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REVIEW

Extracellular Matrix Degradation by Metalloproteinases and Central Nervous System Diseases

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Abstract

Matrix metalloproteinases (MMPs) are a gene family of neutral proteases involved in normal and pathological processes in the central nervous system (CNS). Normally released into the extracellular space, MMPs break down the extracellular matrix (ECM) to allow cell growth and to facilitate remodeling. Proteolysis becomes pathological when the normal balance between the proteases and their inhibitors, tissue inhibitors to metalloproteinases (TIMPs), is lost. Cancer cells secrete neutral proteases to facilitate spread through the ECM. MMPs increase capillary permeability, and they have been implicated in demyelination. Neurological diseases, such as brain tumors, multiple sclerosis, Guillain-Barré, ischemia, Alzheimer's disease, and infections, lead to an increase in the matrix-degrading proteases. Two classes of neutral proteases have been extensively studied, namely the MMPs and the plasminogen activators (PAs), which act in concert to attack the ECM. After proteolytic injury occurs, the process of ECM remodeling begins, which can lead to fibrosis of blood vessels and gliosis. TIMPs are increased after the acute injury and may add to the fibrotic buildup of ECM components. Thus, an imbalance in proteolytic activity either during the acute injury or in recovery may aggravate the underlying disease process. Agents that affect the proteolytic process at any of the regulating sites are potentially useful in therapy.

Index Entries: Blood–brain barrier; brain tumors; cerebral ischemia; extracellular matrix; matrix metalloproteinases.

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Introduction

Brain extracellular matrix (ECM) has multiple roles in central nervous system (CNS) structure and function. Dynamic changes are occurring continually in the ECM through slow turnover under normal conditions, which is accelerated during times of cell growth and injury. Recovery from injury requires remodeling of the ECM, which depends on the balance between proteolytic enzymes and tissue inhibitors. In addition to its physiological involvement in growth and recovery, brain ECM participates in many pathological conditions. Excessive proteolytic activity results in undesirable tissue disruption, whereas too much inhibition prevents normal ECM breakdown, resulting in matrix overgrowth. Astroglial brain tumors use proteases to spread through tissue. Secreted in the invading front of the tumor, a complex cascade of proteases and their inhibitors determines the invasive potential of the tumor cells. Neuroinflammation depends on proteases for the influx of inflammatory cells to the injury site and to prepare the offending agents for removal. However, unwanted proteolytic activity damages normal tissue, further contributing to the damage and interfering with recovery. Proteases are important in the pathogenesis of multiple sclerosis and Guillain-Barré, and as part of the secondary inflammatory response in ischemia, hemorrhage, and infection. Increasing evidence also indicates that MMPs may contribute to tissue damage in neurodegenerative diseases, such as Alzheimer's disease and amyotrophic lateral sclerosis.

Brain cells contain genes that encode for a wide variety of proteases. The best studied of the neutral proteases modulating the ECM are the gene families of the matrix metalloproteinases (MMPs) and the plasminogen activators (PAs). MMPs are neutral, Ca^{2+}-dependent, Zn^{2+}-containing proteases. Secreted by many cell types, MMPs have been shown to degrade all major macromolecules of the brain ECM. Fourteen members of the MMP gene family have been identified. In the brain, the major MMPs are the gelatinases, the stromelysins, and the membrane-type metalloproteinases (MT-MMPs). Several serine proteases, including tissue-type plasminogen activator (tPA), urokinase-type plasminogen activator (uPA), and elastases, have been identified in brain tissue. Cytokines and growth factors regulate the transcription of the MMPs and PAs. Astrocytes, microglia, and vascular endothelial cells secrete latent MMPs, which require activation. Although less is known about the activation of the MMPs in brain than in other tissues, similar mechanisms appear to be involved. Activation may involve free radicals or other proteases. In addition to regulation at transcriptional, translational, and posttranslational levels, MMPs are controlled by storage and secretion. This latter realm of modulation is induced by interaction with filamentous components of the cytoskeleton. Thus, the regulation of the ECM turnover by matrix-degrading proteases is a highly complex process that has both beneficial and detrimental effects on the tissue.

Knowledge obtained from molecular biology studies of the MMPs and PAs in cancer and arthritis has aided in understanding the actions of these enzymes in brain. Current studies of MMPs in brain are aimed at distinguishing favorable ECM remodeling seen in normal development and recovery from detrimental breakdown of the ECM that occurs in cancer cell invasion and inflammatory opening of the blood–brain barrier. Sevéral earlier reviews have addressed the role of MMPs in brain (Romanic and Madri, 1994; Rosenberg, 1995; Yong et al. 1998). This article delineates the role of the MMPs and tissue inhibitors to metalloproteinases (TIMPs) under normal and pathological conditions.

Structure and Function of ECM

Brain ECM provides structure for the tissue, holds water molecules within complex molecular domains, and impedes movement of large or charged molecules. The ECM anchors a

variety of molecules that are important in normal brain function, such as integrins, growth factors, laminin, and other glycoproteins. Matrix macromolecules include the glycosaminoglycans, hyaluronic acid, heparan sulfate, chondroitin sulfate, and large complex glycoproteins (Rutka et al., 1988). The ECM can be defined biochemically as the sum of its component parts, which includes the collagen types, the noncollagenous glycoproteins (laminin, fibronectin, entactin, vitronectin, tenascin), the glycosaminoglycans (dermatan, dermatan sulfate, chondroitin, chondroitin sulfate, keratan, keratan sulfate, heparan, heparan sulfate), and the proteoglycans.

Between the nervous tissue and the epithelial cells, a basal lamina is found that is composed of type IV collagen, entactin, laminin, heparan sulfate, fibronectin, and vitronectin (Giese et al., 1995). Astrocytic foot processes and pericytes abut onto the basal lamina providing a connection among the microvascular endothelium, smooth muscle, and the neuropil. Arterioles have smooth muscle, which is ensheathed in ECM. This ECM is contiguous with the basal lamina between the endothelial cells and the astrocytes. The basal lamina functions as part of the blood–brain barrier by providing a charge and a molecular-size filter that modulates the movement of molecules from the blood into brain across the endothelial cell layer.

Formation of connections and breakdown of other connections are a dynamic process necessary for the growth of cells, the spread of tumors, and the movement of brain cells to a site of inflammation. The ECM components are linked to integrins, which are transmembrane glycoproteins that contain an α- and a β-subunit noncovalently bonded into a heterodimer (Rutka et al., 1988). In vivo studies indicate that normal brain small blood vessels express α1β1, α2β1, α3β1, α5β1, α6β1, α6β4, αVβ3, and αLβ2 (LFA-1) integrin subunits (Gladson, 1996). Integrins mediate and regulate cell-surface signals from the ECM to the intracellular compartment. The mechanisms of the intracellular signal transduction have only begun to be elucidated (Clark and Brugge, 1995). Integrins also attach the various tissue components together. In nonhuman primates, the integrin subunit, α6β4, anchor the astrocytic cytoskeleton to the surrounding ECM.

At the cortical surface the *glia limitans externa* is composed of collagen types I, III, and IV, laminin, fibronectin, and heparan sulfate. This cellular monolayer invests the entire cortical surface of the brain and separates astrocytic foot process from pia-arachnoidal cells (Haines et al., 1993). Thus, it forms an interface between cellular CNS elements derived embryologically from neuroepithelium and leptomeningeal elements presumably derived from the neural crest. Little is known about the function of the glia limitans externa layer, but it is a site of MMP localization as well. This cortical cellular layer may be influenced by the balance between the MMPs and TIMPs, either constraining or facilitating molecular transport into and out of the brain.

Classification of MMPs and TIMPs

MMPs are neutral proteases that are subdivided by their sequence homology and substrate specificity into four subclasses: collagenases, gelatinases, stromelysins, and MT-MMPs. Table 1 shows the current nomenclature, the protein structures, and the preferred substrates. Matrilysin is the smallest of the MMPs with a propeptide and catalytic domain. Stromelysins have hemopexin and hinge domains. Gelatinases have fibronectin and collagen binding sites, whereas the MT-MMPs have a transmembrane region.

Four TIMPs have been identified. All are present in brain. Although they are each able to inhibit the action of multiple MMPs, they have a higher affinity for certain ones. TIMP-1 and TIMP-3 bind mainly to MMP-9, TIMP-1 to MMP-3, and TIMP-2 to MMP-2. The affinity of the recently discovered TIMP-4 has not been published. TIMPs tightly bind to the MMPs, making their purification difficult. Multiple TIMPs can attach to one MMP molecule, creat-

Table 1
Major MMPs Grouped According to Domain Structure[a]

Name and domain structure	Substrates
Minimal domain (Pre-Pro-Cat)	
Matrilysin (MMP-7)	PG, GP, IV, E, G
Collagenases (Pre-Pro-Cat-H-Hem)	
Interstitial collagenase (MMP-1)	FC
Collagenase -3 (MMP-13)	FC
Stromelysins (MMP-3, MMP-10)	PG, PG, IV, G
Gelatinases (Pre-Pro-Cat-Fn-Cat-H-Hem)	
Gelatinase A (MMP-2, 72-kDa)	G, IV
Gelatinase B (MMP-9, 92-kDa)	G, IV
Membrane-bound (Pre-Pro-F-Cat-H-Hem-TM)	
MT1-MMP (MMP-14)	GA, PG, GP

[a] PG = proteoglycan, GP = glycoprotein, I = type I collagen, IV = type IV collagen, E = elastin, G = gelatin, FC = fibrillar collagen, GA = gelatinase A, Pre = preprotein, Pro = proprotein, F = fibronectin, Cat = catalytic site, H = hinge, Hem = hemopexin, C = collagen-like, TM = transmembrane. From Powell and Matrisian (1996).

ing new large complexes. TIMP-2 is required for the activation of MMP-2 by MT1-MMP, but in excess will inhibit the activation (Sato et al., 1994).

Role of MMPs in Normal Development and in Angiogenesis

Secretion of proteolytic enzymes by cells has been implicated in tissue remodeling during embryonic development as well as in invasive neoplastic disease. Interactions between cells and ECM play a central role in the modulation of cell motility, growth, and differentiation during embryogenesis. ECM ligands, their receptors, extracellular proteinases, and proteinase inhibitors all participate in the construction, maintenance, and remodeling of ECM by cells. Growth and movement of peripheral neurons and their supportive cells depend on an equilibrium between stimulatory or inhibitory ECM molecules. The spatiotemporal coordination of this process is governed by the interaction of integrin molecules expressed on the surface of the migrating cell and specific ECM components, such as fibronectins, laminins, tenascins, collagens, and various glycoproteins (Perris, 1997).

During development, endothelial cells in conjunction with astrocytes contribute laminin to the ECM, whereas astrocytes stimulate the formation of the blood–brain barrier (Wagner et al. 1997). Inhibition of MMP-2 and MMP-9 with TIMP-2 and TIMP-1, respectively, decreases tube formation or angiogenesis in vitro (Schnaper et al., 1993). Expression of transmembrane neural cell-adhesion molecule-(NCAM) B downregulated MMP-9 and MMP-1 in a rat glioma cell line, indicating that cellular expression of the recognition molecule NCAM regulates the turnover of the surrounding ECM and MMP expression (Edvardsen et al., 1993).

Regulation of MMP Genes by Cytokines and Growth Factors

Basal transcription of MMP genes, as well as the transactivation by phorbol myrisate acetate

(PMA), cytokines, and growth factors requires specific interaction of AP-1 and PEA-3 sites with other *cis*-acting elements. The AP-1 site plays a dominant role in repression of MMPs by transforming growth factor-β (TGF-β), retinoids, and glucocorticoids, although some AP-1-independent pathways may also play a role.

In contrast, the MMP-2 gene promoter region has AP-2 binding sites and is unresponsive to phorbol ester and most cytokines. MMP-2 is constitutively expressed in brain. Since it lacks a TATA-box in its promoter region, it is similar to many so-called housekeeping genes (Crawford and Matrisian, 1996). The presence of one or more AP-2 binding sites appears critical in tissue-specific expression of pro-MMP-2 (Benbow and Brinckerhoff, 1997). Furthermore, mechanical forces in the ECM may transduce intracellular signals through integrin-mediated focal adhesion complexes (Ruoslahti, 1997). For example, MMP-2 activation has been shown to be regulated by the organization of the polymerized cytoskeleton (Tomasek et al., 1997). Tumor necrosis factor α (TNF-α) has a delayed action on the production of MMP-9. Intracerebral injection of activated TNF-α caused an increase in MMP-9 at 24 h after injection, when blood–brain barrier permeability was increased (Rosenberg et al., 1995). The action of TNF-α on the MMP gene is mediated by the c-Fos/c-Jun heterodimer, which binds to the activator protein-1 (AP-1) site and initiates transcription. Prolonged stimulation of c-Jun induced by TNF-α accounts for the delayed MMP-9 production (Brenner et al., 1989). Glucocorticoids block the production of MMP-9 by forming a complex with c-Fos/c-Jun heterodimer that prevents it from binding to the AP-1 site (Jonat et al., 1990).

Activation of MMPs

Secretion of MMPs in a latent form prevents unwanted proteolysis and self-digestion. However, it also makes activation critical for their necessary actions. Most information on the activation of MMPs has been derived from tissues and cell types other than brain. MMP-2 is activated in two steps (Strongin et al., 1993). Activation of MMP-2 is owing to cleavage in the propeptide domain at Asn^{37}-Leu, generating a 64-kDa intermediate form, and changes in the NH_2-terminal that result in the activated 62-kDa form. The cleavage between Asn-Leu is thought to be mediated by the cell-surface activator, such as MT-MMPs, followed by autocatalytic activation. The COOH-terminal domain of MMP-2 has an essential recognition site, which is activated by the cell-surface activator (Seiki, 1996). ProMMP-2 was purified as a complex with TIMP-2 from the conditioned medium of a human glioblastoma cell line. When the complex was incubated with MMP-3, proMMP-2 was effectively converted to the active MMP-2, increasing its gelatinolytic activity about eightfold. MMP-3 has been shown to be a natural activator of TIMP-2-bound proMMP-2 in tumor cells (Miyazaki et al., 1992). Activation of MMP-2 is thought to occur primarily through the MT-MMPs after binding of TIMP-2 (Sato et al., 1994). Another mechanism of activation involving the PA system was recently demonstrated. Plasmin activates the proMT1-MMP, which then activates the proMMP-2 (Okumura et al., 1997). Plasmin and uPA can activate both MMP-9 and MMP-2 in nonneural tissue (Mazzieri et al., 1997). However, the relevance of these mechanisms in brain cells remains to be demonstrated.

Growth factors generate autocrine and paracrine stimulatory loops that promote tumor proliferation and angiogenesis. The tyrosine kinase receptors are a family of structurally related growth factor receptors that are particularly relevant to tumors of the CNS. This large family includes the receptors for the epidermal growth factor (EGF), the platelet-derived growth factor (PDGF), the fibroblast growth factor (FGF), the insulin-like growth factor (IGF), the neurotropins related to the nerve growth factor (NGF), and the vascular endothelial growth factor (VEGF), as well as several receptors for which no ligand has been identified. They are preferentially expressed in

the embryonic brain and are thought to play a central role in regulating the determination of the cell fate during development of the CNS (Weiner, 1995).

Growth factors also play a significant role in the transcriptional regulation of MMP expression and in their activation. The transcriptional control of MMP and TIMP is regulated by several growth factors with cytokine-like action. Their exact action is partially known from several studies. Growth factors are known to promote the production of MMPs. The locomotion of glioma cells can be modified by basic fibroblast growth factor (bFGF), EGF, PDGF, TNF-α, and NGF (Chicoine et al., 1995).

TGF-β1 upregulates MMP-2 and MMP-9 expression in normal human cervical epithelial cells, but metalloproteinase transcription is unresponsive to TGF-β1 in tumor cell lines. EGF reduces MMP expression in normal and tumor cells by decreasing the stability of MMP mRNA (Delany and Brinckerhoff, 1992; Agarwall et al., 1994). The release of MMP-2 in metastatic cell lines was stimulated by TGF-β1, but EGF had no effect on the release of MMP-2. This observation suggests that EGF acts as a mitogen on all cells, but does not enhance the malignant phenotype. Loss of responsiveness to TGF is an important step toward malignant phenotype (Kawamata et al., 1993). Acting like a growth factor, PDGF was shown to have a mitogenic effect on cultured meningioma cells as well as in astrocytic cells (Black et al., 1994).

bFGF induces expression of MMP-9. Cells transfected with the bFGF gene and fused to a signal sequence are invasive in vitro and *in situ* in the brain (Gately et al., 1995). Transfection with bFGF sense and antisense cDNA modified malignant glioma growth (Redekop and Naus, 1995). bFGF was also shown to act as a meningeal angiogenic factor in the rat dura and arachnoid vessels (Olson et al., 1993). The extracellular sequestration and release of bFGF could be a possible mechanism for the angiogenic effect (Vlodavsky et al., 1992).

Metalloproteinase-dependent neurite outgrowth is induced by NGF within a synthetic extracellular matrix, which can be inhibited by a synthetic peptide inhibitor (Muir, 1994). TNF-α does not decrease proteolytic type IV collagenase activity in the U251 human malignant glioma cell line, but tumor cells are probably protected against TNF-α by high intrinsic manganese superoxide dismutase activity (Del Maestro et al., 1992).

MMPs and TIMPs Control Proteolysis Spatially and Temporally

Gliomas invade locally rather than crossing the blood–brain barrier to form metastases. They rarely disrupt basement membranes, following instead the developmental pathways of astrocytes and oligodendrocytes. Although subpial spread can occur as a route of dissemination, penetration into the subarachnoid space through the *glia limitans externa* is a rare event. Gliomas disseminate mainly along white matter fiber tracts. Ultrastructural analysis of the early events in C6 glioma growth showed that the cells attached to the perivascular endothelial basement membrane. Hemidesmosomal plaque formation occurs along the portion of the cell membrane attached to the endothelium. Several integrins have been associated with desmosomes, which are involved in cell–ECM attachment. Nonneoplastic blood vessels were shown to be surrounded by neoplastic processes rather than the normal sheet of the astrocytic foot processes (Nagano et al., 1993).

The coordinated events involved in a glioma invasion into the normal brain are:

1. Escape of cells from the parent tumor into the surrounding extracellular matrix.
2. Hydrolysis of matrix components.
3. Locomotion through the matrix.
4. Trafficking in the perivascular spaces.

The motility of glioma cells can be modified by EGF, bFGF, PDGF, TNF-α, and NGF. EGF and TNF-α have a random-motility and directional motility-enhancing chemoattractant effect on low-grade human glioma cell cultures (Chicoine et al., 1995).

Reactive astrocytes secrete components of the basal lamina to aid survival of injured neurons. Laminin is known to be secreted by astrocytes after injury (Liesi et al., 1984), which promotes glioma attachment and migration in vivo (Muir et al., 1996). Most studies of different substrates for astrocytoma invasion showed that laminin, tenascin, and type IV collagen promote migration (Giese et al., 1995; Chintala et al., 1996). Whether this laminin is derived from astrocytes or glioma cells is unclear (Muir et al., 1996). Epithelial malignancies induce host cells to synthesize increased quantities of collagen and other ECM components in response to the adjacent malignant tumor. This phenomenon, which is called desmoplasia, is peculiar to certain neuroepithelial malignancies, such as medulloblastoma and glioblastoma multiforme (Rutka et al., 1988). Injury also stimulates astrocytes to secrete matrix macromolecules (Liesi et al., 1984).

Release of MMP-2 and MMP-9 from malignant cells was stimulated by laminin, but not by fibronectin or by EGF (Turpeenniemi-Hujanen et al., 1986). Thus, migration and invasion in astroglial tumors may be owing to MMP activity. Immunohistochemical analysis of human glioma tumor has shown extensive presence of MMPs (Nakagawa et al., 1994). *In situ* hybridization studies indicated that mRNA for MMP-9 is predominantly found in the cytoplasm of glioma cells (Muller et al., 1995). The level is high compared to TIMP-1 transcripts, suggesting excessive proteolysis.

In normal brain tissues, TIMP-2 mRNA expression was high, whereas mRNA for MMP-2 and TIMP-1 was low; MMP-9, MMP-1, MMP-3, and MMP-7 were undetectable (Nakano et al., 1995a). MMP-9 and TIMP-1 were concomitantly overexpressed in tumors and in the neuroinflammatory response. Expression of MMP-2 increased threefold in astrocytomas and anaplastic astrocytomas, and sixfold in glioblastomas as compared to normal brain tissues. These results suggest that the concomitant increased expression of MMP-2, MMP-9, and occasional MMP-7 genes is associated with the malignancy of gliomas and accompanied by the increased expression of TIMP-1 gene. Stromelysin was characteristically not expressed in any gliomas, and the expression level of TIMP-2 did not significantly change in the gliomas (Nakano et al., 1995b).

Malignant brain tumors use proteases to invade tissues. The invasive phenotype of different glioma cell lines appears to be related to their MMP-2 and TIMP-2 expression. The addition of TIMP-2 to the assay system inhibited invasion (Abe et al., 1994). Northern blots of tumor tissue extracts from low-grade gliomas showed high expression of TIMP-2, moderate expression of MMP-2 and TIMP-1, and undetectable expression of MMP-1, MMP-9, MMP-3, and MMP-7 (Nakano et al., 1995a).

Radiation also affects MMP expression. Rat astrocytes increase tPA and MMP-2 expression when exposed to ionizing radiation, which could explain the fibrin deposition, coagulative necrosis, and impaired microcirculation. Blood vessel fibrosis may secondarily follow and is commonly found in postirradiation necrosis in brain tumor patients (Sawaya et al., 1994).

Glioma cells and normal astrocytes have been shown to express various integrins (Brooks et al., 1994, 1995, 1996; Gladson, 1996; Previtali et al. 1996; Friedlander et al. 1996; Mahesparan et al., 1997; Pijuan-Thompson and Gladson, 1997). High-grade gliomas express the integrins, $\alpha v\beta 3$ and $\alpha 6\beta 4$, which bind to laminin and vitronectin of tumor borders and blood vessels (Pedersen et al., 1993). Integrin $\alpha v\beta 3$ may be necessary for angiogenesis, which promotes tumor proliferation (Gladson, 1996). MMP-2 and $\alpha v\beta 3$ are specifically colocalized on angiogenic blood vessels and melanoma cells in vivo. Furthermore, MMP-2 in its active form can directly bind to $\alpha v\beta 3$ on the tumor cell surface. The complex of proteolytically active MMP-2 and integrin promotes collagen type IV degradation in vitro (Brooks et al., 1996).

During development, viable basement membrane consisting of laminin is a barrier to astro-

Table 2
Substrates for Brain MMPs[a]

Substrates	MMP-2	MMP-3	MMP-7	MMP-9	MMP-14
Collagen 1	+	–	–	–	+
Collagen IV	+	+	+	+	–
Collagen V	+	+	–	+	+
Fibronectin	+	+	+	+	+
Laminin	+	+	+	–	–
Proteoglycan	–	+	+	–	–
Elastin	+	–	+	+	–
Entactin	–	–	+	+	–
Vitronectin	+	+	+	+	+
Aggrecan	+	+	+	+	–
–APP	+	–	–	–	–

[a] Data extracted from Romanic and Madri (1994); Powell and Matrisian (1996); Nagase (1997). *See* Table 1 for names of MMPs.

cytes and forms a pathway for astrocyte migration (Bernstein and Karp, 1996). Fetal and glioma cells migrate similarly when transplanted into the adult rat brain. Gliomas are routed by the same ECM components used during brain development. Laminin and fibronectin are found in the border zone between host tissue and implanted tumor or fetal cells produced by the host cells. Spheroids from glioma cells in vitro fail to immunostain for ECM proteins, suggesting that the host-derived ECM production is caused by either neovascularization, repair synthesis, or local production of guiding substrates (Pedersen et al., 1993). On the other hand, laminin supplementation to serum-free medium has been shown to increase both the release of type IV collagenase from malignant cells and their proteolytic capability (Turpeenniemi-Hujanen et al., 1986).

The cytoskeleton is the only cytoplasmic structure that physically connects the nucleus and the plasma membrane (Clubb and Shivers, 1996). Integrins have been shown to colocalize at focal contacts with cytoskeleton fibers (Tawil et al., 1993). Since integrins affect metalloproteinase activation, the same signaling mechanism that affects the cytoskeletal structure also leads to degradation of the ECM. The mechanism involves depolymerization of the cytoskeleton into a migratory state with MT1-MMP expression and gelatinase A activation (Tomasek et al., 1997). One possible mechanism for glioma cell invasion is for tumor cells to attach to laminin along blood vessels and white matter tracts through genetically linked integrin receptors. This results in an enhanced expression of MMP-2 by the host astrocytes. Activation of MT1-MMP on the cell surface of the tumor cell activates MMP-2, which is near the integrin receptor. Loosening of the tumor cell's adhesion to the ECM alters integrin expression and promotes cytoskeleton rearrangement. The freed tumor cell can then round itself up and migrate.

Migrating cells from astrocytomas have long, slender processes that are bipolar, rather than spread over the substrate (Friedlander et al., 1996). Motility depends on the substrate and correlates with the degree of malignancy of the tumors (Table 2). Antibodies to the integrin $\alpha v\beta 1$ inhibit migration of astrocytoma cells completely, and an antibody toward the laminin receptor has been shown to decrease the degradation of collagen IV (Turpeenniemi-Hujanen et al., 1986; Friedlander et al., 1996). Integrin $\alpha v\beta 3$ antagonists reduce tumor growth in a breast cancer model and induce

apoptosis of angiogenic blood vessels in the chick chorioallantoic model of angiogenesis (Brooks et al., 1994, 1995). Therefore, integrin antibodies may offer a potential therapy to modulate metalloproteinase activation of tumor cells.

Molecular Treatment of Gliomas with TIMPs

In response to injury, reactive astrocytes synthesize ECM components. Laminin, fibronectin, and type IV collagen are expressed by astrocytes when endothelial cells proliferate during neovascularization (Liesi et al., 1984). Implantation of fetal brain or glioma cells into the rat brain induced laminin deposition in the zone between normal and implanted brain tissue. Specific regions of the large 800-kDa laminin molecule may have both stimulatory and inhibitory effects on tumor cells (Pedersen et al., 1993, 1995).

In vitro studies of particular cell lines show a wide range of MMP and TIMP expression rather than a particular pattern of expression in vivo (Mohanam et al., 1995). The aberrant expression of TIMPs is believed to represent an important modulating factor in the invasive capacity of human tumors. Quantitation of TIMP-1 and TIMP-2 by enzyme-linked immunosorbent assay (ELISA) demonstrated low levels of TIMP-1 and TIMP-2 proteins in glioblastomas, and moderate levels in anaplastic astrocytomas compared with normal brain tissues, low-grade gliomas, and metastatic tumors. TIMP-2 mRNA expression was lower in glioblastoma and anaplastic astrocytoma than in meningioma, normal brain tissues and metastatic tumors (Mohanam et al., 1995). Transfection of TIMP-1 into the astrocytoma cell line SF-188 decreased its invasiveness in vitro (Matsuzawa et al., 1996). Transfection of TIMP-2 into brain tumor cell decreased tumor growth, invasion, and angiogenesis in vivo (Imren et al., 1996). These findings suggest that downregulation of both TIMP-1 and TIMP-2 significantly contributes to the invasive potential of human glioblastoma multiforme and anaplastic astrocytomas.

Role of MMPs in Neuroinflammatory Blood–Brain Barrier Damage

MMPs have been implicated in many neurological diseases that involve the neuroinflammatory response. During the inflammatory response, transcription factors, c-FOS/c-JUN and nuclear factor-κB (NF-κB), are formed. These bind to the AP-1 and NF-κB sites in the proinflammatory genes. Several MMPs, such as MMP-3 and MMP-9, have AP-1 and NF-κB binding sites. Once activated, the normally latent enzymes attack the ECM. Inflammation leads to the production of cytokines, chemokines, growth factors, and hormones that modulate MMP production. Activation of proteases (MMPs and PAs) is an important regulatory step in the inflammatory response. Proteases activate the MT1-MMP in the presence of TIMP-2. Activation of MT1-MMP can follow several pathways. An intracellular proprotein convertase, furin, has been suggested as a potential activator of MT1-MMP (Sato et al., 1996), and plasmin is another potential activator (Okumura et al., 1997). Once activated, the MMP-2 alters the endothelial wall, promoting the recruitment of proinflammatory factors. MMP-3 and MMP-9 are induced at the injury site after several hours. Most likely, the MMP-3 is activated and in turn activates the MMP-9. Further damage is done by enzymes released by the invading neutrophils, including additional MMPs and elastases (Armao et al., 1997).

Immune cells are recruited to the site of injury as part of the inflammatory response. T-cells, neutrophils, and monocytes release proteases to facilitate migration across the blood–brain barrier (Leppert et al., 1995). Evidence for the role of the proteases in capillary damage comes from studies involving the intracerebral injection of MMP-2, which

showed that the capillary permeability was increased and that the increase could be inhibited by TIMP-2. Ultrastructural studies showed disruption of the basal lamina around the capillary (Rosenberg et al., 1992). TNF-α injected intracerebrally was shown to increase MMP-9 production around 24 h after injection with opening of the blood–brain barrier at that time (Rosenberg et al., 1995). When lipopolysaccharide was directly injected into brain, it caused an earlier increase in proMMP-9 along with the activated 84-kDa species, which reached maximum by 8–12 h, the time when the capillary injury was also maximal (Mun-Bryce and Rosenberg, 1998). Synthetic hydroxamate-based MMP inhibitors blocked the blood–brain barrier opening after both TNF-α and lipopolysaccharide injections.

Studies have been done in an animal model for inflammatory demyelination, experimental allergic encephalomyelitis (EAE), which is a monophasic illness induced by the injection of myelin with Freund's adjuvant (Gijbels et al., 1994; Kieseier et al., 1998b). Prior to onset of the demyelination, an increase is seen in the permeability of the blood vessel (Leibowitz and Kennedy, 1972). Fibrin deposition around cerebral blood vessels is prominent in EAE, and the fibrinolytic toxins in snake venom reduced the severity of the illness (Paterson, 1976). The cerebral dysfunction in EAE is owing to blood vessel disruption with cerebral edema accumulation and the more permanent damage caused by protease-mediated demyelination.

Proteases are important in the pathogenesis of EAE. Myelin damage is initiated by the release of neutral proteases, including uPA, from macrophages activated during the immune response (Cammer et al., 1978). Inhibitors to uPA, such as aminomethylcyclohexane carboxylic acid, protect rats against EAE (Brosnan et al., 1980). MMPs are increased in cerebrospinal fluid (CSF) of rats with EAE (Gijbels et al., 1993). Animals with EAE treated with a hydroxamate MMP inhibitor showed reduced permeability of the blood–brain barrier and had fewer clinical symptoms, but the inflammatory responses were unaffected (Gijbels et al., 1994). Quantitative polymerase chain reaction (PCR) of rats with EAE shows production of mRNA for several of the MMPs, including MMP-3, MMP-7, and MMP-9 (Wells et al., 1996).

Elevated levels of MMP-9 have been found in the CSF of patients with multiple sclerosis (Gijbels et al., 1992). Acute attacks of multiple sclerosis, characterized by blood–brain barrier opening to the magnetic resonance imaging (MRI) contrast agent gadolinium showed elevated levels of MMP-9, whereas patients with nonenhancing lesions had lower levels. (Rosenberg et al., 1996) When patients with enhancing lesions were given high-dose methylprednisolone, the levels of MMP-9 in the CSF dramatically decreased.

MMPs are involved in the Guillian-Barré-like illness induced in rats by the injection of fragments of peripheral nerve in Freud's adjuvant (Hughes et al., 1998). Experimental autoimmune neuritis, an animal model of Guillain-Barré syndrome, has an increase in the levels of MMPs in the affected nerves (Redford et al., 1997; Kieseier et al., 1998a). Treatment with an inhibitor to TNF-α processing and the MMP activity, BB-1101, reduced the injury to the peripheral nerves. Neuroinflammatory neuritis may, therefore, respond to treatment with MMP inhibitors.

MMPs in Cerebrovascular Diseases

Cerebral ischemia and intracerebral hemorrhage (ICH) cause cellular damage and increased capillary permeability, and disrupt the ECM. The subsequent cerebral edema compresses tissue and further alters cellular function around the lesion. Activated MMPs and serine proteases have been localized to the site of insult, and are believed to participate in the secondary injury that accompanies the neuroinflammatory response of cerebrovascular diseases.

Proteases have been detected in ischemic stroke, resulting from permanent occlusion

and in reperfusion of ischemic tissue once the obstruction has been removed from the involved cerebral vessel. Studies in human tissues have confirmed the increase in MMPs after stroke (Anthony et al., 1997; Clark et al., 1997). An extended latency between the ischemic stroke and reperfusion inevitably leads to tissue damage and increased cerebral edema (Yang and Betz, 1994). After 2 h of carotid artery occlusion, blood–brain barrier permeability increased 3 h after reperfusion and then again at 48 h (Belayev et al., 1996). Elevated levels of MMP-2 corresponded to the earlier 3-h blood–brain barrier opening, and the MMP-9 protease peaked at the later 48-h blood–brain barrier opening. This biphasic barrier opening was interpreted as the activation of constitutively expressed MMP-2 by MT-MMPs at the 3-h reperfusion time-point, and an induction of MMP-9 production resulting from neuroinflammatory signaling (Rosenberg et al., 1998).

ICH or hemorrhagic transformation after stroke is a common neurological condition. Proteases have been implicated in tissue breakdown after a stroke that leads to hemorrhagic conversion. Collagenase-induced ICH is a new model of experimental ICH, which has been characterized both physiologically and behaviorally (Rosenberg et al., 1990). An initial accumulation of neutrophils in brain tissue has been associated with white matter hyperintensity in T_2-weighed images, indicating edema formation by 12 h after collagenase-heparin injection (Del Bigio et al., 1996). Leukocytes, which accumulate in the sites of hemorrhagic damage, are known to produce MMPs and free radicals of oxygen and nitrogen (Birkedal-Hansen, 1993). In addition, resident cells of the CNS, including microglia and astrocytes, are also activated by inflammatory mediators and release cytokines (Sawada et al. 1989; Lee et al. 1997), which amplify the production of MMPs and the extent of proteolytic damage (Colton et al., 1993; Gottschall, 1995; Yamada et al., 1995).

Induction of MMP-9 was observed beginning 12 h after the collagenase-induced hemorrhage. By 24 h the MMP-9 production was maximum and an activated species was also seen. Concomitant production of uPA suggested that these proteases may act together. Treatment of rats with a collagenase-induced hemorrhage with an inhibitor to MMPs, BB-1101, resulted in a decrease in cerebral edema at 24 h (Rosenberg and Navratil, 1997). Although the response to treatment with an MMP inhibitor suggests the MMPs are involved in the generation of cerebral edema, which is consistent with their role in blood–brain barrier inquiry, the sources of the increased MMP-9 in brain remain to be determined.

MMPs and TIMPs in Alzheimer's Disease

MMPs have been linked to the metabolism of amyloid in Alzheimer's disease. Amyloid deposition is found in brains of patients with Alzheimer's disease. Plaques are the pathological hallmark of the amyloid deposits, and these are found around blood vessels. Amyloid-β peptide (A-β) is a 40–43 amino acid fragment of the β-amyloid precursor protein. This A-β peptide forms an insoluble fibrillar molecule that is thought to be toxic to neurons and endothelial cells. Hippocampal tissues from patients with Alzheimer's disease showed metalloproteinase activity at 70, 100, and 130 kDa, which appears to correspond to MMP-2 and MMP-9 (Backstrom et al., 1992). Activation of the enzymes with an organomercurial resulted in a greater activation of the 70-kDa species in the Alzheimer's brains than in the controls. Furthermore, a higher concentration of the 100-kDa was found in those with Alzheimer's disease. It was proposed that the normal action of the MMPs is to degenerate amyloid and that MMP inhibition prevents the normal breakdown of amyloid. In a more recent report, brains of aged beagle dogs with amyloid deposits were analyzed for MMPs. Amyloid-containing brains had higher levels of a MMP at 95 kDa that reacted with antibodies against MMP-9 (Lim et al., 1997).

Studies in cell cultures have shown that β-amyloid stimulates the production of MMP-2, MMP-3, and MMP-9 in astrocytes (Deb and Gottschall, 1996). Microglial cells responded to amyloid precursor proteins with activation of NF-κB through a cGMP-Interleukin-independent mechanism, and they showed increased transcription and levels of interleukin-(IL) 1β and inducible nitric oxide (Barger and Harmon, 1997).

Glial hyaluronic acid binding protein, which is a fragment generated from proteolytic cleavage of a versican-like, aggregating proteoglycan in brain, has been found in brains of patients with Alzheimer's disease (Bignami et al., 1994). Stromelysin (MMP-3) may be involved in generating the hyaluronic acid fragment. Another line of evidence suggesting involvement of the MMPs in Alzheimer's disease is the finding of increased TIMP-1 in the brain of Alzheimer patients. Several TIMP-positive regions were localized to the neuritic senile plaques, neurofibrillary tangles, and Purkinje cells, and the pattern was similar to that observed with anti-tau and SP18 antibodies (Peress et al., 1995). Increased collagen content and abnormalities of the basal lamina are seen in cerebral capillaries from patients with Alzheimer's disease, suggesting an abnormality of the blood–brain barrier in the illness (Kalaria, 1992). At the present time, the manner in which MMPs and TIMPs interact in Alzheimer's disease in unclear, but current evidence suggests an inhibition of the MMPs by an excess of TIMPs or by another protein, such as the amyloid precursor protein (Miyazaki et al., 1993).

Future Directions

Altered levels of MMPs and TIMPs have been implicated in many diseases of the brain. Brain tumors often oversecrete MMPs as compared to TIMP production, but generalizations are not possible because of the variability between tumor types. The fact that turnover of the ECM by matrix-degrading proteases is a highly complex and regulated process allows for several possible avenues of therapeutic intervention. As illustrated in Fig. 1, MMP activation mechanisms are protease- and disease-dependent. Tumor cells that migrate from the primary site of the malignant glioma are responsible for local recurrence, which creates spatial ECM degradation and promotes further tumor progression. Invasion is not linked directly to the end stage of malignancy in these tumors, but represents an early event in glioma progression. Alternatively, neuroinflammatory signaling of immune and resident brain cells can lead to ECM breakdown when protease production and activation are altered.

Treatment of malignant brain tumors has proven difficult. Aggressive treatment protocols that can kill tumor cells almost invariably are associated with devastating side effects. Although less rigorous approaches are not as damaging to the patient, they are almost invariably associated with local or diffuse recurrent tumors. Gene therapies, such as overexpression of TIMPs in tumor cells and the use of antisense MMPs, may in the long run control tumor growth. Comprehensive studies of the factors that cause tumor cell differentiation and invasiveness are of paramount importance.

Multiple sclerosis is associated with increased levels of MMP-9 in brain and CSF. EAE is characterized by increased levels of MMP-9 and the severity of the blood–brain barrier injury, and the clinical signs are reduced by inhibitors to metalloproteinases. Stroke causes an early increase in MMPs both acutely and after a long delay. Intracerebral hemorrhage results in an increase in MMPs, and treatment with MMP inhibitors reduces the edema secondary to hemorrhage. Alterations in MMPs have been implicated in the pathophysiology of Alzheimer's disease. Various synthetic inhibitors of MMPs may be useful in treatment of autoimmune diseases of the nervous system, such as multiple sclerosis and Guillian-Barré. Emphasis is currently focused on developing inhibitors that both target-specific proteases and reduce their toxic side

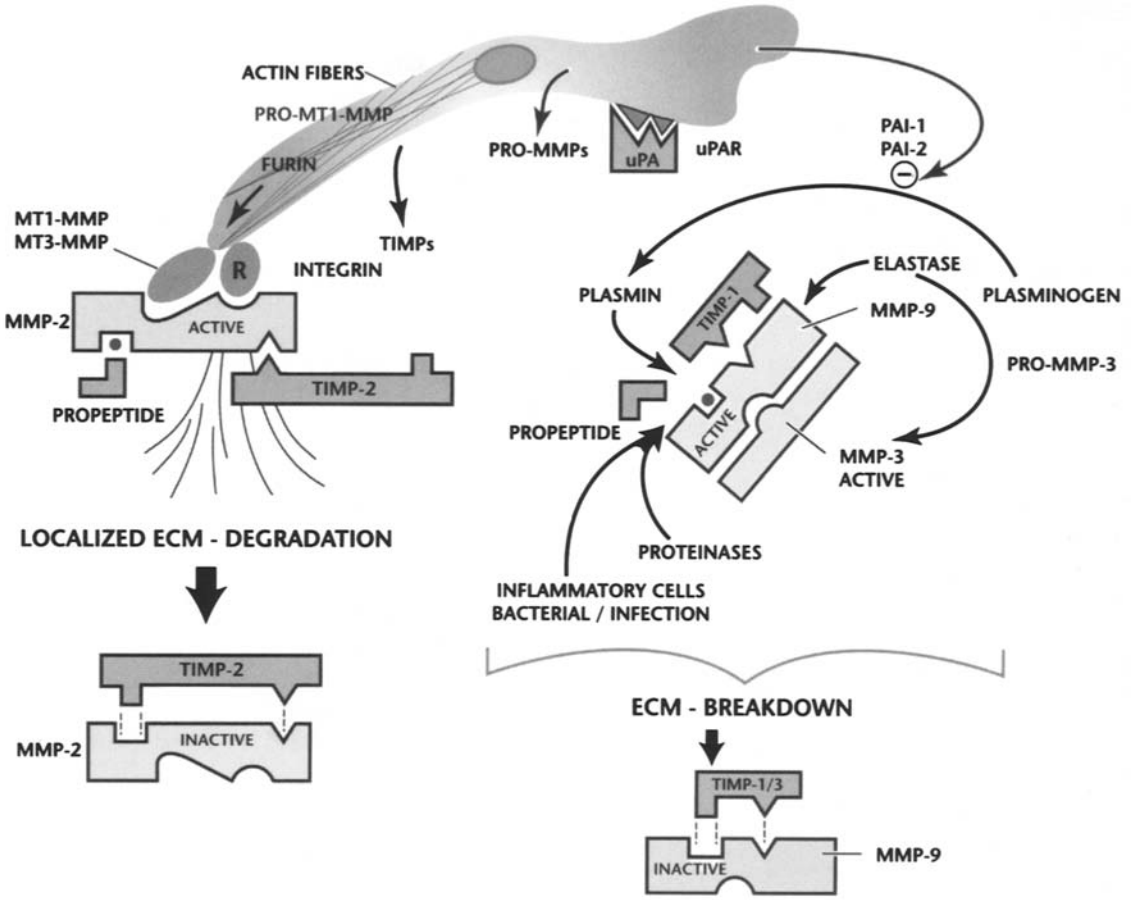

Fig. 1. Schematic representation of the proposed mechanisms for MMP action in the ECM degradation. The right side of the figure depicts the spatial ECM degradation involving the integrins and MMP-2. The binding of the MMP-2 to TIMP-2 and to MT-MMP at the cell surface is needed for the activation of the MMP-2. The active MMP-2 enzyme remains within the spatial region where it is activated. Inactivation occurs when another TIMP-2 binds the MMP-2 molecule in the extracellular space. Another potential pathway of MMP activation is shown on the left. The inflammatory response probably involves plasmin and MMP-9, and also elastases. MMP-3 as shown participates in the activation. Proteolysis continues as the enzymes are secreted in the ECM until the TIMP-1 or another TIMP blocks the reaction. Plasminogen activator inhibitors (PAI) may also block the formation of plasmin and interfere with the activation of the MMPs.

effects. In addition, the same therapeutic approaches used for brain tumors at the gene level should also benefit other neurological diseases that involve unregulated proteolytic activity. Thus, MMPs are important in development, tumor growth, neuroinflammation, and recovery, making it critical to understand the varied and complex roles that these ubiquitous enzymes play in the CNS.

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