REGULATION AND FUNCTION OF MATRIX METALLOPROTEINASES IN NERVOUS SYSTEM INJURY AND NEUROPATHIC PAIN

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Abstract

The balance between expression of matrix metalloproteases (MMPs) and their endogenous tissue inhibitors (TIMPs) has important implications for the healthy nervous system as well as pathogenesis and recovery in disease or injury. The functional consequences of MMP activity depend on the cellular localization, tissue distribution, and temporal pattern of MMP expression. MMP expression and activity is controlled by intracellular and extracellular signaling molecules, cell surface receptor activity, transcription factors, proteolytic activation of inactive MMP proforms, and inhibition by TIMPs. Evidence that MMPs are not only effectors of tissue damage, but may be operational in repair mechanisms has indicated the need to re-examine the role of MMPs after acute processes such as stroke and traumatic spinal cord injury, as well as in chronic conditions such as neuropathic pain. Here we review recent evidence for a beneficial and harmful role for MMPs following central and peripheral nerve injury, as well as their involvement in neuropathic pain following such injury. Finally, therapeutic potential of modifying MMP activity is discussed and considerations for future directions.

Key Words: MMP, TIMP, Peripheral nerve injury, Brain injury, Spinal cord injury, Neuropathic pain.

Introduction

Matrix metalloproteases (MMPs) are a family of zinc-dependent extracellular proteases that participate in the modification of the extracellular matrix (ECM). In addition to cleavage of a wide range of ECM components including collagen, proteoglycan and laminin , MMPs are able to process a number of cell surface and soluble proteins including growth factors, adhesion molecules, receptors, cytokines and chemokines (1). The large number of available substrates indicates the complex role that MMPs play in development, remodeling, signaling, and inflammation within the nervous system.

Tissue inhibitors of matrix metalloproteases (TIMPs) are endogenously expressed proteins that can prevent the activation of the proforms of MMPs and block the proteolytic activity of all known MMP isoforms. The TIMP family includes TIMP-1 through TIMP-4, all of which are able to inhibit MMP activity. The different numbers correspond other properties such as expression patterns, regulation, and efficiency of specific MMP-isoform inhibition (2). Inhibition of MMP activity by TIMPs is one of the primary means of MMP regulation, and the expression of these two families of proteins may be intricately involved in the modulation of numerous processes within the nervous system, especially the response to injury.

Structural Description of MMPs

MMPs are one family within a large superfamily of zinc endopeptidases known as metzincins. Other members of the superfamily are astacins, serralysins, and adamalysins. Metzincins are referred to as endopeptidases because they cleave internal peptide bonds of their substrates. Metzincin proteinases share a highly conserved zinc-binding motif and the common ability to degrade components of the ECM and other bioactive molecules (3). Many members of the metzincin superfamily function in similar roles as MMPs; for example, adamalysins, also known as ADAMs (a disintegrin and metalloproteinase domain), are extensively involved in shedding of growth factors and receptor domains on the cell surface, modulating intracellular and extracellular signaling pathways crucial for development, normal physiology, and disease pathology (4). To date, 24 MMPs have been discovered in humans and most are secreted as inactive zymogens that require activation by extracellular proteases or free radicals (1, 4).

They are composed of a specific domain structure that typically consists of propeptide, catalytic, and hemopexin-like, four bladed β-propeller domains. The propeptide domain maintains MMPs in the inactive form until proteolysis and the catalytic domain contains the zinc binding motif. The hemopexin-like domain is important for cleavage of substrates and appears to be an important site for TIMP inhibition. Some MMPs also possess transmembrane domains and function as membrane bound proteases (MMP-14, MMP-15, MMP-16 and MMP-24). MMP-2 and MMP-9, also referred to as gelatinases, contain fibronectin-like domains that allow them to bind to their collagen substrates.

TIMPs primary role is to limit MMP proteolysis, although there is evidence that they are involved in the activation of pro-MMPs. TIMPs are six-loop disulphide-bonded proteins that bind in the active site cleft of the catalytic domain, blocking MMP proteolytic activity (5). In addition to TIMP inhibition, MMP proteolytic activity is regulated by intracellular signaling cascades, transcription, post-transcriptional events, zymogen activation, and intra/extracellular localization.

Cell and Tissue Distribution of MMPs

MMPs are expressed by a vast array of cell types and tissues throughout the body. This is evidenced by the participation of MMPs in both normal and pathological processes including but not limited to: embryonic development, organ morphogenesis,

nerve growth, ovulation, cervical dilation, hair follicle cycling, bone remodeling, angiogenesis, apoptosis, arthritis, cancer, CV disease, periodontal disease, skin ulceration, gastric ulcers, liver fibrosis, emphysema and neurological diseases (6). This section will focus on the cell and tissue specific distribution of MMPs in the healthy nervous system. An analysis of how this distribution changes in states of disease and injury will appear in later sections about the brain, spinal cord, and PNS.

MMP baseline expression is generally at very low levels in the normal physiological state, making detection with traditional immunohistochemical techniques difficult (7,8). It has been shown that small amounts of MMP-2 and MMP-9 are constitutively expressed in neurons (1). Studies have also demonstrated that microvessel endothelial cells, some astrocytes, and microglia within the CNS are able to express pro-MMP-2 and pro-MMP-9 (9, 10) MMP-2 is typically expressed in astrocytes, while MMP-9 is expressed in microglia (11). In fact, the primary sites of MMP expression in the normal human CNS are the perivascular and parenchymal microglia of the white matter (12). When MMPs are expressed in astrocytes, they are usually localized to the end-feet (13). MMP-2 and MMP-9 levels vary among brain regions with the highest levels of expression in the hippocampus, supporting their role in memory formation, and the lowest levels in the cerebellum (14). Additionally, TIMP production is very strong in the deep cortex and white matter of the brain. This may explain why axon regeneration is inhibited in this region (15). Studies of the spinal cord have shown MMP-9 expression in motoneurons and the meninges (16). The participation of MMPs in neuron growth within the PNS is supported by the identification of MMP-2 and MMP-9 in the Schwann cell basal lamina where it is believed to participate in the degradation of CSPG, an inhibitor of endoneurial laminin (17). MMPs and TIMPs are expressed by endothelial cells, neurons, glial cells, and Schwann cells of the CNS and PNS. They are important in ECM maintenance, synaptic modification, endothelial barrier integrity, and nerve growth.

Transcriptional Regulation of MMPs

Stress and injury to the nervous system results in a complex signaling cascade beginning with the release of inflammatory cytokines (IL-1β and TNF-α) from macrophages, neurotransmitters from neurons, and many other molecules from diverse cell types (13). All of these molecules are able to bind specific cell surface receptors and initiate intracellular cascades that eventually result in the activation of transcription factors and the expression of specific genes. While some MMP expression is constitutive, for the purpose of this review, we will focus on activated receptors and downstream signaling that is relevant for the transcription of inducible MMPs and TIMPs (4).

Harmful stimuli such as mechanical disturbances and reactive oxygen species (ROS) are capable of directly activating intracellular transcription factors such as activator protein 1 (AP-1) and nuclear factor-κB (NF-κB). These transcription factors represent the first line of defense because their activation results in the transcription of inflammatory mediators (IL-1β, TNF-α, MMPs, etc.) and other signaling molecules. AP-1 is a heterodimeric protein composed of proteins from the c-Fos and c-Jun families. MMP-2, MMP-9, and TIMP-1 genes contain AP-1 transcription factor sites and their expression is enhanced by active AP-1. Furthermore, MMP-2 and MMP-9 are upregulated by NF-κB pathways during the inflammation response of microglia and astrocytes (9,18).

AP-1 and NF-κB can also be activated by mitogen activated protein kinase (MAPK) pathways, eventually leading to MMP expression. Activation of MAPK pathways begin with binding of ligands to cell surface receptors. Ligand binding to cannabinoid (CB), transient receptor potential vanilloid 1 (TRPV1), IL-1β and TNF-α receptors has been shown to modulate numerous intracellular signaling processes including MAPK pathways. Extracellular signal-regulated kinase (ERK 1/2) and p38 MAPK have been shown to be upregulated following mechanical and ischemic brain injury (19). Inhibition of ERK 1/2 and p38 kinase pathways significantly reduces MMP-9 expression after injury. It has also been demonstrated that inhibition of p38 and ERK in rat cortical astrocyte culture reduces TNF-α-induced MMP-9 expression (18). IL-1β is a key regulator of neuronal MMP-9 levels in cell culture and MMP-9 upregulation that occurs following mouse CNS trauma (20). Alcohol and ROS induce activation of protein tyrosine kinase receptors, which can also activate ERK and p38 MAPK pathways and stimulate MMP expression (21). It has also been shown that alcohol reduces TIMP-1 and TIMP-2 levels possibly via a similar mechanism (22).

TRPV1 receptors are activated by many different stimuli including inflammatory cytokines and their activity has been linked to increased MMP expression. Direct activation of TRPV1 by capsaicin, a TRPV1 agonist, showed increase in MMP-1 expression. Furthermore, heat shock induced calcium influx through TRPV-1 and extracellular calcium is required for MMP-1 expression in HaCaT cells indicating that TRPV-1 mediated MMP-1 expression is calcium dependent (23). Activation of TRPV-1 results in increased intracellular calcium, from both extracellular and intracellular stores. This rise in intracellular calcium serves to trigger variable signaling cascades and feedback mechanisms, of which many are still not fully understood (24). One possible pathway involves the rapid increase of intracellular calcium leading to increased adenylate cyclase activity, which in turn causes increased levels of cAMP (24). Increased cAMP is able to activate PKA, which further sensitizes TRPV-1 and activates the transcription factor cAMP response element-binding, CREB, by phosphorylation. CREB has been shown to induce the transcription of MMP-2 (25).

CB1 and CB2 are two homologous, cannabinoid activated, G-protein coupled receptors, whose activation impacts a broad range of intracellular signalling pathways including intracellular Ca-dependent pathways, adenylyl cyclase mediated cAMP production, and MAPK activity. Agonist binding to the CB1 receptor has been shown to reduce Ca levels in the cell and increase inward-rectifying K currents through direct coupling with Ca-Q and inward-rectifying K channels [24, 26]. These effects tend to reduce neuronal excitability and neurotransmitter release in the CNS. CB receptors also modulate adenylyl cyclase activity through interactions with Gs and Gi/o proteins. CB1 can couple

with either Gs or Gi/o depending on the specific ligand, ligand concentration, cell type, and availability of Gs/Gi/o, thus, Agonist binding to CB1 has complex effects on cAMP levels. Conversely, CB2 only couples with Gi/o and has an inhibitory effect on adenylyl cyclase cAMP production (26).

Another important downstream signaling effect of CB receptor activation is the modulation of MAPK pathways. Experiments in various cell lines have shown that agonist binding to the CB1 receptor has a stimulatory effect on p38 and ERK1/2 MAPK pathways, while activation of the CB2 receptor solely induces activation of p38 MAPK. There are well established links between MAPK activation and the expression c-Fos and c-Jun which form the AP-1 transcription factor complex (27). Additional evidence links CB receptor binding and MAPK activation to MMP and TIMP gene regulation. While baseline MMP expression is low in unstimulated cells, many studies have shown that upon stimulation following injury, MMP and TIMP gene transcription is modulated by MAPK activated c-Jun and c-Fos transcription factors (28). Treatment of fibroblast cell lines with Oncostatin M (OSM), an inflammatory cytokine, stimulated TIMP-1 mRNA expression, and inhibition of p38 and ERK1/2 phosphorylation abrogated this effect, indicating that MAPK phosphorylation is required for OSM-induced TIMP-1 expression. Additionally, OSM rapidly induces c-Jun expression which directly drives TIMP-1 promoter activity in NIH 3T3 cells (29). Other studies have shown that treatment of cancer cells with anandamide, a CB1/2 agonist, reduces cancer cell invasiveness by increasing expression TIMP-1/2 (30). Increased TIMP-1/2 expression induced by cannabinoids may be an effective mechanism for reducing the secondary pathogenesis of the inflammation response by blocking the activity of MMPs. These observations suggest cannabinoid and TRPV-1 receptor signalling may be important targets in chronic pain mechanisms because of their downstream modulation of calcium, cAMP, and MAPK signaling pathways that induce AP-1 (Fig. 1).

The transcriptional regulation of MMPs and TIMPs is very complex and still not well understood. It is becoming apparent that the major players are inflammatory cytokines (IL-1β and TNF-α), ROS, and cell surface receptors (TRPV1 and CB) which modulate transcription factor (AP-1, NF-κB and CREB binding protein) activity via MAPK and Ca-dependent downstream signaling pathways.

Pro-MMP Activation

After transcription of MMP mRNAs, post-transcriptional modification, and translation, the next major step in MMP regulation is activation of pro-MMPs. With the exception of MMP-23, which lacks the pro-peptide domain, and MMP-11 and MP-14, which are activated intracellularly by furin, MMPs are released as inactive zymogens that require proteolytic activation (6). Most MMPs are activated by extracellular cleavage of an approximately 80 amino acid pro-peptide sequence at C-terminal end of the protein. Activation requires disruption of zinc binding in the catalytic domain followed by removal of the pro-peptide domain. Tissue and plasma proteinases are the usual agents of MMP activation *in vivo*, but other compounds including sulfhydril reactive agents, mercurial compounds, oxygen ions, free radicals, and peroxides can activate pro-MMPs (21, 31, 32).

There is considerable evidence implicating membrane bound MMPs as major activators of pro-MMPs beginning with the identification of membrane-type 1 MMP (MT1-MMP) as an activator of pro-MMP-2 (33). Recent studies have also shown that TIMP-2 plays a role in pro-MMP-2 activation. TIMP-2 binds to MT1-MMP on the cell surface and then recruits pro-MMP-2 to the cell membrane through interactions between the TIMP-2 C-terminal domain and the pro-MMP-2 hemopexin domain. Once pro-MMP-2 is co-localized near the active MT1-MMP, its pro-peptide sequence is cleaved and it becomes an active protease (6). Pro-MMP-2 can also be directly activated by MT3-MMP and indirectly by urokinase through its proteolytic conversion of plasminogen to plasmin. Plasmin is able to directly activate pro-MMP-2 (34). These studies demonstrate that the regulation of MMPs is dependent on the levels and activity of many different proteases and other reactive species.

MMP Inhibition by TIMPs

TIMP inhibition of MMP activity is critical in order to maintain a healthy balance of ECM turnover because it prevents rampant breakdown of ECM proteins. The most common mechanism of TIMP inhibition of MMP activity involves binding of the N-terminal amino acid of the TIMP protein and the zinc ion coordinated to the MMP. This interaction between TIMPs and the catalytic domain of MMPs results in conformational changes that prevent MMP proteolytic activity (6). Four homologous TIMPS have been identified to date. TIMPs 1-4 appears to have very little specificity for binding the catalytic domains of specific MMPs. TIMP-4, similar to the other TIMP isoforms, has been shown to inhibit multiple MMPs including MMP-1,-2,-3,-7, and -9. There is evidence that TIMP-1 and -2 can bind the hemopexin domain of pro-MMP-9 and -2, respectively, resulting in complex effects on the activation process. TIMP-4 is similar to TIMP-2 in that it can also bind and inhibit activation of pro-MMP-2 (35). The balance between MMPs and TIMPs is important in normal physiology and pathology, and it is becoming clear that the role of TIMPs in this balance is much more expansive than solely *in vivo* inhibition.

MMPs in Normal Physiology

In a healthy, functioning nervous system MMPs primary role involves maintenance of the ECM by facilitating the turnover of the primary components of the ECM. MMP-2 and MMP-9 have been specifically implicated in the degradation of Type IV collagen, the major component of the basement membrane (36). In the CNS, ECM maintenance involves clearance of inhibitory chondroitin sulfate proteoglycans (CSPGs) and components of the endothelial basement membrane of cerebral and spinal microvasculature (37). MMPs are also important because they prevent the accumulation of numerous other substrates such as cytokines and growth factors in the ECM (38). Studies of CNS injury in MMP knockout mice have demonstrated decreased myelinogenesis, decreased angiogenesis, and abnormal CNS cell morphology indicating that MMPs may play an integral role in

angiogenesis, myelin turnover, and axonal physiology (39, 40). MMPs are also involved in numerous developmental processes of the nervous system (39) and participate in synaptic plasticity associated with long term learning and memory. Studies in a rodent model of inhibitory-avoidance learning and memory tasks have shown that MMP-9 expression is required for proteolysis that accompanies synaptic modification suggesting that MMPs are very important for long-term memory formation (41). Another significant aspect of MMP function in normal physiology is their ability to act as sheddases on extracellular portions of transmembrane proteins. They can activate transmembrane receptors by cleaving extracellular domains or they can remove agonist bound portions of the protein. By releasing growth factors, death receptors, and death-inducing ligands such as tumor necrosis factor, MMPs are involved in the signaling processes that regulate cell survival and death in the nervous system (4).

TIMPs also have an important role in normal nervous system development and function. They are expressed in similar patterns to MMPs in neurogenesis and neuronal migration within the developing CNS. The regional and cell specific expression of TIMP isoforms is important for every stage of postnatal cerebellar development in a rat model. This suggests that the ratio of MMPs to TIMPs and the resulting balance of ECM proteolysis is a critical component in the development of a healthy CNS (42). Studies in TIMP-1 knockout and overexpressing mice have also demonstrated that TIMP-1 expression is important in other adult CNS functions including neuronal death, axonal sprouting, and hippocampus dependent memory and learning (43, 44). Taken together, this evidence suggests that the expression ratio of MMPs to TIMPs is crucial for a variety of physiological processes in the nervous system including ECM proteolysis-dependent CNS development, synaptic plasticity, cognitive function, neuronal structure, and survival/death related cell signaling.

MMPs in Disease and Injury

The role of MMPs in the pathology of disease and injury is multifaceted. MMP action following disturbances of the nervous system varies in acute versus chronic phases and MMPs can serve both neurotoxic and neuroprotective functions depending on temporal and cell specific gene expression patterns, tissue distribution, MMP isoforms, activation of inactive pro-MMPs, and the level of TIMP inhibition. MMPs have been implicated in neuroinflammatory processes that accompany most pathologies of the central and peripheral nervous system. Additionally MMPs are linked to neuropathic pain (NP), inflammatory pain as in osteoarthritis, and even the metastatic potential of cancers [39]. MMP-9 overexpression is associated with the early-phase development of NP in a rat sciatic nerve ligation model, while MMP-2 overexpression coincides with late-phase development of chronic NP (45). The acute inflammatory phase of multiple sclerosis is characterized by MMP involvement in injury to blood vessels, along with disruption of the myelin sheath and axons [46,47]. MMP activity has also been implicated in BBB disruption, edema, and hemorrhage in animal brains following middle cerebral artery occlusion (mCAO) (1, 10, 34, 48-50). A study in MMP-9 knockout mice concluded that MMP-9 expression limited functional recovery following spinal cord injury (16). There is a large body of evidence tying MMP expression to BBB disruption, ischemia, edema, hemorrhage, demyelination, neural death, and pain.

It is important to note that roles of MMP activity can be destructive during certain phases of disease and injury, yet fruitful in other phases. MMP inhibition reduces the number of neurons and new vessels formed during revascularization (1). This indicates that MMPs are involved in angiogenesis and neurogenesis following mCAO in rats. The capacity for regeneration in the nervous system is controlled by ECM molecules present in Schwann cell basal lamina. Laminin (51), fibronectin (52), and type IV collagen (53) are all powerful stimulants for axonal regrowth and elongation (54, 55). Other molecules present in nerve cells such as chondroitin sulfate proteoglycans (CSPG) act to block these regenerating effects and inhibit neural growth (56). However MMP-2 has been shown to counter the inhibitory action of CSPG and restore the neurite-promoting actions of laminin. Studies have demonstrated that induction of MMPs improves neuronal regeneration and functional recovery following injury to peripheral nerves and the spinal cord by promoting wound healing (40, 57, 58). Although there is a lot of conflicting evidence concerning the protective versus detrimental roles of MMP activity, it appears that MMP dependent remodeling of the ECM is very important for processes like inflammation, blood vessel formation, and neurogenesis in disease and injury states of the nervous system. There is a need to better understand the significance of temporal-, tissue-, and cell-specific regulation of MMP expression along with post-trancriptional modification of MMP activity in order to fully appreciate the role of MMPs in the pathogenesis of disease and injury.

MMPs and Peripheral Nerve Injury

While MMPs are crucial to normal nervous system functioning and development, their biological significance is complicated by their involvement in and activation following nervous system injury and disease. Injury to the peripheral nervous system results in a well-documented upregulation of MMPs. Emphasis has been placed on MMP-2 and MMP-9, as overexpression of these specific metallaproteinases are consistently observed following peripheral nerve injury (PNI). These two predominant MMP isoforms exhibit time-dependent upregulation following injury, with peak levels of MMP-9 and MMP-2 associated with the acute and chronic phases of injury, respectively (45, 57, 59-62).

In a rat spinal nerve ligation (SNL) model of injury, MMP-9 expression was increased robustly within one day of injury [45]. The authors reported similar findings for MMP-9 activity; peak levels were reached within one day of injury and progressively declined after day 3 of injury. Because MMP-9 induction occurs within hours following injury, it has been suggested that this isoform is most important during the acute phase of PNI. However, the role of MMP-9 during the acute phase of injury is complex and appears to have both beneficial and harmful effects on recovery. Studies analyzing patterns of MMP expression in a rat sciatic nerve crush model found MMP-9 transcription significantly increased within one day of injury, with MMP-9 mRNA

levels showing up to a 90-fold increase over baseline (59, 60). This rapid induction of MMP-9 is predominantly regulated by Schwann cells and appears to play a central role in Wallerian degeneration (59). Wallerian degeneration is vital for the eventual repair and regeneration of the injured nerve, and involves the breakdown of the axonal cytoskeleton and myelin sheath followed by their subsequent removal by macrophages (54, 63). The early rise in MMP-9 during the acute phase of injury correlates with Wallerian degeneration, and treatment with metalloproteinase inhibitors delays the onset of Wallerian degeneration. Taken together these findings suggest a significant role for MMP-9 in the neuronal repair pathway (63).

Of considerable interest are inflammatory cytokines such as TNF-α, IL-1β, and IL-6 in inducing the production of MMPs following injury. It has been proposed that a primary mechanism for the secondary pathogenesis that often follows PNI involves the pro-inflammatory cytokine activation of Schwann cells. These pro-inflammatory cytokines are rapidly produced after tissue injury and are known to activate Schwann cells. Studies investigating the inflammatory response after PNI in rats found that in response to axonal injury, activated Schwann cells secrete TNF-α, and that these increased TNF-α levels correspond to increased gelatinase activity both temporally and spatially as early as 3h after injury (57, 61, 62). Additionally, studies utilizing MMP-9 inhibitors and MMP-9 knockout mice show substantial reduction in macrophage infiltration following injury (57). The control that these pro-inflammatory cytokine pathways have over MMP-9 expression is also evidenced by the dramatic increase in MMP-9 production by Schwann cells following injection of TNF-α and IL-1β into the rat sciatic nerve (59). This subsequent activation of MMPs by Schwann cells induces cleavage of the surrounding ECM and perpetuates the inflammatory response following injury. Together these findings indicate direct involvement of MMP-9 in the recruitment of macrophages to the injured PN with TNF-α acting as an upstream mediator in this pathway. A debilitating role for MMPs in inflammatory pathologies of the PNS is evidenced by the observation that MMP-9 levels are upregulated in patients with Guillain-Barre's syndrome (61). Despite extensive data indicating a primarily harmful role of MMPs following PNI, evidence also exists to support a neuroprotective role of MMPs. Early induction of MMP-9 helps to regulate ECM turnover and allows for important physiological processes such as cell migration and cell survival. As previously mentioned, macrophage recruitment to the lesion site following injury is imperative to the repair process by clearing away cellular debris to allow for axonal regeneration and remyelination. Thus, the rapid induction of MMP-9 may play a crucial role in PNI repair (60).

Interestingly, Shubayev (2004) found that MMPs are beneficial in promoting regeneration of peripheral nerve fibers up to 8 days following sciatic nerve injury in rats (54). MMP-2 was also found to be transported to the axonal growth cone, further suggestive of a beneficial role in peripheral nerve repair (56). Taken together, this evidence suggests that MMP-9 may be beneficial in the early response to injury, but may later contribute to secondary inflammatory processes and neuropathy. In contrast to MMP-9, MMP-2 appears to play an important role in the late-phase of injury and may help to facilitate axonal regeneration and repair after PNI.

MMPs and Brain Injury

Considering that baseline levels of MMP expression in the brain are very low, brain injuries and diseases cause significant changes in the spatial and temporal expression of MMPs. There is considerable evidence in various animal models of brain injury pointing to MMPs as mediators of pathophysiology, yet there is also support for the role of MMPs as facilitators of recovery and wound healing following injury (1). The balance between the neurotoxic and neuroprotective properties of MMPs depends on many complex factors such as the level, location, timing, and specific isoforms of MMP expression.

MMPs have been implicated in the pathology of a wide range of brain injuries and disease (ischemic stroke, thermal injury, inflammatory injury, hemorrhage, dementia, mechanical injury, etc.) demonstrated by immunoreactive and mRNA based studies of their expression patterns. In a rat model of middle cerebral artery occlusion (MCAO) followed by reperfusion, an early and progressive increase in MMP-9 was observed in the endothelium, and a transient increase in MMP-2 expression by astrocytes occurred in the first few hours after reperfusion. In the later stages of injury, there was a massive wave of MMP-9 expression from macrophages/microglia and MMP-2 in neurons and astrocytes. It has been suggested that the initial breakdown of endothelial barriers after injury allows circulating matrix proteins like vitronectin and fibronectin to enter the neuropil and activate microglial MMP expression (9). Rats exposed to 3rd degree burns over 70% of their body demonstrated an early and rapid increase of MMP-2 and -9 expression (64). Costanzo et al confirmed the temporal pattern of MMP expression seen in ischemic injury in a mouse model of olfactory nerve injury. This study used knockout mice to show an early elevation of MMP-9 followed by a later, independent peak in MMP-2 activation. Other studies have shown that MMPs are also upregulated in animal models of lipopolysaccharide induced neuroinflammation, collagenase induced hemorrhage, kainic acid induced seizures, surgically induced brain injury, and scratch mechanical injury (13, 14, 19, 65, 66). TIMP expression is similarly modulated by brain injuries demonstrated by the increased TIMP-1 expression observed after reperfusion in a mouse model of MCAO (50, 67).

Acute multiple sclerosis lesions are known to express very high levels of MMP-2 where it is believed to play a role in myelin degeneration (68). MMP inhibitors have demonstrated effectiveness in treatments of multiple sclerosis by reducing the rate of new plaque formation (69). Alcohol has been shown to induce MMP-1,-2, and -9 activity in brain microvascular endothelial cell culture, indicating that MMP expression may be involved in brain pathologies associated with alcoholism (22). The HIV Tat protein is able to produce elevated expression of MMP-2 and MMP-7 in brain macrophages. Increased MMP expression may help explain the neurological problems seen in HIV-infected patients (70). Elevated MMP-2 levels in astrocytes, microglia, and macrophages may participate in the pathogenesis

of vascular dementia in humans (71). MMP-2 and MMP-9 are overexpressed in the acute phase moderate and severe traumatic brain injury demonstrated by elevated gelatinase levels in plasma and brain ECF (72).

Changes in gene expression imply that MMPs have important functional consequences on post-injury processes, but the role of MMP activity is complex. There is evidence that MMPs contribute to numerous mechanisms of secondary pathogenesis following brain injury principally through their involvement in neuroinflammation, yet it is also clear that MMP activity is essential for processes like the sprouting of new neurons and vascular remodeling that are important for recovery (1). The mechanisms by which MMPs induce brain damage include, but are not limited to the following: disruption of the blood-brain barrier (BBB), hemorrhagic transformation, myelin degradation in white matter, neuronal cell death, activation of inflammatory cytokines, and scar formation (1,48,73,74). Active MMP-2 is able to degrade the basal lamina of endothelial cells causing a loss of vascular integrity in areas of ischemia (34). The localization of MMPs to cell nuclei following MCAO also supports their role in processing of IL-1β and facilitation of neuroinflammation (1).

Multiple MCAO animal models have shown that treatments which are able to lower MMP activity/levels, such as saline flushing, hypothermia, and synthetic or natural MMP inhibitors, have neuroprotective properties by reducing ischemic area, brain edema, and BBB disruption (1, 10, 49, 50, 72, 75-79). Studies have also shown that treatment with MMP inhibitors can reduce mortality associated with the risk of hemorrhage in patients given thrombolytic agents following stroke (80).

MMP function is not simply detrimental and there is considerable evidence that MMPs serve an important function in the recovery from injury by facilitating the formation of new neurons and blood vessels. Multiple studies of nerve injury have found a pattern of elevation of MMP-9 within hours of injury followed by a much later elevation of MMP-2 (at least 7 days after injury). These studies suggest that MMP-9 participates in acute phase post-injury processes such as inflammation, BBB disruption, and neuronal degeneration, while MMP-2 is more important in the chronic phase where recovery processes like angiogenesis and axonal regeneration are occurring (37, 45). A beneficial role of MMP-2 in recovery is supported by a study that used pre-treatment with resveratrol in a mouse MCAO model. Resveratrol produced elevated MMP-2 levels and had significant neuroprotective effects such as reduced infarct size and improved scores on neurological examination (81). Interestingly, an experiment using MMP-9 knockout mice in a collagenase –induced model of intracerebral hemorrhage found higher mortality, enhanced edema, and more significant neurological defects in the MMP-9 deficient mice when compared to wild-type (82). Clearly MMPs play a complex role in the response to injury that cannot be easily categorized as good or bad. Therefore, more research is needed to elucidate how temporal, spatial, and MMP isoforms expression patterns affect the course of both pathological and beneficial post-injury processes.

MMPs and Spinal Cord Injury

Ample evidence exists that links MMP activity after spinal cord injury (SCI) to a diverse range of effects such as inflammation, demyelination, angiogenesis, and axonal growth (16, 31, 40, 83-85). Here we examine the potentially beneficial and detrimental roles of the two gelatinases, MMP-2 and MMP-9, in the pathophysiology of SCI.

MMP-2 and -9 demonstrate different temporal upregulation and cellular localization following injury, with MMP-9 induction associated with the acute phase, and MMP-2 induction associated with the late phase of injury (16, 84, 86, 87). Most studies report scarce MMP-9 activity in the uninjured spinal cord (7), and immunohistochemistry has found evidence of MMP-9 in uninjured meninges and motorneurons (39). However this tissue distribution changes within the 24 hours following SCI, as increased MMP-9 activity is observed in blood vessels, macrophages, and astrocytes surrounding the lesion site (16). Studies utilizing a contusional SCI model in rats, and studies examining MMP expression in human spinal cords have reported maximal MMP-9 activity 24 hours after injury (7,16). Goussev (2003) (84) also found that MMP-9 activity was influenced by the severity of SCI, with moderate and severe-SCI groups showing higher levels of MMP-9 activity compared to a mild-SCI group. In contrast to the temporal and spatial patterns of MMP-9, MMP-2 exhibits a delayed upregulation following injury, with levels increasing 5 days post-injury and the highest levels occurring between 7 and 14 days post-injury [40,84,87]. Interestingly, a study examining MMPs in human spinal cords following traumatic injury reported a transient early induction of MMP-2 in macrophages at the lesion epicenter (7). Increased MMP-2 activity is found predominantly in reactive astrocytes bordering the lesion within the spinal cord (40).

The activation and activity of MMP-9 during the acute phase of injury is complex and appears to have both beneficial and harmful effects on recovery. Studies implicating the detrimental effect of MMP-9 following SCI have mostly focused on the role of MMP-9 in secondary pathogenesis, including disruption of the blood-spinal cord barrier (BSCB) and subsequent neutrophil invasion leading to additional tissue damage and demyelination. Disruption of the blood-spinal cord barrier is thought to play a central role in secondary inflammatory processes, as the breakdown of this barrier allows for uncontrolled leakage of immune cells and endogenous proteins into the spinal cord. The infiltrating cells are able to activate resident microglia and astrocytes, as well as pro-inflammatory cytokines. Recent studies have shown that the acute upregulation of MMP-9 promotes inflammation and barrier disruption, (84), with the compromise of the BSCB being a key trigger of these secondary degenerative events (85). This is important because secondary pathogenesis determining the amount of functional recovery after injury.

MMP-2 and MMP-9 may be especially important in mediating the inflammatory response following SCI, as both have been shown to be involved in the process of immune cell and macrophage infiltration into tissues (88). In this regard, MMP-9 is not only highly expressed during immune cell infiltration after

SCI, but has also been shown to play a crucial role in the transmigration of neutrophils to the lesion site from surrounding vasculature (16). Neutrophils may, in turn, cause additional necrotic tissue damage, lesion expansion, and functional impairment via the generation of reactive oxygen species, proteases, nitric oxide, and pro-inflammatory cytokines (16, 85, 89). Noble (2002) (16) found that almost two-thirds of invading neutrophils were localized to white matter within the lesion, suggesting this mechanism may be responsible for loss of locomotor activity following SCI. There is also evidence that MMP-9 produced by the infiltrating macrophages contributes to the demyelination of neurons that were spared by the original injury (16). MMP-9 is able to cleave myelin basic protein, providing a possible mechanism for this secondary myelin degradation that occurs following SCI. Moreover, neutrophils entering the spinal cord following injury were found to be most numerous 1-3 days postinjury (89), consistent with the temporal upregulation of MMP-9. Despite these destructive effects, neutrophil invasion following injury may be necessary for recovery by sterilizing and clearing the injury site of debris. There is also evidence that neutrophils migrating to the injury may help promote wound healing, tissue repair, and neural regeneration by releasing protective cytokines (89).

Based on the activity of MMPs in SCI, numerous studies employing knockout mice and specific MMP inhibitors have been undertaken. Results have again pointed to both beneficial and harmful effects of MMPs. Genetic knock-out studies further emphasize the detrimental role of MMP-9 in SCI, as MMP-9-deficient mice show improved stabilization of the BSCB, reduced infiltration of neutrophils and macrophages, enhanced tissue sparing and significant improvement in locomotor recovery when compared to wild-type mice (16,40,85). Administration of the MMP-9 inhibitor atorvastatin following SCI in rats demonstrated neuroprotective effects [85]. Rats receiving atorvastatin showed reduced breakdown of the BSCB and reduced neutrophil and macrophage infiltration to the spinal cord compared to controls. This inhibition of MMP-9 also resulted in reduced secondary tissue damage by inflammatory mediators and a corresponding increase in functional recovery, suggesting that suppressing MMP-9 expression may have neuroprotective effects (85).

Despite extensive data indicating a primarily harmful role of MMPs following SCI, evidence also exists to support a neuroprotective role of MMPs. One study examining the acute phase of SCI recovery found increased MMP-9 activity in elongating nerve processes [83], suggestive of a positive role for MMP-9 in axonal regeneration. Additional evidence for a beneficial role of MMPs comes from their involvement in ECM remodeling, glial scar formation, and revascularization, all important events in the wound healing process [84]. Following SCI, revascularization and glial scar formation occur between 7-14 days after injury, correlating the increase in MMP-2 activity (84). Following injury activated astrocytes undergo massive proliferation to form a dense matrix at the lesion site and begin to secrete molecules into the surrounding ECM. Although the formation of a glial scar serves a protective role by reestablishing a physical barrier across the injury site to suppress further damage, cells within the scar also secrete inhibitory molecules that act to arrest neuroregeneration. CSPG is one of these molecules that has been shown to inhibit axonal regeneration (90). Glial scarring is more extensive in MMP-2-knockout animals (40), and numerous studies have demonstrated that MMP-2 is able to degrade CSPGs and thus inhibit glial scarring (40, 84). Moreover, at all time points examined, gelatinase activity was highest at the epicenter of the lesion, with a gradual decrease peripherally. Conversely, activated astrocytes were localized primarily at the border surrounding the lesion, with only those astrocytes immediately bordering the epicenter showing gelatinase activity (84). These findings suggest that the limited regeneration and recovery seen after SCI may be partly attributed to the lack of gelatinase activity throughout the glial scar, resulting in the accumulation of glial inhibitory molecules. Consequently, MMP-2 activity may attenuate glial scarring and thus improve functional recovery following SCI.

MMPs and Neuropathic Pain

Neuropathic pain (NP) can occur from any number of etiologies and is defined as pain secondary to a primary lesion or dysfunction of the nervous system (91). Evidence exists for the involvement of a diverse range of processes in the development of NP following injury including inflammation, cannabinoid receptor signaling, neuron regeneration, and ion channel activity (92), and there is increasing evidence among researchers investigating the role of MMPs in the development of NP (45). The role of MMP-2 and MMP-9 in the inflammatory response may be a key factor in the development of NP, as inflammation is capable of causing increased damage to nervous tissue following traumatic injury. Kawasaki et al (2008) revealed that following SNL, upregulation of MMP-9 and MMP-2 was consistent with the early and late phases of pain, respectively. Their studies also suggested that MMP-9 induces NP in the acute phase of injury through IL-1β cleavage and microglial activation and MMP-2 maintains NP in the chronic phase through IL-1β cleavage and astrocyte activation. Both TIMP-1 and TIMP-2 inhibit MMP-2 and exogenous administration of TIMP-2 was shown to reduce NP in the chronic phase of injury (30,45). Upregulation of TIMP-1 is observed in rats with NP when compared to rats with inflammatory pain, thus further implicating TIMP and MMP activity in chronic pain conditions (93). Treatment with synthetic inhibitors of MMPs has also reversed thermal hyperalgesia, a behavioral measure of NP, in chronic nerve constriction pain models (94, 95). Studies employing MMP-5-knockout mice reported the absence of NP following sciatic nerve injury (96). This evidence suggests a central role of MMP-2 in the development of chronic NP by mediating tissue remodeling, inflammation, and neuron regeneration.

Additionally, studies examining NP following SCI have found significant evidence for altered gene expression in both the acute and chronic phases of NP (97,98). TRPV1 and CB receptor activity have both been implicated in NP following SCI and may play important roles in regulating MMP activity. TRPV1 activation has been shown to play a role in heat-shock-induced MMP-1 expression in human epidermal keratinocytes (99), and TRPV1 receptors are upregulated in animals demonstrating NP following

SCI (100). TRPV1 antagonists have also shown significant anti-hyperalgesic effects in rats following SCI (101), however the signaling pathways of TRPV1 in chronic NP and effects on MMPs have not been elucidated clearly. The cannabinoid system is also implicated in NP, as CB1/CB2 receptors are overexpressed following injury, and spinal or systemic administration of CB agonists attenuates thermal hyperalgesia in animal models of chronic pain (102,103). CB receptors are also found to induce the production of TIMPs, suggesting a mechanism for MMP inactivation and attenuation of NP. The mechanism by which TRPV1 and CB receptor signaling affects MMP levels in NP may involve the p38 MAPK in microglia surrounding the injury and calcium dependent intracellular signaling pathways (Fig.1). Tsuda et al. (2004) (104) found that following PNI, the activated and phosphorylated form of p38 MAPK was increased in microglia in the dorsal horn. Moreover, the development of NP was suppressed by the administration of a p38 MAPK inhibitor. These findings suggest an important role for the p38 signaling pathway in the development of NP following PNI. Additional investigation is needed to characterize TRPV1 and CB receptor signaling following injury and their roles in modulating MMP activity in NP following SCI.

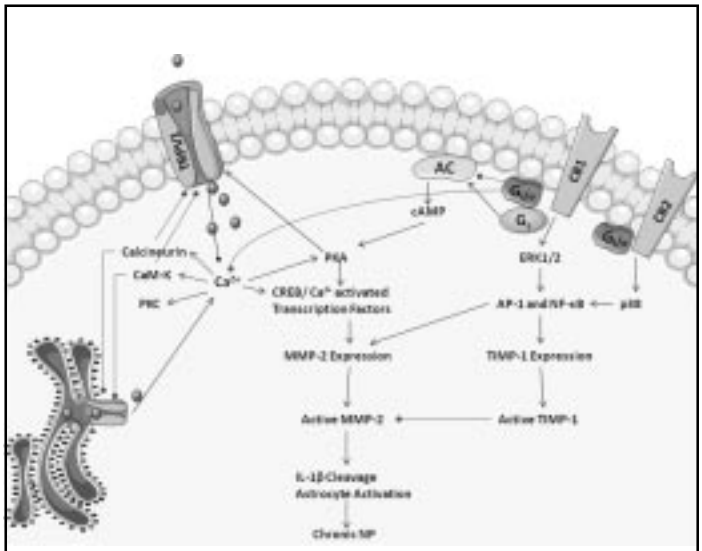

Figure 1: Possible mechanism of transcriptional regulation of MMP-2 and TIMP-1 by TRPV1 and CB1/2 receptor signaling and implications for chronic neuropathic pain. TRPV1 and CB1/2 modulate Ca^{2+} -dependent and MAPK intracellular signaling pathways which are capable of activation CREB, AP-1 and NF-κB transcription factors which control expression of MMP-2 and TIMP-1.

Therapeutic Potential of MMP Inhibitors

Numerous studies have shown that there is a close association between MMP activity and induction of secondary pathogenesis following nerve injury, resulting in loss of functional recovery and NP. TIMPs are normally responsible for maintaining the balance between tissue formation and degradation by MMPs. However the upregulation of MMPs after central nerve injury is not consistently observed with TIMPs, which could allow for the uninhibited activity of MMPs on surrounding tissue (7). Both animal and human investigations have confirmed a lack of TIMP induction corresponding to MMP upregulation post-injury. Evaluation of interventions following SCI indicate that attenuating the post-injury inflammatory response may be the most effective mechanism to prevent additional tissue damage by secondary pathogenesis (105). Studies evaluating the effects of MMP inhibitors following acute SCI have reported encouraging findings including reduced inflammatory response, decreased tissue damage, and improved locomotor recovery (16, 40, 84, 85, 89). Neuroprotective effects have also been reported in the literature regarding the use of MMP inhibitors following cerebral damage (19, 66, 78, 88). As a result of these findings MMPs have recently generated considerable interest as possible therapeutic targets.

Despite these encouraging studies in experimental settings suggesting a neuroprotective effect of MMP inhibition, synthetic compounds having the ability the inhibit MMPs have not shown promising therapeutic benefits in clinical trials. One possible reason for these conflicting findings is that compounds that inhibit MMPs also have the capability to inhibit other metalloendopeptidases and MMP-mediated processes, such as the release of TNF-α and the shedding of the IL-6 receptor (106). Inhibition of other proteases may not offer therapeutic benefit and may even be harmful. For example, one study suggested that broad-spectrum MMP inhibitors that impede the release of TNFα may play a role in exacerbating liver injury (107). It has also been demonstrated that administration of MMP inhibitors may lead to joint pain in some patients, further highlighting the complex of therapeutic MMP inhibition (108). The therapeutic effects of broad-spectrum MMP inhibitors reported in the literature may be in part attributed to the regulation of non-MMP related processes (80,109). MMP inhibitors may offer significant clinical benefits by improving functional recovery and attenuating the development of neuropathic pain in nervous system injury and disease. In order to effectively use these drugs therapeutically, we must take into account temporal and spatial patterns of MMP expression. MMP inhibitors should be used on limited time courses and great care must go into determining the specific isoform of MMP that should be inhibited.

Future Directions

The findings reviewed here provide the basis for constructing further hypotheses regarding MMPs involvement in the pathogenesis of nerve injury. They also provide potential targets for therapeutic intervention.

There is also evidence that MMPs may be modulated in the signaling cascades initiated by TRPV1 and CB1/CB2 receptor activation. TRPV-1 receptor activation has been linked to an upregulation of MMPs (99). This is suggestive of a causative mechanism for inflammation and inflammatory mediated pain following injury. One possible pathway involves the rapid increase of intracellular calcium following TRPV-1 activation leading to increased adenylate cyclase activity, which in turn causes increased levels of cAMP (110). Increased cAMP is able to activate PKA, which further sensitizes TRPV1, and activates CREB by phosphorylation. CREB has been shown to induce the transcription of MMP-2 (25) and thus may help explain the overexpression of MMP-2 and TRPV1 observed in SCI.

CB receptors are typically expressed in presynaptic membranes where they inhibit the release of neurotransmitters (111). CB1 and CB2 receptors are upregulated in the DRG and ipsilateral spinal cord following saphenous nerve injury (112), and administration of endogenous and synthetic CBs have been shown to decrease hyperalgesia in both central and peripheral injury models of chronic pain (Guindon 2007) (102,113-116). It has also been discovered that CB receptors are able to induce the production of TIMPs which serve to inhibit MMP activity. We hypothesize that this increased expression of TIMPs may mediate the anti-hyperalgesic effect of CB receptor activation by counteracting the TRPV-1 induced upregulation of MMP-2. While there is strong support for a role of the cannabinboid system in NP, there are mixed findings on which receptor mediates the antihyperalgesic effects. This is complicated by the different models of pain being studied, such as acute v chronic pain, peripheral v central injury, or thermal v mechanical hyperalgesia. In one study using heat to produce cutaneous injury, a CB1/2 agonist, WIN, was found to attenuate both mechanical and thermal hyperalgesia by acting through the CB1 receptor (117). However in a carrageenan model of inflammatory pain, Nackley et al (2003) (118) found that CB2 was also involved in mediating the antihyperalgesic effects of WIN. Thus the specific role of these receptors in NP is complex and demands further inquiry.

We suspect that CB1/CB2 activity may induce the production of TIMPs, which then serve an anti-hyperalgesic effect by inhibiting elevated MMP levels resulting from TRPV-1 activation (Fig.1). However, to explore further the relationship between TRPV-1 and CB1/CB2 receptors and the development of secondary inflammation and NP, it is necessary to gain a better understanding of signaling cascades and downstream effects of receptor activation. More examination is needed into the specific roles and interactions of CB1/CB2 and TRPV1 receptors and their signaling pathways in the development of inflammation and chronic NP through modulation of MMP levels. Information is limited regarding this relationship; however the specific role of each receptor in the regulation of MMPs and secondary pathogenesis following nerve injury is complex and deserves additional focus.

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