ADAMTSs. It is not known if more specific, endogenous non-TIMP ADAM inhibitors exist.

Metalloproteinases and CNS diseases

In the adult CNS, most MMPs are expressed at low or undetectable levels, although there are some exceptions. For example, RNASE PROTECTION ASSAYS have revealed a high constitutive expression of **MMP11** and **-14** in the adult mouse brain¹⁹. Similarly, the more sensitive polymerase chain reaction (PCR) technique has revealed the expression of **MMP2**, **-3**, **-7**, **-9** and **-13** in the normal rat spinal cord²⁰. But, overall, MMPs are largely absent from the normal CNS and their upregulation has been reported in several neurological disorders and after injury. By contrast, in the case of ADAMs, over 17 family members are normally expressed in the adult CNS (REF. 9). For example, ADAM17 has been localized by immunohistochemistry to astrocytes and endothelial cells in adult human brain²¹, whereas ADAM8 has been detected in neurons and oligodendrocytes in the uninjured adult rat CNS (REF. 22). However, the literature on the expression of ADAMs in CNS pathology is limited.

We will divide our discussion of the role of MMPs and ADAMs in pathology into three parts: their role in neuroinflammation and MS, their involvement in malignant gliomas, and their participation in other neurological conditions such as stroke, viral infections and Alzheimer's disease.

Metalloproteinases in neuroinflammation and MS. MS is an immune disorder characterized by demyelination and axonal loss. The presence of proteinases in the cerebrospinal fluid (CSF) of patients with MS has been known for over 20 years; some of these proteinases were recently identified as MMPs. Specifically, MMP9, which is absent in the CSF of normal individuals, is upregulated in MS and in other inflammatory neurological diseases²³. MMP profiles in serum and in leukocytes are also altered in MS. So, when compared to healthy controls, MS patients show increased MMP9 messenger RNA in leukocytes and elevated MMP9 levels in serum. Similarly, the MMP9:TIMP1 ratio in the serum of people with MS is higher than normal²⁴. The serum MMP9 content in MS patients is higher during relapse than in an off-period²⁵. By using GADOLINIUM-ENHANCED MAGNETIC RESONANCE IMAGING (MRI) techniques, it was found that MS patients with high MMP9 and low TIMP1 levels tended to worsen²⁶. Several groups have shown increased post-mortem expression of various MMP members (**MMP2**, **-3**, **-7** and **-9**) in the brains of patients with MS (REFS 27,28). Cellular sources of MMPs in the diseased brain include infiltrating leukocytes (lymphocytes and macrophages) and intrinsic CNS cells (perivascular and parenchymal microglia, astrocytes and even neurons). Although it has been less thoroughly investigated, most studies indicate that TIMP1 and -2 might not be altered in MS compared with controls²⁹. No data are available for TIMP3 and -4.

MMPs are also dysregulated in experimental autoimmune encephalomyelitis (EAE), an animal model of MS. Increased expression of **MMP7**, **-9** and **-12** is observed in the rat CNS immediately preceding, and in parallel with, the development of symptoms. By contrast, transcripts encoding **MMP2**, **-3**, **-11** and **-13** were unchanged²⁰. Surprisingly, the elevation of **MMP7** is not noted in mouse EAE, in which a prominent increase in **MMP3**, **-9** and **-12** occurs³⁰. So, overall, a common elevation of **MMP9** and **-12** is found in MS, and in mouse and rat EAE models.

The correlation between elevated MMPs and disease activity is probably causal, as inhibitors of metalloproteinase activity (for example, GM6001, Ro-9790 and BB1101) alleviate or prevent EAE (REFS 1,31). Also, 3–4-week-old mice that lack **MMP9** are less impaired after the induction of EAE when compared with wild-type animals³². In humans, interferon- β , a drug used against MS, attenuates **MMP9** production by T cells *in vitro*, in agreement with the decreased capacity of T cells to traverse ECM barriers^{33,34}. In addition, MS patients treated with interferon- β show a decrease in the content of serum **MMP9** and a lowering of the number of **MMP9**-expressing leukocytes³⁵. Together, these observations indicate that the antagonism of MMP function might contribute to the efficacy of interferon- β in MS.

Studies of MS and EAE have revealed several mechanisms that help to account for the involvement of MMPs in CNS pathology (FIG. 2). First, there is good evidence that T cells and other leukocytes use MMPs to penetrate ECM barriers, including the basement membrane that surrounds cerebral capillaries. In this regard,

RNASE PROTECTION ASSAY

Technique for the quantitation of RNAs in solution. It is based on the use of a complementary radiolabelled probe that is hybridized to the target RNA, after which the mixture is treated with RNase to degrade all remaining single-stranded RNA. The hybridized target will be protected from digestion and can be later visualized on a polyacrylamide gel.

GADOLINIUM-ENHANCED MAGNETIC RESONANCE IMAGING

Imaging technique in which gadolinium is introduced as a contrast agent, allowing for short data-acquisition times, large anatomical coverage and improved image quality.

FAS LIGAND

Ligand for the Fas antigen, a transmembrane protein that mediates apoptosis and might be involved in negative selection of autoreactive T cells in the thymus.

GLIOBLASTOMA MULTIFORME

Aggressive glioma, commonly of astrocytic origin, that infiltrates adjacent normal brain tissue. It accounts for about 10% of childhood brain tumours.

the capacity of macrophages from MMP12-null mice to penetrate basement membranes is markedly diminished *in vitro* and *in vivo*³⁶. Also, when T cells are applied onto a monolayer of endothelial cells that overlie a barrier of collagen matrix, the addition of GM6001, an metalloproteinase inhibitor, did not prevent the T cells from traversing the endothelial barrier. However, the entry of T cells into the collagen matrix is abolished³⁷. One result of disrupting the basement membrane is the breakdown of the blood–brain barrier (BBB). This has been shown experimentally by the intracerebral injection of MMPs and the subsequent leakage of microvessels^{31,38}. In addition, there is a good correlation between gadolinium-enhanced MRI activity in humans, an index of BBB dysfunction, and the serum content of MMP9 (REFS 25,26).

MMPs have other undesirable consequences in the CNS parenchyma (FIG. 2). When injected into the CNS, MMPs can disrupt myelin and cause demyelination³⁹. Furthermore, some of the fragments of the MMP-mediated digestion of myelin basic protein (MBP) can induce EAE when injected into rodents^{31,40}. Also, human MMP9 cleaves human MBP into peptide fragments, one of which is the immunodominant epitope in humans⁴⁰. Another mechanism by which MMPs might promote an inflammatory response is by the conversion of precursor, inactive molecules into their activated forms. Tumour-necrosis factor (TNF)- α — a pro-inflammatory cytokine with several actions that include oligodendrocyte toxicity — is first made as a 26-kDa membrane-associated protein that requires proteolytic conversion to the fully active 17-kDa protein. Although TACE is known to be the protease that mediates the physiological maturation of TNF- α (REF. 41), it is clear that MMP7 and MT4-MMP can also efficiently mediate this conversion⁴². Other molecules, such as transforming growth factor (TGF)- α , IL-6, TNF receptors, L-selectins and FAS LIGAND (FasL), are also synthesized as precursors that require processing by MMPs or ADAMs for maturation. So, in pathological conditions in which local levels of MMPs are high, these molecules might significantly modulate inflammation in the CNS.

Another pathogenic effect of MMPs in the CNS is neurotoxicity. Vos *et al.*⁴³ reported that MMP1 is toxic to spinal cord neurons *in vitro*, and we found a similar neurotoxic effect of MMP2 (REF. 44). An alternative mechanism for MMP-mediated death might involve the phenomenon known as ‘anoikis’ (Greek for homelessness); cells that are attached to an ECM substrate survive through integrin signalling and death ensues when the cell is detached⁴⁵. In principle, MMPs can indirectly cause cell death by degrading ECM and therefore interfering with cell attachment and integrin signalling. Indeed, neuronal death in the hippocampus after kainate-induced seizures in animals has been attributed to proteases that degrade laminin⁴⁶. Finally, it has been noted that MMP7, but not MMP2, -3 or -9, acts on cell-associated FasL to convert it into soluble FasL, which induces apoptosis of epithelial cells⁴⁷. Conversely, MMP7-mediated cleavage of FasL protects sarcoma and colon carcinoma cells from chemotherapy-induced

cytotoxicity. Given the presence of Fas and FasL in the CNS, MMPs could affect cell survival or death by similar mechanisms. In summary, the excessive production of MMPs in the CNS can be neurotoxic through several mechanisms (FIG. 2).

The literature on the upregulation of ADAMs in the CNS of MS patients is still in its infancy. Cells of the immune system produce various ADAMs (REF. 48), and it is likely that several ADAMs will be elevated in the CNS during neuroinflammation and MS. Also, with the exception of ADAM17 described above, much remains to be explored about the consequences of the aberrant expression of ADAMs in the CNS.

Metalloproteinases in malignant gliomas. An extensive literature links MMPs to tumour invasiveness and metastasis, as remodelling of the ECM is thought to be necessary for a tumour cell to advance. More recent studies have extended the roles of MMPs to other features of tumour progression, notably proliferation, angiogenesis and survival⁴⁹. Essentially, all MMP members have been linked to cancers of various origins. So, it is not surprising that there are many reports of the increased expression of MMPs in brain tumours *in situ* and *in vitro*. Specifically, the upregulation of MMP2, -9 and all the MT-MMP members has been noted in high-grade specimens of GLIOBLASTOMA MULTIFORME (GBM) as compared to lower-grade cases or to non-transformed control brains^{50–52}. The finding that AG3340 — an inhibitor of metalloproteinase activity — inhibits glioma growth, invasion and angiogenesis in animals⁵³ highlights the importance of MMPs in the progression of brain tumours.

MMP2, MT1- and MT2-MMP have been localized to glioma cells in addition to CNS elements by *in situ* hybridization. By contrast, MMP9 is predominantly expressed in blood vessels at proliferative margins⁵¹. A simplistic interpretation of these results is that MMP2 and MT-MMPs might regulate glioma invasiveness, whereas MMP9 might be crucial for angiogenesis. In this regard, the capacity of various glioma lines to migrate across a reconstituted basement membrane *in vitro* is correlated with the levels of MMP2 expression⁵⁴. Furthermore, the ability of the C6 gliosarcoma line to migrate along myelin is related to MT1-MMP activity⁵⁵. As mentioned earlier, MT1-MMP facilitates the activation of MMP2, and an excessive MT1-MMP activity was recently noted in GBMs (REF. 52). Similarly, factors that stimulate glioma motility, such as hepatocyte growth factor/scatter factor, increase MMP2 or MT1-MMP levels. Conversely, inhibitors of MMP activity, including BB94 and BB2516, reduce glioma invasiveness in tissue culture.

Several MMPs can be activated by the serine protease plasmin⁵⁶. The plasmin cascade starts with binding of urokinase plasminogen activator (uPA) to its receptor molecule uPAR. This results in uPA activation and the subsequent conversion of plasminogen to plasmin by uPA. It is noteworthy that the uPA and uPAR are upregulated in GBMs (REF. 57), endowing the tumour with the machinery to activate its own MMPs

effectively. In support of this idea, downregulation of uPAR in a glioma cell line led to decreased capacity to invade brain aggregates⁵⁸. The expression of MMPs by glioma cells highlights a difference between CNS and systemic cancers. In the latter (for example, breast cancers), MMPs are often expressed by the stroma, rather than by the tumour itself. So, several mechanisms seem to exist for bestowing glioma cells with the capacity for autonomous, sustained growth.

TIMPs are also altered in gliomas. A decrease in TIMP2 content in GBM compared to lower-grade tumours has been noted in some⁵², but not all⁵⁰, studies. This decrease would potentially remove a counterbalance for MMP activity. Although earlier studies noted a decrease of TIMP1 with increasing grades of glioma, later studies noted that it was actually upregulated in GBM (REFS 50,52). It is worth noting in this context that TIMPs have several properties that can contribute to the pathophysiology of cancer cells. Indeed, TIMPs have mitogenic action, and regulate survival and apoptosis independently of their inhibitory functions on MMPs (REF. 14).

Finally, elevated expression of various MMPs has been shown for other CNS tumour classes, including childhood astrocytomas, neuroblastomas and meningiomas. Overall, an excess of MMP activity seems to characterize many brain tumours. The development of metalloproteinase inhibitors to treat gliomas seems warranted and some are already being tested in clinical trials. A role for ADAMs in glioma biology remains to be defined and the related literature is limited at present⁵⁹.

Metalloproteinases in other neurological conditions. A role for metalloproteinases in stroke is indicated by the finding that MMP2 and -9 are rapidly upregulated after focal cerebral ischaemia in rats^{60,61}. In humans, elevation of brain MMP9 is detected post mortem within days of infarction and, interestingly, this protein remained elevated in patients that died months after the event⁶². In another report, MMP9 was strongly expressed by neutrophils in tissues from patients up to one week after an infarct, whereas the expression of MMP2 and -7 was less marked. From one week to five years, neutrophils were absent in the lesions and the large number of macrophages present were immunoreactive for MMP2 and -7 (REF. 28). The elevated MMP expression might contribute to the tissue destruction in stroke. As noted earlier, MMPs have the capacity to kill neurons (FIG. 2). Indeed, the intravenous treatment of rats with a neutralizing MMP9 antibody one hour before vessel occlusion reduced infarct size by 28% (REF. 61). In addition, the size of infarcts after ischaemia in MMP9-null mice was less than that observed in wild-type controls⁶³.

Viral infections of the CNS have been increasingly associated with the production of MMPs. Elevated expression of MMP9 has been detected in the CSF of HIV-infected patients⁶⁴. MMP2, -7 and -9 are increased in the CSF of patients with HIV-associated dementia compared to that of non-demented AIDS patients⁶⁵. When brain-derived sequences of the HIV-transactivating protein TAT (tyrosine aminotransferase), obtained

from demented patients, were expressed in U937 monocytoid cells and human macrophages, elevated MMP2 and -7 expression was obtained compared to TAT sequences from non-demented patients. These elevated MMPs resulted in neuronal death⁴⁴. Collectively, the results indicate that HIV infection induces MMP expression in macrophages and that the latter are the possible sources of MMPs and neurotoxicity in the CNS of people with HIV-associated dementia. Other viruses, including EPSTEIN-BARR VIRUS, HTLV-1 and CORONAVIRUS⁶⁶ have also been reported to increase the expression of MMPs in susceptible cell types. Of interest, these viruses have been associated with MS pathology, raising the possibility that viral-induced demyelination might involve MMP intermediaries.

MMPs are also associated with Alzheimer's disease, although the history has been full of false leads. Alzheimer's disease is characterized by plaques that are formed mostly by the deposition of amyloid- β (A β) — a peptide derived from cleavage of the amyloid precursor protein (APP), an integral membrane molecule. Three proteases — α -, β - and γ -secretase — are involved in APP cleavage at different sites to generate A β peptides of various lengths⁶⁷. The predominant cleavage is mediated by α -secretase, and this mode of cleavage is thought to be non-amyloidogenic. By contrast, the combination of β -secretase and γ -secretase activities releases the amyloidogenic A β peptide. The aspartyl protease BACE (β -site APP cleavage enzyme) is the best candidate to be the β -secretase⁶⁸, whereas the γ -secretase activity depends on proteins known as presenilins.

It was initially suggested that MMP2 was the α -secretase⁶⁹, but this idea was quickly disputed. Moreover, it was also suggested that MMP2 had β -secretase-like activity⁷⁰. Furthermore, the presence of immunoreactive TIMP in plaques of Alzheimer's disease led to the speculation that an MMP was excessively produced or activated in the plaques, as TIMPs have high affinity for MMPs and would therefore localize to sites of protease activity⁷¹. It was subsequently suggested that the α -secretase activity was mediated by a non-MMP metalloproteinase, largely on the basis of studies using pharmacological inhibitors⁷². Indeed, there is now good evidence for ADAM10 (REF. 73) and ADAM17 (REF. 74) as α -secretases. If this proves to be the case, these ADAMs could have a protective action against Alzheimer's disease, as they would funnel APP towards the non-amyloidogenic pathway. It is worthwhile investigating whether the function of ADAMs is deficient or altered in Alzheimer's disease. Recently, hippocampal neurons that are immunoreactive for ADAM1 and -2 were detected in Alzheimer's disease patients but not in age-matched controls⁷⁵. In addition, levels of these proteins were also elevated⁷⁵.

MMPs are also implicated in other diseases of the nervous system, including inflammatory myopathies⁷⁶ and peripheral nerve axotomy. The involvement of MMPs in degenerative diseases of the CNS, including AMYOTROPHIC LATERAL SCLEROSIS, is an area of increasing interest. It is likely that the list of neurological disorders associated with aberrant MMP or ADAM expression will grow over the years.

EPSTEIN-BARR VIRUS

Virus that binds the complement receptor CD21 and causes infective mononucleosis and, in the presence of other factors, tumours such as Burkitt's lymphoma and nasopharyngeal carcinoma.

HTLV-1

Human T-cell leukaemia/lymphoma virus type I. A retrovirus that causes leukaemia and mild immunodeficiency.

CORONAVIRUSES

A group of RNA viruses that include the causative agent of the common cold.

AMYOTROPHIC LATERAL SCLEROSIS

Progressive neurological disease that is associated with the degeneration of central and spinal motor neurons. This neuron loss causes muscles to weaken and waste away, leading to paralysis.

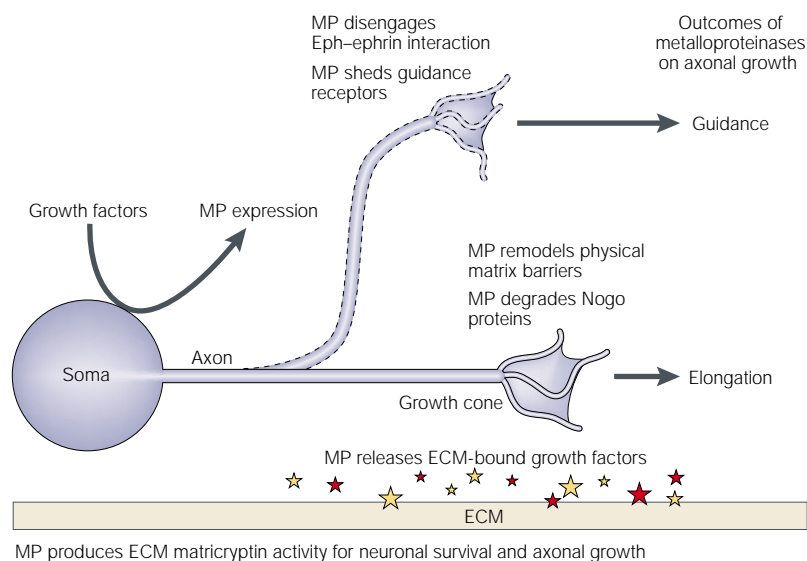


Figure 3 | Metalloproteinases regulate axonal growth. Several growth factors, including nerve growth factor (NGF), increase the expression of metalloproteinases (MPs) by neurons. MPs, in turn, regulate neurotrophic factor activity. One mechanism involves the release of growth factors that are anchored to the extracellular matrix (ECM). MPs that are located in the vicinity of growth cones can promote the elongation of axons owing to their ability to remodel the ECM and degrade inhibitory molecules such as the Nogo proteins. By interacting with molecules implicated in axonal guidance, for example, by shedding DCC (deleted in colorectal carcinoma) receptors or by breaking Eph receptor–ephrin bonds, MPs can affect the directional growth of axons.

Metalloproteinases in CNS ontogeny and repair
 Although our discussion has so far revolved around the detrimental roles of metalloproteinases, it must be stressed that some of the functions of MMPs in the CNS might be beneficial. For example, some MMPs and TIMPs are expressed in the CNS during development^{77,78}, pointing to their possible importance in brain maturation. Furthermore, MMPs are rapidly upregulated after nearly all types of injury to the CNS, including trauma^{19,79}, indicating their possible relevance in tissue repair. In this section, we focus on the lesser-known beneficial aspects of metalloproteinases in the CNS.

In concordance with the classic role of MMPs in modulating the motility of cells across tissue matrices, metalloproteinases might regulate the migration of precursor cells to their destinations during neural development. Neural stem cells express MMP2 and all four TIMPs (REF. 80), and the migration of an oligodendrocyte progenitor requires MMP activity *in vitro*. The protein NOTCH affects cell-fate decisions in neurogenesis, and both ADAM10 (REF. 81,82) and ADAM17 (REF. 83) have been shown to activate the notch signalling cascade. Another role for MMPs in CNS development might lie in myelinogenesis, the process whereby oligodendrocytes extend several processes from their soma that reach and enwrap axons to form myelin. The initial expansion of oligodendroglial processes is immense and could require remodelling of the brain matrix by MMPs. This hypothesis has been tested and oligodendrocytes were found to express MMP9 during the period of myelinogenesis. Furthermore, the inhibition of MMP activity *in vitro* prevented the extension of oligo-

dendroglial processes⁸⁴. Other metalloproteinases could also regulate myelinogenesis, but this remains to be tested. The rabbit corpus callosum expresses MMP1 and -3 before and during myelination⁸⁵, whereas ADAM10 is expressed in oligodendrocytes before and during myelinogenesis⁸.

In parallel with myelinogenesis, metalloproteinases also participate in axon elongation (FIG. 3). Early studies noted the presence of proteolytic activity at neuronal growth cones during attachment and reattachment events⁸⁶. Some of the activity is probably contributed by metalloproteinases, as interference with MMP activity inhibited growth-cone motility⁸⁷. Inducers of neuronal differentiation and axonal outgrowth, such as nerve growth factor, laminin or retinoic acid, enhanced the expression of MMP2, -3 and -9 by dorsal root ganglion (DRG) neurons, PC12 and neuroblastoma cells^{88,89}. Furthermore, growth cones of PC12 cells that stably expressed MMP3 had a reduced capacity to penetrate a reconstituted basement membrane. *In vivo*, *Drosophila melanogaster* flies that carry mutations in the protein Kuzbanian (the mammalian homologue is ADAM10) show axon stalling during development⁹⁰. In a study in which neuritic outgrowth of DRG neurons that grow on top of normal adult nerves was evaluated, the slow neurite elongation was further reduced by treatment with metalloproteinase inhibitors⁹¹. By contrast, pre-treating the nerves with recombinant MMP2 accelerated neurite growth⁹¹. Further studies led to the conclusion that DRG neurons expressed MMP2 that degraded and inactivated the neurite-inhibiting activity of CHONDROITIN SULPHATE PROTEOGLYCANs present on nerves, leading to the exposure of permissive laminin for neurite outgrowth⁹¹. Indeed, cleavage of a specific peptide bond in an ECM molecule leads to profound functional changes in other systems. For example, the cleavage of the Ala586–Leu587 bond in the $\alpha 2$ chain of laminin-5 by MMP2 induced migration of breast epithelial cells by exposing a cryptic pro-migratory site on laminin-5 (REF. 92). The biologically active sites in matrix molecules that become exposed after structural or conformational alterations have been called ‘matricryptic sites’, and the name matricryptins has been used to describe the resulting ECM fragments that have biological activity. Last, in concordance with the activity of MMP2 on proteoglycans described above, metalloproteinases might be used in the CNS to destroy other inhibitory proteins. C6 glioma cells and fibroblasts transfected with MT1-MMP could digest NI250 (REF. 55), a Nogo protein identified as one of the most potent inhibitors of axonal elongation. In this way, some MMPs might act by neutralizing inhibitory proteins for axonal outgrowth.

Although these data implicate metalloproteinases in the creation of penetrable paths for axonal elongation (FIG. 3), metalloproteinases can also regulate guidance cues for growth cones. Ephrins are guidance molecules that bind to receptor tyrosine kinases of the Eph family. When the growth cone of a neuron that expresses Eph receptors encounters ephrin ligands on the surface of another cell, this facilitates the adherence of the cells to each other and bidirectional signalling to occur. The

CHONDROITIN SULPHATE PROTEOGLYCANs
 Important components of the extracellular matrix and connective tissue. These proteins contain hydrophilic, negatively charged polymers of glucuronic acid and sulphated *N*-acetyl glucosamine residues.

Box 1 | Metalloproteinases in the CNS

Beneficial activities

- Cell-fate specification
- Angiogenesis
- Myelinogenesis
- Survival of cells
- Axonal growth
- Signaling
- Repair processes
- Migration of neuronal precursors to final sites
- Terminate inflammation

Detrimental activities

- Tumorigenesis
- Disruption of blood–brain barrier
- Inflammation
- Demyelination
- Neuronal death
- Diseases, including glioma, stroke and Alzheimers disease

growth cone then overcomes these adhesive forces and breaks away from the ephrin surface. Hattori *et al.*⁹³ showed that the adhesive ephrin–Eph interaction is broken *in vitro* by ADAM10, which becomes activated after engagement of the Eph receptor. Another guidance molecule is **netrin 1**, which binds a receptor known as **DCC** (deleted in colorectal carcinoma). When axon outgrowth from embryonic dorsal spinal explants was evaluated *in vitro*, the facilitatory activity of netrin 1 was potentiated by IC3 and GM6001, hydroxamate metalloproteinase inhibitors⁹⁴. It was found that DCC was shed from the cell surface by the activity of an unidentified MMP; preventing the ectodomain shedding of DCC with a metalloproteinase inhibitor resulted in responsiveness to netrin 1 (REF. 94). The recent report of a phenotype-based GENE-TRAP screen to identify genes that control wiring patterns in the mouse CNS further implicates metalloproteinases in axonal guidance. ADAM23 was one of the genes identified in this screening; its inactivation *in vivo* led to neurological defects, tremor and ataxia⁹⁵.

So, a beneficial role for metalloproteinases in neurogenesis and myelinogenesis during development seems to be supported by several lines of evidence. By contrast, the favourable functions (if any) of the MMPs that are upregulated in CNS disorders in adulthood are less clear. We have highlighted the detrimental effects of metalloproteinases in the injured adult CNS (FIG. 2) but some beneficial functions are also possible after injury, although they remain speculative at this point. What could they be? First, it is possible that metalloproteinases enable the migration of precursor cells to injured sites to replenish lost cells. A role in facilitating axonal regrowth, remyelination and angiogenesis is also possible on the basis of the functions of these proteases in development, as described earlier. Another possible role

of MMPs in the adult CNS might involve their potential to affect signal transduction by, for example, linking G-protein-coupled-receptor signalling with the EGF pathway. In this context, the engagement of G-protein-coupled receptors has been shown to activate a membrane-associated metalloproteinase that converts pro-heparin-binding EGF to its mature form, triggering EGF signalling⁹⁶. Regulation of synaptic plasticity could also be another function of metalloproteinases in CNS injury⁷⁷. Of interest, MMP1, -2, -3 and -9 were upregulated after spinal-cord transection in the AXOLOTL during the period of regeneration and subsided when recovery was near completion⁹⁷.

MMPs could also participate in terminating inflammation in the CNS, in contrast to the perpetuation of inflammation that we discussed earlier (FIG. 2). The prolonged incubation of IL-1 β with MMP3, and to a lesser extent MMP2 and -9, resulted in IL-1 β degradation⁹⁸. Furthermore, MMP2 binds to the chemokine **MCP3** (macrophage chemoattractant protein 3) and cleaves its first four amino-terminal amino acids. The cleaved MCP3 binds to several chemokine receptors, acting as an antagonist⁹⁹. So, MMPs expressed in the CNS might serve to abolish a chemotactic gradient for leukocyte entry. Interestingly, MMP9 has been reported to truncate the amino terminus of IL-8, leading to a 30-fold potentiation of IL-8 activity¹⁰⁰. Conversely, MMP9 degraded chemokines, such as gro- α , leaving **RANTES** (also known as SCYA5) and MCP2 intact¹⁰⁰.

In summary, metalloproteinases in the CNS have clear beneficial functions during development and might also have positive effects after injury. These effects contrast with the better-described detrimental roles of metalloproteinases in the CNS (BOX 1).

The challenges ahead

The challenges associated with defining the roles of MMPs in the CNS are several-fold. First, it is obvious that metalloproteinases can be both friends and foes in the CNS. We will therefore have to understand the balance between these states and the context that swings the pendulum between beneficial and detrimental roles. Second, a given metalloproteinase might have different properties at a given condition, dependent on factors such as spatial localization and cellular source. For example, MMP9 expressed by a macrophage in the vicinity of myelin might produce demyelination, whereas the same MMP elaborated by oligodendrocytes at the tip of their processes might promote remyelination. In this context, an inhibitor of MMP9 would simultaneously curb both detrimental and reparative processes. So, the challenge is to identify all the functions of a given metalloproteinase in a particular situation and to evaluate if the net result of its inhibition is worthwhile. A third challenge is related to establishing the identity of the metalloproteinase involved in a given function. Many of the pharmacological inhibitors that are available at present lack specificity towards members of a subfamily and will often antagonize the activity of members of different metalloproteinase families^{10,11}. So, when functions are ascribed to particular metalloproteinases,

GENE TRAPPING

A mutation strategy that uses insertion vectors to trap or isolate transcripts from flanking genes. The inserted sequence acts as a tag from which to clone the mutated gene.

AXOLOTL

Mexican salamander. A useful model to study nerve repair, as the spinal cord of this animal shows marked regeneration after transection.

it has to be clear, not only which metalloproteinase is involved, but also whether one is dealing with the right subfamily of metzincin metalloproteinases. Clearly, the next generation of pharmacological inhibitors must be able to discriminate between subfamilies and between members of each subfamily. Mice with null mutations for specific MMPs and ADAMs are already available and can help to discriminate functions of specific metalloproteinases. However, these valuable resources often show compensatory upregulation of activity of other MMPs as a result of the gene deletion and the analysis of these animals must therefore be interpreted with caution.

The identification of their physiological substrates is intimately related to the task of defining the functions of metalloproteinases in the CNS. Although it is clear that various MMP members can act on certain ECM components *in vitro* (TABLE 1), much remains to be discovered about their natural substrates *in vivo*. In the case of ADAMs, their CNS substrates are largely unknown and research needs to focus on this area. Similarly, the ways in which metalloproteinases are transcribed and interact with other molecules, such as chemokines, remain to be elucidated.

Can any consensus be adopted at this point as to whether metalloproteinases should be targeted for inhi-

bition to treat specific CNS diseases? Given the data that aberrant expression of MMPs is correlated with conditions such as MS, we suggest that the use of metalloproteinase inhibitors is warranted. If factors such as inflammation, which cause the disease to worsen, can be curbed, then the side effects of impairing reparative activities might be a reasonable trade-off. However, much more specific inhibitors and a clearer view of which metalloproteinase member to antagonize would help towards rational therapeutics.

In summary, much remains to be learned about the biology and pathology of metalloproteinases in the CNS. We welcome the explosion of this field, as the rewards of an increased understanding would seem to be immense.

 Links

DATABASE LINKS [Multiple sclerosis](#) | [Alzheimer's disease](#) | [Hemopexin](#) | [MMP7](#) | [MMP26](#) | [MT4-MMP](#) | [MT6-MMP](#) | [ADAM1](#) | [ADAM2](#) | [ADAM12](#) | [ADAM9](#) | [ADAM10](#) | [ADAM17](#) | [src](#) | [ADAM11](#) | [ADAM28](#) | [ADAM15](#) | [ADAM19](#) | [ADAM8](#) | [ADAM23](#) | [TIMP2](#) | [MT1-MMP](#) | [MMP9](#) | [TIMP3](#) | [TIMP1](#) | [TIMP4](#) | [MMP11](#) | [MMP14](#) | [MMP3](#) | [MMP13](#) | [MMP12](#) | [uPAR](#) | [TAT](#) | [NOTCH](#) | [Ephrins](#) | [Netrin 1](#) | [DCC](#) | [MCP3](#) | [RANTES](#)

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Acknowledgements

We thank the Canadian Institutes for Health Research and the Multiple Sclerosis Society of Canada for operating funds. V.W.Y. acknowledges the past and present trainees in his laboratory who have contributed to his understanding of metalloproteinases in the CNS: V. Brundula, S. Chabot, N. Dooley, J. Dowden, F. Giuliani, U. Ladiwala, P. Larsen, D. M. Le, L. Y. S. Oh, O. Stuve, J. H. Uhm, G. Vecil, C. Verhaghe and Y. Zhou. Because of space limitations, important contributions by several authors could not be included. We apologize for this omission.