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Matrix Metalloproteinases and Neurotrauma: Evolving Roles in Injury and Reparative Processes

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Abstract

Matrix metalloproteinases (MMPs) are involved in a wide range of proteolytic events in fetal development and normal tissue remodeling as well as wound healing and inflammation. In the CNS, they have been implicated in a variety of neurodegenerative diseases ranging from multiple sclerosis to Alzheimer disease and are integral to stroke-related cell damage. Although studies implicate increased activity of MMPs in pathogenesis in the CNS, there is also a growing literature to support their participation in events that support recovery processes. Here the authors provide a brief overview of MMPs and their regulation, address their complex roles following traumatic injuries to the adult and developing CNS, and consider their time- and context-dependent signatures that influence both injury and reparative processes.

Keywords

traumatic brain injury; spinal cord injury; angiogenesis; oxidative stress; blood-brain barrier; glial scar

An Overview of MMPs

The matrix metalloproteinase (MMP) family includes 23 human (24 murine) MMP members (Page-McCaw and others 2007). Based on structure and substrate specificity, MMPs are categorized into subgroups that include collagenases (MMP-1, -8, -13, and -18), gelatinases (MMP-2 and -9), stromelysins (MMP-3, -10, and -11), membrane-type MMPs (MMP-14 (MT1-MMP), MMP-15 (MT2-MMP), MMP-16 (MT3-MMP), MMP-17 (MT4-MMP), MMP-24 (MT5-MMP), and MMP-25 (MT6-MMP)), and other MMPs. Structurally, most MMPs share a conserved domain structure consisting of a prodomain, catalytic domain, hinge region, and hemopexin domain. Unlike the other MMPs, the 6 MT-MMPs are membrane proteins, linked to the plasma membrane either by a trans-membrane domain (MT1-, MT2-, MT3-, MT5-MMP) or by a glycosylphosphatidylinositol linkage (MT4- and MT6-MMP), attached to the hemopexin domain.

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MMPs are best known for their ability to cleave constituents of the extracellular matrix. However, in more recent years, the list of substrates has greatly expanded to include peptide growth factors, tyrosine kinase receptors, cell adhesion molecules, cytokines, and chemokines. Processing of these molecules influences cell functions as diverse as cellular differentiation, migration, regulation of growth factor activity, survival or apoptosis, angiogenesis, inflammation, and signaling (Yong 2005; Page-McCaw and others 2007).

Current methods for detecting MMPs involve gelatin, in situ gelatin zymography, Western blotting, in situ hybridization, quantitative PCR, immunohistochemistry, enzyme-linked immunoassays, and enzyme activity assays (e.g., using fluorogenic peptide-, radiolabeled-, and fluorescent dye-linked substrates; Fig. 1). Each of these approaches has both strengths and weaknesses (Table 1). The merging of these approaches provides the ideal scenario whereby MMPs are described by activity, mRNA, protein levels, and expression at the cellular level.

Regulation of MMPs

Here we briefly describe regulation of MMPs (Fig. 2). For a more in-depth description, refer to the review by Sternlicht and Werb (2001). MMPs are regulated at the transcriptional level and by posttranslational modification. In addition, overall activity is influenced by endogenous inhibitors. Most MMPs are not constitutively expressed at detectable levels. They are subject to transcriptional regulation when exposed to a variety of transcriptional regulators that include growth factors, cytokines, chemokines, and components of the extracellular matrix.

Posttranslational modifications provide a second level of regulation. MMPs are initially expressed as an inactive zymogen in which the cysteine residue at the propeptide region binds the zinc ion present at the catalytic site. Activation requires removal of the propeptide domain to expose the active catalytic site. Although most MMPs are secreted as inactive zymogen, MMP-11, MMP-27, and the MT-MMPs contain an RXX/RR furin-like enzyme recognition motif, which allows them to be activated intracellularly by subtilisin-type serine proteinases. The extracellular activation of most MMPs is mediated by other activated MMPs, the plasmin-plasminogen cascade, and a range of proteinases and nonproteolytic agents. MMP-2 is activated at the cell surface through a unique and complex mechanism of activation involving MMP-14 (MT1-MMP) and TIMP-2 (Sternlicht and Werb 2001).

Following activation, MMPs are modulated by physiological inhibitors. The four known tissue inhibitors of matrix metalloproteinases (TIMPs), differentially expressed by cells in various tissues, TIMP-1, -2, and -4, are secreted, whereas TIMP-3 is bound to the extracellular matrix. TIMPs inhibit MMPs by binding to the catalytic site of MMPs and forming tight 1:1 noncovalent complexes with MMPs (Yong and others 1998; Sternlicht and Werb 2001). However, the TIMPs differ in their affinity for specific MMPs, and their interaction does not always lead to inhibition. For example, TIMP-1 binds pro-MMP-9, which protects MMP-9 from MMP-3 cleavage. TIMPs may also participate in MMP activation. For example, TIMP-2 is involved in the activation of pro-MMP-2 by MMP-14 (MT1-MMP) (Webster and Crowe 2006).

TIMPs are not the only endogenous MMP inhibitors. Indeed, α 2-macroglobulin is an inhibitor of the MMPs. α 2-macroglobulin is an abundant plasma protein. In contrast to the TIMPs, which act at a local cellular level, α 2-macroglobulin represents the major inhibitor of MMPs in tissue fluids. Moreover, because α 2-macroglobulin/MMP complexes are removed by scavenger receptor-mediated endocytosis, α 2-macroglobulin plays an important

role in the irreversible clearance of MMPs, whereas TIMPs inhibit MMPs in a reversible manner (Sternlicht and Werb 2001).

A membrane-anchored glycoprotein, RECK, widely expressed in various human organs, inhibits MMP-2, -9, and -14. RECK probably has other functions because a null mutation in the gene is embryonic lethal in mice (Oh and others 2001). Another class of MMP inhibitors, protein subdomains, has structural similarity to the TIMPs.

A primary sequence alignment search also uncovered similarities between the TIMPs and the noncollagenous NC1 domain of type IV collagen. Moreover, functional analyses indicate that the NC1 domain has MMP inhibitory activity. Although their activity against MMP-2 is substantially lower than that of the TIMPs, other MMPs may be their true physiologic targets (Sternlicht and Werb 2001).

Finally, it should be noted that MMPs typically act close to the cell surface. MT-MMPs are inserted in the plasma membrane. Although other MMPs are generally secreted from cells, they attach to the cell surface by binding to MT-MMPs, cell adhesion molecules, cell surface proteoglycans, and integrins (Yong 2005). This is exemplified by the binding of MMP-2 to cell membranes and to the integrin $\alpha_v\beta_3$, MMP-9 to CD44, and MMP-7 to heparan sulfate proteoglycans (Dumin and others 2001). The MT-MMPs, single-pass transmembrane proteins that are fixed and active at the cell surface, also provide docking sites for other MMPs. Pro-MMP2 forms a complex consisting of MT1-MMP and tissue inhibitor of metalloproteinase 2 (TIMP-2), which allows a neighboring MT1-MMP to activate it on the cell surface (Dumin and others 2001).

Expression and Distribution of MMPs in the CNS

MMPs are expressed at basal levels in the healthy adult CNS (Table 2). In rodents, MMP-2 and -9, the most studied of the MMPs in the CNS, are immunolocalized in neurons, astrocytes, endothelial cells, and myelinated fibers (Planas and others 2001; Rosenberg and others 2001a; Noble and others 2002). By real-time PCR, most if not all members of the MMP family are expressed in the healthy adult murine spinal cord (Wells and others 2003).

Several studies have immunolocalized MMPs in the human brain. Astrocytes (Cuzner and others 1996) express both MMP-2 and MMP-9. Microglia express these gelatinases (Cuzner and others 1996; Rosenberg and others 2001b), as well as MMP-7 (Cossins and others 1997), MT1-MMP (Yoshiyama and others 1998), and MT3-MMP (Yoshiyama and others 1998). Finally, MMP-1 has been localized in neurons (Buss and others 2007).

Temporal Expression of MMPs following Neurotrauma

Although it is clear that MMPs are present in the CNS, their overall level of activity is relatively modest. In many instances, activity is not detected by zymography (Lee and others 2004). However, in response to trauma, activity is markedly up-regulated with different MMPs exhibiting unique temporal profiles (Table 3).

Several studies have examined MMPs in experimental models of spinal cord injury (SCI). One of the earliest studies was in a rat model of SCI (de Castro and others 2000) where gelatin zymography revealed prominent MMP-9 activity by 12 to 24 hours postinjury followed by a rise in MMP-2 by 5 days postinjury. Similar findings have been reported in a murine model of spinal cord contusion injury where MMP-9 is markedly up-regulated at 1 day postinjury and thereafter declines to negligible levels by 14 days. Although MMP-9 is a signature of the acutely injured cord, MMP-2 activity is most robust during wound healing, with prominent activity between 7 and 14 days postinjury (Goussev and others 2003).

Evaluation of mRNA transcripts offers added insight into injury-related changes in expression of MMPs. In a murine model of spinal cord compression, there is up-regulation of mRNA transcripts encoding MMP-2, -3, -7, -10, -11–13, -19, and -20 within 24 hours of injury, whereas increased expression of MMP-2, -12, and -13 is delayed in onset until 5 days after injury (Wells and others 2003).

Expression at the cellular level (in situ zymography and immunocytochemistry) varies according to time postinjury. MMP-9 is localized in the acutely injured cord to blood vessels, macrophages, and astrocytes (Xu and others 2001; Noble and others 2002), whereas MMP-1 is expressed in neurons (Xu and others 2001). The temporal profile for MMP-2 is characteristically delayed in onset, localizing in reactive astrocytes at 7 to 14 days postinjury.

Most recently, there has been an effort to assess MMPs in the human spinal cord (Buss and others 2007). At 2 days after traumatic SCI, MMP-1, -2, -9, and -12 are immunolocalized in CD68-positive macrophages. Although MMP-1 is expressed in macrophages in the acutely injured spinal cord, it is also detected in perilesional activated astrocytes in the more chronically injured cord. The time course for MMP protein expression varies somewhat according to the MMP studied. MMP-9 progressively increases from 1 week to 3 weeks, whereas MMP-2 peaks within the first week. Compared with other MMPs, MMP-12 shows limited immunostaining at and around the lesioned site up to 11 days postinjury and markedly decreases thereafter.

As might be expected, there is also up-regulation of MMPs in experimental models of traumatic brain injury (TBI) to the adult (Wang and others 2000) and immature brain (Sifringer and others 2007). Zymograms show that MMP-9 is elevated as early as 3 hours after contusion to the adult murine brain, reaching a maximum at 24 hours. Increased MMP-9 levels persist for up to 1 week. It is notable that such a time course is similar to that reported for spinal cord injury (Noble and others 2002), suggesting common pathways leading to up-regulation of MMP-9. Neutrophil infiltration is common to both brain and spinal cord injury (Stirling and Yong 2008), and these leukocytes are sources of MMP-9 (Rosell and others 2008). Thus, it is conceivable that the early rise in MMP-9 activity is dictated by this pool of infiltrated leukocytes.

The interaction between extracellular matrix and regulatory MMPs is important in establishing and maintaining synaptic connectivity (Yong and others 2001; Dityatev and Schachner 2003). Recent studies show that MMP-3 degrades many chondroitin sulphate proteoglycans (CSPGs) (Pizzi and Crowe 2007), which are critical to axonal growth and synaptic reorganization. Growing evidence shows MMP-3 is implicated in reactive synaptogenesis and functional recovery. MMP-3 immunoreactivity following TBI and TBI and bilateral entorhinal cortical lesioned rats (TBI + BEC, a model for assessing the interaction between neuroexcitation and deafferentation) is increased within the first several weeks (Falo and others 2006) and is localized to astrocytes within the deafferented neuropil (Kim and others 2005; Falo and others 2006). The distinct temporal pattern of expression during periods of reactive synaptogenesis suggests that MMP-3 may actively modify local extracellular matrix-related proteins and thus may play an important role in the restoration of functionally active synapses after brain injury.

Recent efforts have begun to explore the involvement of MMPs in the injured immature brain. MMPs are developmentally regulated and participate in neuronal migration, dendritogenesis and synaptogenesis, and myelination (Luo 2005; Larsen and others 2006). When seven-day-old rats are subjected to a contusion injury, MMP-2 and MMP-9 protein

levels increase in both the ipsilateral cortex and thalamus between 6 and 24 hours postinjury (Sifringer and others 2007).

In summary, both rodent models of neurotrauma and specimens from human spinal cord show parallels in the sequential protein expression of MMP-9 and MMP-2 within the first several weeks after SCI. That is, MMP-9 shows an early up-regulation followed by increased MMP-2 expression thereafter. In rodents, MMP-9 immunolocalizes in neurons (Yamaguchi and others 2007), macrophages, astrocytes, and blood vessels (Noble and others 2002), whereas MMP-2 localizes to neurons (Yamaguchi and others 2007) and astrocytes (Hsu and others 2006). In the human spinal cord, initial studies suggest that MMP-2 and MMP-9 are limited in expression to macrophages (Buss and others 2007). This limited cellular localization in the human cord, relative to that of rodents, may reflect differences in severity and/or type of injury, species studied, and specificity of the antibodies and their related antigens.

MMPs and the Blood-Brain Barrier

Disruption of the blood-brain barrier accompanies traumatic injury to the brain and spinal cord (Cortez and others 1989; Fukuda and others 1995; Barzo and others 1996; Unterberg and others 1997; Whetstone and others 2003; Suehiro and others 2004; Buss and others 2007). There is both direct and indirect evidence that implicate MMPs in barrier breakdown. Constituents of the barrier, including tight junction-related proteins and the basal lamina, are substrates for MMPs. MMP-2, -3, and -9 are responsible for degradation of the tight-junction proteins, occludin and claudin-5, which form the endothelial barrier, as well as basal lamina proteins, including fibronectin, laminin, and heparan sulfate (Rosenberg and Yang 2007).

Elevated levels of MMPs, particularly MMP-9, parallel the time course for maximal disruption of the barrier. In a murine model of spinal cord contusion injury, barrier disruption to the protein luciferase is maximal at 24 hours postinjury (Whetstone and others 2003), a time point that corresponds to peak activity of MMP-9 (Noble and others 2002; Fig. 1). Moreover, barrier disruption is reduced after spinal cord injury in MMP-9 null mice as well as mice treated with the general MMP inhibitor, GM6001 (Noble and others 2002). Similar findings supporting greater stabilization of the barrier have been reported in spinal cord-injured MMP-12 null mice (Wells and others 2003). Spinal cord-injured MMP-12 null animals show attenuation of blood-spinal barrier breakdown as well as indices of reduced inflammation (decreased infiltration of macrophages and reduced microglial activation). Thus, early vascular disturbances and the emerging proinflammatory state are early signatures of MMP activation.

MMP-mediated barrier disruption has likewise been reported in experimental models of TBI. After partial frontal lobe resection, treatment with MMP inhibitor-1 (an inhibitor of MMP-9 and MMP-2 administered) attenuates barrier disruption and edema (Yamaguchi and others 2007). Following focal cerebral contusion, MMP-9 activity is increased within hours after injury, and treatment with a general MMP inhibitor likewise reduces the acute disruption of the barrier and edema formation (Shigemori and others 2006).

Collectively, these studies provide strong evidence for pathological MMP-directed disruption of the blood-brain barrier after CNS trauma. Although the molecular basis is unclear, studies suggest that dysregulation of MMP activity following trauma is mediated by the mitogen-activated protein (MAP) kinase pathway. Supporting this position is the observation that a MAP-kinase inhibitor significantly reduces MMP-9 activity, as well as MMP-mediated ZO-1 degradation and edema (Mori and others 2002; Maddahi and others 2009).

MMPs as Mediators of Leukocyte Infiltration

Shortly after SCI, infiltrating leukocytes invade the area of damage. The time course of infiltration varies according to the type of leukocyte, and there may be species/strain-related differences (Kigerl and others 2006). In spinal cord-injured rodents, neutrophils appear at the primary lesion site within the first 24 hours postinjury (Carlson and others 1998; Kigerl and others 2006). Blood-borne monocytes/macrophages infiltrate the lesion within the first several days, achieve their highest density by about 1 week postinjury, and persist thereafter for weeks to months (Carlson and others 1998).

Both neutrophils and macrophages are implicated in secondary damage by releasing proinflammatory cytokines, reactive oxygen species, nitric oxide, and proteases (Wang and Feuerstein 2000; Popovich and others 2002). Moreover, macrophages mediate axonal dieback after CNS injury (Busch and others 2009). In contrast to these destructive effects, macrophages also release protective cytokines and growth factors and remove injured tissue debris, which collectively define an environment that is supportive of neuronal regeneration and tissue repair (Popovich and others 2002).

MMPs, particularly MMP-9, are thought to be used by leukocytes to transmigrate across blood vessels. Pharmacologic and genetic approaches support this position. There are fewer neutrophils infiltrating within the lesion epicenter of MMP-9-null compared with the wild-type mice at 24 hours after injury (Noble and others 2002). Neutrophil infiltration is likewise abrogated in spinal cord-injured mice treated with an MMP inhibitor (Noble and others 2002). Rats, when immunologically depleted of neutrophils prior to SCI, show reduced MMP-9 activity in the injured cord, suggesting that these leukocytes are the principal source of this protease in the acutely injured cord (de Castro and others 2000).

Prometalloproteinases (pro-MMPs), particularly pro-MMP-9, are potent ligands of the leukocyte β_2 integrins. The pro-MMP-9/ $\alpha_M\beta_2$ complex on resting neutrophils is primarily detected in intracellular granules, but after cellular activation, it becomes localized to the cell surface. Peptides that bind to the α_M integrin-I domain and inhibit its complex formation with pro-MMP-9 prevent neutrophil migration in an in vitro transendothelial assay. These results suggest that the translocating pro-MMP-9/ $\alpha_M\beta_2$ complex may be part of the cell surface machinery guiding neutrophil migration (Stefanidakis and others 2004).

Following endothelial transmigration, monocytes traverse the subendothelial basement membrane and the interstitial matrix of collagens and fibronectin. This is achieved via MMP-induced cleavage of cell surface molecules, regulation of cellular receptors, and degradation of components of the basement membranes. The requirement of MMP-14 (MT1-MMP) during human monocyte migration and endothelial transmigration has been demonstrated. MT1-MMP is up-regulated by monocytes following their attachment to fibronectin and to tumor necrosis factor α (TNF α)-activated endothelial cells, and anti-MT1-MMP monoclonal antibody impairs monocyte chemotactic protein-1-stimulated monocyte migration on fibronectin, vascular cell adhesion molecule-1, and inter-cellular adhesion molecule-1. In addition, monocyte transmigration across TNF α -activated endothelium is inhibited by anti-MT1-MMP mAb (Matias-Roman and others 2005).

MMP-12, also known as macrophage metalloelastase, is critical for the migration of blood-borne macrophages across the endothelial basement membranes into inflammatory sites. In vivo studies demonstrate the dependency of macrophages on MMP-12 to transmigrate across barriers. This is exemplified in a study in which Matrigel-impregnated sponges were inserted either subcutaneously or into the peritoneal cavity. Ten days later, MMP-12-deficient macrophages were unable to effectively invade the sponges relative to wild-type controls (Shipley and others 1996). It is likely that MMP-12 also influences the migration of

macrophages into the injured cord. Comparisons of cell density of Iba-1-positive elements reveal fewer macrophages and microglia in MMP-12 null mice compared with wild-type (WT) animals (Wells and others 2003).

MMPs and Oxidative Stress

MMPs are regulated by reactive oxygen species (ROS; Alexander and Elrod 2002). Studies have shown that ROS, such as hydrogen peroxide and nitric oxide, increase MMP-2 and -9 activity and decrease TIMPs in an in vitro model of the blood-brain barrier (Rajagopalan and others 1996; Haorah and others 2007).

MMPs, released by polymorphonuclear leukocytes, culminate in neurotoxicity via a ROS-dependent mechanism. When cultured with neurons, toxicity is dependent on MMP activity, ROS, and cytokine release. Conversely, treatment with either an MMP inhibitor or media conditioned by MMP-9 null neutrophils leads to decreased oxidative stress and neurotoxicity (Nguyen and others 2007). Thus, pathways leading to MMP-9 activation and ROS generation are related and interact cooperatively to mediate this neutrophil-driven neurotoxicity.

ROS including nitric oxide or hypochlorous acid also leads to activation of MMPs (Morita-Fujimura and others 2000; Maier and others 2006). This finding is further strengthened by the observation that in transgenic rats overexpressing the antioxidant enzyme superoxide dismutase 1, oxidative stress and MMP-9-mediated blood-brain barrier disruption are attenuated (Morita-Fujimura and others 2000). Increased oxidative stress after injury to the spinal cord also leads to MMP-9 up-regulation, blood-brain barrier disruption, and apoptosis (Yu and others 2008). Thus, increased oxidative stress following trauma appears to be a common junction point, working both downstream of MMP activation to cause greater barrier disruption and neurotoxicity and upstream to feed back into the cycle by increasing MMP activation.

MMPs and Angiogenesis

Angiogenesis is the process of forming new blood vessels from existing ones and requires degradation of the vascular basement membrane and remodeling of the extracellular matrix to allow endothelial cells to migrate and invade into the surrounding tissue. It has become increasingly clear that MMPs contribute more to angiogenesis than simply degrading the extracellular matrix to facilitate invading endothelial cells. MMPs regulate angiogenesis by making angiogenic chemokines/cytokines (such as soluble Kit ligand, interleukin-8) bioavailable, as well as releasing angiogenic inhibitors, including tumstatin, endostatin, and angiostatin. In addition, MMPs are also implicated in cell mobilization, including inflammatory cells, endothelial cells, and stem cells. Therefore, MMPs finely tune the biologic process of angiogenesis (Heissig and others 2003).

Angiogenesis has been well characterized in the rodent spinal cord after contusion injury (Casella and others 2002; Loy and others 2002; Whetstone and others 2003). Endothelial cell breakdown and death, as well as blood-spinal cord barrier breakdown, are evident 1 to 3 days after injury. Between 3 and 7 days, the early appearance of vascular elements, associated with increased laminin staining, define the early angiogenic period (Whetstone and others 2003). By 14 days, there are an increased number of vessels, including large-diameter vessels. No increases in blood vessels are noted between 14 and 28 days, suggesting completion of revascularization of the injured cord (Whetstone and others 2003).

Several lines of indirect evidence suggest that MMPs are integral to angiogenesis after SCI. Gelatinase activity is noted in blood vessels with an angiogenic phenotype within 1 to 3 days

postinjury, coinciding with maximal active expression of MMP-9 (Goussev and others 2003). The subsequent increase in vascular-related gelatinase activity parallels the gradual emergence of MMP-2 activity (Goussev and others 2003). Although not studied in CNS trauma, MT1-MMP is another member of the MMP family that may be integral to angiogenesis. This is suggested by studies showing that tips of platelet endothelial cell adhesion molecule (PECAM)-positive blood vessels contain MT1-MMP and that this protease regulates cell migration and invasion (van Hinsbergh and Koolwijk 2008).

MMPs, Glial Scar Formation, and Axonal Regeneration

MMPs modulate the formation of a glial scar and its composition. In a rat hemisection model, in situ fluorescent zymography reveals MMP-related gelatinase activity in the injured site, which is spatially and temporally correlated with scar formation (Duchossoy and others 2001). Such a finding raises the possibility that MMPs facilitate migration of astrocytes. In vitro and in vivo data support this possibility. In vitro scratch wound assays show attenuated migration of cultured MMP-9 null astrocytes or astrocytes treated with an MMP-9 inhibitor (Hsu and others 2008). Moreover, glial scar formation is abrogated in spinal cord-injured MMP-9 null animals (Hsu and others 2008).

In addition to facilitating migration of astrocytes to form a glial scar, MMPs also influence its composition. The glial scar is an interface that is inhibitory to axonal regeneration. Accordingly, scar-modulating treatments have become a main therapeutic goal in the field of SCI (Steinmetz and others 2005). The glial scar consists predominately of reactive astrocytes, microglia/macrophages, and extracellular matrix molecules, especially chondroitin sulfate proteoglycans (CSPGs; Silver and Miller 2004). In response to injury, astrocytes, oligodendrocyte progenitors, and macrophages increase the expression of CSPGs, which in turn inhibit neurite outgrowth in vitro and regeneration in vivo (Fitch and Silver 1997; Bradbury and others 2002; Jones and others 2002). CSPGs, such as neurocan and versican, are degraded by MMP-2, whereas tenascin-C, brevican, neurocan, NG2, phosphacan, and versican are degraded by MMP-3 (Pizzi and Crowe 2007). There is also evidence that MMP-9 degrades CSPGs as spinal cord-injured mice deficient in MMP-9 who show reduced CSPG immunoreactivity at the lesioned epicenter (Fig. 3; Hsu and others 2008). By degrading inhibitory CSPGs, MMPs support axonal regenerative potential in the injured CNS (Yong 2005; Pizzi and Crowe 2007).

In vitro evidence supports the hypothesis that MMPs facilitate regeneration of axons. When primary dorsal root ganglion neurons are grown on sections of normal adult nerves, neurite outgrowth is enhanced in the presence of active MMP-2, which degrades inhibitory CSPGs to expose permissive laminin (Zuo and others 1998). The inhibitory effect of CSPGs on neurite extension of dorsal root ganglia neurons is also effectively eliminated by recombinant MT5-MMP (Hayashita-Kinoh and others 2001).

In vivo data likewise implicate MMPs in plasticity. For example, spinal cord-injured MMP-2 deficient mice show fewer serotonergic fibers caudal to the injured site compared to wild-type mice (Hsu and others 2006). Such a finding may result from reduced sprouting across the lesioned site.

Besides the axon inhibitory properties of CSPGs, the other major group of molecules involved in inhibiting axonal regeneration is myelin-associated inhibitory proteins, including Nogo, myelin-associated glycoprotein (MAG), and oligodendrocyte myelin glycoprotein. These proteins inhibit neurite outgrowth by binding the neuronal glycosylphosphatidylinositol-anchored Nogo-66 receptor (NgR), which then transduces the inhibitory signal intracellularly via a transmembrane coreceptor, p75 neurotrophin receptor (p75^{NTR}). Recent studies demonstrate that N-terminal fragment cleavage of the NgR

generated by MMPs binds to the Nogo66 domain of NogoA, thus blocking its binding to intact NgR (Walmsley and others 2004). Furthermore, membrane-type 1-MMP (MT1-MMP) directly degrades bNI-220 (now known as Nogo). MT1-MMP-transfected fibroblasts are able to convert the nonpermissive substrate into a permissive one for cell spreading and migration. Western blots demonstrate disappearance of the bNI-220 band when bNI-220 is preincubated with MT1-MMP-transfected fibroblasts (Belien and others 1999). Taken together, MMPs alter inhibitory signaling via NgR or directly by removing the ligands to NgR. There are no data to show that MMPs remove oligodendrocyte myelin glycoprotein, but a recent report has shown that MMP-2, MMP-7, and MMP-9 degrade recombinant human MAG, and the fragment generated by these MMPs inhibits axonal growth (Milward and others 2008). In this context, activities of MMPs could enhance inhibitory signaling for axonal regeneration.

Finally, the complexity of MMPs in the setting of neurotrauma is further realized in the context of axonal dieback. After spinal cord injury, infiltrated macrophages mediate long-distance axonal retraction from the initial site of injury. The involvement of MMPs in dieback is supported by in vitro studies showing that an MMP inhibitor or a specific MMP-9 inhibitor prevents macrophage-induced axonal retraction (Busch and others 2009).

In summary, MMPs limit the formation of an inhibitory glial scar and the expression of myelin-associated inhibitory proteins, thus supporting recovery processes, and they also mediate adverse responses, including axonal dieback and the generation of degradation products that are inhibitory to axonal growth.

MMPs and Myelin

Several lines of evidence link MMPs to demyelination. MMP-9 degrades myelin basic protein, the major protein component of the myelin sheath (Kobayashi and others 2008). MMPs, particularly MMP7 and MT4-MMP, are involved in the conversion of precursor TNF α to its active form that is toxic to oligodendrocytes (English and others 2000). Finally, genetic and pharmacologic studies demonstrate greater preservation of myelin basic protein in the injured brain and spinal cord (Wang and others 2000; Noble and others 2002).

MMPs are also implicated in remyelination. As a first step in this process, oligodendrocytes extend multiple processes toward the axons. Oligodendrocytes express and use MMP-9 to facilitate process extension along an astrocyte-derived extracellular matrix in vitro (Uhm and others 1998). Extension is significantly retarded in oligodendrocytes cultured from MMP-9 null mice or when wild-type oligodendrocytes are treated with either TIMP-1 or a neutralizing MMP-9 antibody (Oh and others 1999).

In vivo studies demonstrate the dependency of remyelination on MMP-9. Remyelination after lysolecithin-induced demyelination is impaired in MMP-9 null mice (Larsen and others 2003). This may be explained at least in part by the failure to clear NG2, an inhibitory CSPG deposited in the extracellular matrix during the demyelination phase. NG2 may prevent the maturation and differentiation of oligodendrocytes that are needed for remyelination (Larsen and others 2003). In vitro evidence has also shown a role for MMP-12 in myelination. The maturation of oligodendrocyte precursor cells to oligodendrocytes is significantly reduced in cultures from MMP-12 null mice compared with wild-type controls (Larsen and Yong 2004).

Therapeutics

Very few studies have examined efficacy of MMP inhibitors in CNS trauma. The overall impression is that early blockade of MMPs stabilizes the barrier, reducing edema formation,

and confers both early and long-term neuroprotection. In rodent models of brain and spinal cord injury, treatment with MMP inhibitor-1 (an inhibitor of MMP-9 and MMP-2) or a general MMP inhibitor attenuates early barrier disruption and edema (Noble and others 2002; Shigemori and others 2006; Yamaguchi and others 2007). Moreover, short-term treatment with a general MMP inhibitor results in a significant and dose-dependent neuroprotection in the injured immature brain (Sifringer and others 2007) and enhanced long-term motor recovery and greater sparing of white matter in the injured spinal cord (Noble and others 2002).

Despite the promising results of these initial studies, there are concerns about MMPs as therapeutic targets, particularly with regard to the window of intervention. This is best illustrated in a study where an MMP inhibitor was administered over a period of one week after SCI (Trivedi and others 2005). In contrast to the beneficial outcomes when given for the first three days, no improvement was seen when treatment was extended to one week. It has become increasingly clear that MMPs are modulators of both early injury and recovery processes. In their absence, favorable wound-healing events supporting neurovascular plasticity may be substantially impaired (Rosell and Lo 2008).

Summary

How MMPs influence injury and recovery processes is dependent on a number of factors that include when and where they are expressed and the profile of available substrates. As such, they are implicated in both injury and recovery processes (Fig. 4). In general, studies of CNS trauma support the view that early MMP activity is detrimental in part by promoting barrier dysfunction and early inflammation. Consistent with this position, pharmacologic strategies need to block early MMP activity, stabilize the barrier, attenuate the early inflammatory response, and result in improved neurologic outcomes. Involvement of MMPs during wound healing is more complex. Both genetic and pharmacologic studies suggest that MMPs modulate key events that likely influence neurologic outcome, including glial scar formation, demyelination and remyelination, angiogenesis, and axonal plasticity. Thus, although broad-spectrum MMP inhibitors given acutely after injury support recovery, longer term treatment may result in no or poorer recovery. The development of more specific MMP inhibitors together with genetic approaches that allow for the temporal expression/deletion of specific MMPs will better define how members of the MMP family exert multifunctional roles in injury and recovery processes.

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Figure 1.

Immunolocalization of MMP-9 in the contused spinal cord. MMP-9 is immunolocalized in the uninjured (A, B) cord and 24 hours after injury (C, D). Note that MMP-9 is expressed in motoneurons in the ventral horn (arrowheads, B) of the uninjured cord. After injury, this protease is noted in structures that resemble blood vessels (arrows, D) and inflammatory cells (arrowheads, D). Scale bars A, C, 500 μ m; B, D, 50 μ m. (E) Gelatin zymography of the cord reveals prominent activity of MMP-9 by 24 hours after injury in wild-type (WT) mice, whereas no changes are seen with regard to MMP-2 activity. As might be expected, activity is not noted in spinal cord-injured MMP-9 null animals. 1,10 Phenanthroline, a general inhibitor of matrix metalloproteinases (MMPs), completely blocks activity of MMP-9 and MMP-2, thus confirming specificity. Modified from Noble and others (2002) with permission.

Figure 2.

Regulation of matrix metalloproteinases (MMPs). MMPs are regulated at several levels. Diverse biological signals (a), such as soluble factors, cell-cell interactions, and cell-matrix interactions, initiate a cascade of events that lead to RNA transcription (b), posttranscriptional mRNA processing (c), mRNA degradation (d), protein synthesis (e), intracellular activation of furin-susceptible MMPs (f), intracellular trafficking and secretion (g), extracellular localization (h), activation of the zymogen form by other activated MMPs or by several serine proteinases that cleave peptide bonds within MMP prodomains (i), endogenous protein inhibitors (j), and endocytosis and intracellular degradation (k).

Figure 3.

Chondroitin sulfate proteoglycans (CSPGs) and the injured murine spinal cord. CSPGs are immunolocalized using clone CS-56 in spinal cord-injured wild-type (A) and MMP-9 null (B) animals. By 2 weeks postinjury, there is a more prominent expression of CSPGs in the wild-type relative to the null animal. Scale bar B, 100 μ m. Modified from Hsu and others (2008) with permission.

Figure 4.

Matrix metalloproteinases (MMPs) in pathogenesis and wound healing. MMPs have multiple outcomes in the CNS after traumatic injury. In the acute period, leukocytes express and use MMPs to facilitate their entry into the damaged tissue. This process involves disruption of tight junctions between adjacent endothelial cells and the subsequent degradation of the surrounding basement membrane that collectively culminate in breakdown of the blood-brain barrier. MMPs are up-regulated in activated microglia, astrocytes, and infiltrated leukocytes. The resultant elevated local levels of MMPs are thought to contribute to demyelination and neuronal and oligodendrocyte death during a subacute period of hours to weeks after injury. Although they are perhaps best known for these detrimental effects, there is growing evidence that MMPs also support recovery processes during wound healing by promoting remyelination and angiogenesis. They may also facilitate the regenerative potential of injured axons by degrading various inhibitory extracellular proteins associated with the glial scar, transforming the injured CNS into a more growth-permissive environment. NO, nitric oxide; ROS, reactive oxygen species; IL-1, interleukin-1; TNF α , tumor necrosis factor α ; BBB, blood-brain barrier.

Table 1**Methods for Detecting MMPs: Pros and Cons**

Methods	Strength	Limitations
Gelatin zymography	Both pro-form and active forms detected	Limited to those MMPs expressing gelatinase activity; lacks cellular resolution
Western immunoblots	Protein expression in homogenates	Dose not measure activity; lacks cellular resolution
Immunohistochemistry	Protein expression at cellular level	Antibodies may cross-react with other MMPs; does not discriminate active from inactive MMPs
In situ hybridization	mRNA expression at cellular level	Is not necessarily predictive of protein or activity
Quantitative PCR	mRNA for multiple MMPs in homogenates	Is not necessarily predictive of protein or activity
Enzyme-linked immunoassays	Protein in blood and tissue fluids	Antibodies may cross-react with other MMPs; does not discriminate active from inactive MMPs
Activity assays	Proteinase activity in homogenates	Lacks specificity for a particular MMP; subject to sample interference
In situ gelatinase zymography	Cellular resolution of activity	Limited to gelatinases; subject to substrate specificity

Each of the current methods for detecting matrix metalloproteinases (MMPs) has its advantages and disadvantages. Development of a comprehensive picture of MMP involvement in neurotrauma or other in vivo disease models requires a clear understanding of where and when a given MMP is active. There are complementary tools that provide tissue to cellular resolution. These include RT-PCR and in situ hybridization to measure mRNA; immunoblotting, enzyme-linked immunosorbent assays, and immunohistochemistry to assay protein levels; and activity assays and in situ zymography, which are indices enzymatic activity.

Table 2**Immunoexpression of MMPs in Normal Adult CNS**

MMP	Species	Localization	Antibody (Source)	References
MMP-1	Human	Spinal cord: Motoneurons	Monoclonal (Chemicon, USA)	Buss and others (2007)
MMP-2	Rat	Brain: Neurons, astrocytes, endothelium	Polyclonal (Chemicon, USA)	Planas and others (2001)
	Rat	Spinal cord: Dorsal root ganglion neurons	Not specified	Kawasaki and others (2008)
	Human	Brain: Astrocytes and microglia	Polyclonal (Biogenesis Ltd., UK)	Cuzner and others (1996)
	Human	Brain: Microglia	Monoclonal (R&D Systems, USA)	Rosenberg and others (2001b)
MMP-3	Human	Brain: Microglia	Gift from H. Nagase (Imperial College, UK)	Rosenberg and others (2001b)
MMP-9	Mouse	Spinal cord: Meninges and ventral horn motoneurons	Gift from Zena Werb (University of California, San Francisco, USA)	Noble and others (2002)
	Rat	Spinal cord: Dorsal root ganglion neurons	Polyclonal (Chemicon, USA)	Kawasaki and others (2008)
	Rat	Brain: Neurons, myelinated fiber tracts	Monoclonal (Chemicon, USA)	Planas and others (2001)
	Human	Brain: Astrocytes and microglia	Polyclonal (Biogenesis Ltd., Poole, UK)	Cuzner and others (1996)
MMP-12	Human	Spinal cord: Microglia and astrocytes	Monoclonal (R&D Systems, USA)	Vos and others (2003)
MMP-19	Human	Brain: Microglia in white matter	Polyclonal (Affinity BioReagent, USA)	Van Horssen and others (2006)
MT3-MMP (MMP-16)	Human	Brain: Microglia in white matter and gray matter	Monoclonal (Fuji Chemical Industries, Japan)	Yoshiyama and others (1998)
MT5-MMP (MMP-24)	Mouse	Brain: Neurons in granular layer of cerebellum and dentate gyrus of hippocampus	Polyclonal (generated by authors)	Hayashita-Kinoh and others (2001)

Matrix metalloproteinases (MMPs) have been immunolocalized in the human and rodent brain and spinal cord. What has emerged from this collective effort is the finding that MMPs are most consistently localized in glia (microglia, astrocytes), and there is limited evidence for their expression in neurons. MMP-2 and -9, the most studied of the MMPs in the CNS, show this localization based on a variety of both monoclonal and polyclonal antibodies.

Table 3

Summary of MMPs Studied in Experimental Models of Neurotrauma

Type	Common Name	Location of Injury	Type of Injury	Change in MMP	Peak Time for Change	Localization	References
MMP-1	Collagenase 1	Spinal cord	Contusion	Increased activity	24 hours	Neurons, glia	Xu and others (2001)
MMP-2	Gelatinase A	Spinal cord	Contusion	Increased activity	7–14 days	Astrocytes	Hsu and others (2006)
		Adult brain	Closed head acceleration impact	Increased activity	24–48 hours	ND	Ding and others (2009)
		Adult brain	Surgical excision	Increased activity	72 hours	Neurons, neutrophils	Yamaguchi and others (2007)
		Immature brain	Contusion	Increased activity	24 hours	ND	Sifringer and others (2007)
MMP-3	Stromelysin 1	Spinal cord	Compression	Increased transcripts	24 hours	ND	Wells and others (2003)
		Adult brain	Cortical contusion	Increased activity	1–24 hours	ND	Shigemori and others (2006)
		Adult brain	Fluid percussion	Increased protein	2 days	Astrocytes	Falo and others (2006)
		Adult brain	Fluid percussion + bilateral entorhinal lesion	Increased protein	2 and 7 days	Astrocytes	Falo and others (2006)
MMP-9	Gelatinase B	Spinal cord	Contusion	Increased activity	24 hours	Astrocytes, macrophages, blood vessels	Noble and others (2002)
		Adult brain	Fluid percussion	Increased activity	4 hours to 5 days	ND	Truettner and others (2005)
		Adult brain	Intracerebral hemorrhage	Increased activity	72 hours	Neurons and blood vessels	Wang and Tsirka (2005)
		Adult brain	Closed head acceleration impact	Increased activity	1–48 hours	ND	Ding and others (2009)
		Adult brain	Surgical excision	Increased activity	72 hours	Neurons, neutrophils	Yamaguchi and others (2007)
		Immature brain	Contusion	Increased transcripts	24 hours	ND	Sifringer and others (2007)
MMP-7	Matrilysin				24 hours		
MMP-10	Stromelysin 2				24 hours		
MMP-11	Stromelysin 3				24 hours		
MMP-12	Metalloelastase	Spinal cord	Compression	Increased transcripts	5–14 days	ND	Wells and others (2003)
MMP-13	Collagenase 3				5 days		
MMP-19	Enamelysin				24 hours		
MMP-20					24 hours		
MMP-23	Femalysin				1–2 days		
MMP-24	MT5-MMP	Spinal cord	Compression	Decreased transcripts	2–5 days	ND	Wells and others (2003)

The changes in matrix metalloproteinase (MMP) expression or activity have been studied in several models of injury to the CNS. The results collectively describe a cell-type and temporally specific pattern for various MMPs during the development of traumatic injury and its resolution. ND, not determined.