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# INVASION OF HUMAN GLIOMA: ROLE OF EXTRACELLULAR MATRIX PROTEINS

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## 1. ABSTRACT

The invasion of glioma into normal brain tissue is a major challenge to clinical intervention because these tumors often highly infiltrate the surrounding brain tissue. Total surgical resection of gliomas is impossible, and recurrence of tumor growth is a common phenomenon; patients have a mean survival time of 8 - 12 months. Although in recent years substantial progress has been made toward understanding the invasive behavior of gliomas *in vitro* and *in vivo*, the factors responsible for the extensive infiltration are still poorly documented. This review focuses on recent research concerning the invasion of gliomas, as well as the extracellular matrix components, and the proteolytic enzymes involved. A better understanding of cell-matrix interactions will help in developing therapeutic strategies to decrease the invasion of gliomas.

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## 2. INTRODUCTION

The extracellular matrix (ECM) is a dynamic milieu that plays a pivotal role in regulating cellular functions during normal and pathological remodeling processes such as embryonic development, tissue repair, inflammation, tumor invasion, and metastasis. Although the ECM contains mainly collagens and noncollagenous glycoproteins such as glycosaminoglycans and proteoglycans, its composition is probably unique for each cell type within an organ. In the nervous system, in contrast to other organ systems, the structure and function of the ECM have not yet been completely determined. The brain is largely free of a well-defined ECM, except where mesodermally derived endothelial cells invade the CNS to establish vasculature. The parenchyma of the CNS, however, appear to be filled with a relatively amorphous matrix that contains mainly hyaluronic acid (1) and little collagen and other fibrous proteins.

**Table. 1. Major extracellular matrix components of the brain**

| | |
|---|---|
| Glial limitans externa | Collagen (I,III,IV)<br>Fibronectin<br>Laminin<br>Heparan sulfate |
| Vascular basement membrane | Collagen (IV, V)<br>Laminin<br>Heparan sulfate<br>Vitronectin<br>Fibronectin |
| Brain parenchyma | Hyaluronic acid<br>Dermatan sulfate<br>Chondroitin sulfate<br>Hyaluronectin<br>Tenascin |

References: 2, 3, 4, 84

A well-defined ECM exists in the form of a true basement membrane, cerebral vasculature, and the glial limitans externa. The latter is a basement membrane that covers the brain's entire cortical surface and also separates astrocyte foot processes from pial cells and the subarachnoid space (2). The cerebral vascular basement membrane, which surrounds the blood vessels of the brain, contains type I, III, and IV collagens, fibronectin, laminin, and heparan sulfate proteoglycans (Table 1). Recent *in vitro* studies have shown that both immature astrocytes and normal leptomeningial cells can synthesize basement membrane molecules. For this review we limit our focus to the ECM components of the human brain that have been proposed as potential candidates for mediating glioma cell invasion.

## 3. EXTRACELLULAR MATRIX COMPONENTS

### 3. 1. Proteoglycans

Proteoglycans and glycosaminoglycans (GAG) are abundantly present in the brain parenchyma. Proteoglycans contain a core protein with one or more covalently bound glycosaminoglycan side chains. The major proteoglycans in the CNS are chondroitin sulfate (CS) and heparan sulfate. CS is a polymer consisting of alternating units of N-acetylgalactosamine and glucuronic acid. In the mature brain, CS, is located in the cytoplasm of some neurons (5-7) and astrocytes, and in myelinated and unmyelinated axon fibers, but not in oligodendrocytes and myelin (8). In contrast, CS proteoglycans are the major proteoglycans of the white and gray matters of the brain, located predominantly in the extracellular spaces of granular and molecular layers in the immature cerebellum, and they are believed to be associated with cell differentiation and migration in the central nervous system (9). Both heparan sulfate and chondroitin sulfate are present in the basement membrane (10). Heparan sulfate is found as membrane protein in synaptic vesicles and in the ECM of the neuromuscular junction (11, 12), and it is also present in the basement membranes of the Schwann's cells (13). Heparan sulfate proteoglycans have been found to induce cell motility (14).

Hyaluronic acid (HA or hyaluronan), a high molecular-weight proteoglycan found in the extracellular matrix, is the only proteoglycan that does not contain a core protein. A polysaccharide comprised of repeating disaccharide units of D-glucuronic acid and N-acetyl glucosamine, HA is found in most extracellular matrices and at the cell surface. Brain parenchyma contain several other proteoglycans, such as tenascin and cytotactin, in addition to glial hyaluronate binding protein (GHAP or hyaluronectin).

Glycosaminoglycans are found mainly within tumor tissue (15); their hyaluronic acid content has been shown to increase transiently during tumor cell migration and is usually found at the interface between tumor mass and host tissues (16). Apart from its major role in tumor cell invasion, HA has been implicated in many cell functions including neural crest migration (17). Elevated levels of HA have been correlated with tumor cell invasiveness (18). HA interacts with other extracellular matrix proteins via hyaluronan binding proteins and receptors such as cluster differentiation 44 (CD44). Recent studies have demonstrated that suppression of CD44 expression decreases migration and invasion of human glioma cells (19). Further, intracranial injection of CD44-suppressed cells led to relatively localized tumor growth and with a reduced malignant behavior, whereas control cells showed extensive invasion typical of highly malignant gliomas (17). Koochekpour *et al*. (20) demonstrated that HA-

mediated cell detachment involved its high-affinity receptors, CD44. They also showed that HA induces cell detachment, stimulates migration, and promotes invasion through interaction with CD44. In 1994, Merzak *et al*. (21) showed for the first time that CD44 is involved in glioma cell invasion. Thiery *et al*. (22) had reported that antisense CD44 oligonucleotides inhibited the invasion of glioma cells *in vitro*, and that several components of basement membrane molecules, such as fibronectin, laminin, vitronectin, and collagen I, might be involved in CD44-mediated invasion of human glioma cells. The proposal is that once synthesized, HA becomes hydrated, opening up the extracellular space to invading tumor cells. This process is suggested to be facilitated through CD44-HA interaction. The role of HA in glioma cell invasion is complicated however, by the fact that astrocytes synthesize HA themselves (23), rather than tumor cells stimulating host fibroblasts to make HA (24). Our own results showed that invasion of glioblastoma cells was significantly increased in the presence of HA (25).

**3.2. Myelin**

Gliomas also disseminate along the myelinated fiber tracts of white matter, which results in distant spread of tumor cells through the corpus callosum into the contralateral hemisphere (26-28). Caroni and Schwab (29) showed that C6 rat glioma cells attach to and spread on crude extracts of CNS myelin, and that the spreading of C6 glioma cells on myelin extracts depends on the expression of a membrane-bound metalloendoprotease (30). More recently, Giese *et al*. (31) found that established glioma cell lines as well as primary cells isolated from glioblastoma biopsy specimens, attach and migrate on crude myelin extracts. Although these findings indicated that glioma cells migrate and spread along existing anatomical structures such as myelin, the responsible component/molecule of the crude myelin extract has not been identified. As Giese *et al*. (31) suggested, the three-dimensional conformation of the associated proteins could be changed during the migrating process, and this in turn could influence the behavior of the glioma cells. It seems, however, that neither neural cell adhesion molecules nor integrins mediate the adhesion of glioma cells to crude myelin extracts.

**3. 3. Fibronectin**

Fibronectin is an $M_r$ 500,000 glycoprotein found in most extracellular matrices as aggregates or fibrils. Fibronectin consists of two polypeptide chains of approximately $M_r$ 250,000 linked by interchain disulfide bonds. Fibronectin has three homologous repeats type I (about 45 amino acids long with two disulfide bonds), type II (about 60 residues with two disulfide bonds), and a type III (about 90 residues with no disulfide bonds). The first major adhesive protein identified, fibronectin has many biological functions involving cell adhesion, migration, and invasion. Fibronectin mediates a variety of adhesive events by binding to fibrinogen/fibrin, collagen, heparan sulfate, and hyaluronic acid. Fibronectin is found at the gliomesenchymal junction of tumors and in tumor-associated blood vessels, and it is expressed by glioblastoma cell lines *in vitro* (32, 33). An arg-gly-asp (RGD) sequence in the third fibronectin repeat functions as an integrin receptor for most cells (34). Kochi *et al*. (35) showed that astrocytomas and glioblastomas do not express fibronectin and that fibronectin was confined to proliferating vessel walls and leptomeninges.

**3. 4. Vitronectin**

Vitronectin, originally known as S-protein and primarily found in the liver, is a multicellular serum protein that promotes cell adhesion, spreading, and migration of a variety of cell types (36). Vitronectin is found mainly in serum, skin, and wound tissue (37, 38). Vitronectin is absent in normal brain and early-stage glioblastoma but late-stage glioblastomas have recently been shown to express vitronectin (39). Vitronectin supports cell adhesion through integrins. Apart from modulating plasminogen (40) and plasminogen activator inhibitor (PAI-I) (40-43) vitronectin promotes cell adhesion mediated by integrins $\alpha v\beta 3$, $\alpha v\beta 5$, $\alpha IIb\beta 3$ and $\alpha v\beta 1$. To date, $\alpha v\beta 3$ integrin appears to be specific for vitronectin, by the finding that low to high metastatic potential of human melanoma was correlated with an increased expression of vitronectin receptor $\alpha v\beta 3$ (44). Gladson and Cheresh (39) recently showed that vitronectin may play a role during local invasion of glioblastoma. A recent study showed that, compared to fibronectin, laminin, and collagen IV, vitronectin was a poor adhesive and migratory protein for glioblastoma cells U-251 and SF-767 (45).

**3. 5. Tenascins**

Tenascins are large disulfide-linked heterodimeric extracellular glycoproteins. Tenascin-C, the original member of a family of heterodimeric extracellular matrix proteins, is implicated in adhesion and migration of human glioma cells (46). However, the recent discovery that tenascin knock-out mice develop normally (47) and the tenascin's contradictory effects on adhesion and migration (48-50) raised a controversy concerning their basic role. To date, three members of the tenascin family have been identified, tenascin-C, tenascin-R, and tenascin-X. All known forms of tenascins consist of heptad repeats, epidermal growth factor (EGF)-like repeats, fibronectin type III repeats and a fibrinogen domain. Tenascin-C (also known as myotendinous antigen, glioma mesenchymal extracellular matrix, hexabrachion, J1-200/220, and cytotactin) is mainly found during normal embyrogenesis; it is prominent in the developing central nervous system and in developing connective tissues, and it is overexpressed in tumors (51, 52). Tenascin-C is expressed in malignant breast carcinomas (53), in wound healing (54, 55) and during newt limb regeneration (56). Tenascin-C is upregulated in most types of

carcinomas including melanomas and gliomas (57-59). In the central nervous system, tenascin-C is found to be synthesized by glial and neural crest cells (60-62) and by satellite cells of the peripheral nervous system (63). Tenascin-R (also known as J1-160/180, januscin, and restrictin), so far identified in rat and chicken, seems to be specific to the central and peripheral nervous system (64-65). Tenascin-X mainly expressed in skeletal and heart muscles, was originally reported as a partial sequence encoded by gene X (66), but little is known about its function. Although tenascin was categorized as an antiadhesive protein (67-69), recent data show that tenascin supports cell adhesion involving the RGD sequence in the third fibronectin type III repeat that it seems to be mediated by V3 integrin (70, 71). A recent study showed that tenascin enhances U251.3 glioma cell migration by an RGD independent mechanism (46).

### 3.6. Laminins

Laminins are a large group of adhesion glycoproteins found in all basement membranes and in hyperplastic blood vessels in gliomas, gliosarcomas, and menigiomas as an integral part of the glial limitans externa (72,74). Laminin is a structural glycoprotein found predominantly in basement membranes (75-77). It plays a role in migration, neurite outgrowth, proliferation, and differentiation (78, 79). The first identified laminin was purified from Engelbreth-Holm-Swarm sarcoma, and recent literature identifies at least six other forms. All the seven known laminins are composed of three subunits designated as $\alpha$, $\beta$, and $\gamma$ and association of disulfide-linked subunits give rise to different laminins (80). Laminin interacts with a variety of basement membrane components such as entactin/nidogen, type IV collagen, and heparan sulfate (80). The other laminin isoforms known are laminin-2 (merosin), laminin-3 (S-laminin), laminin-4 (S-merosin), laminin-5 (kalinin/nicein/epiligrin), laminin-6 (K-laminin), and laminin-7 (Ks-laminin) (78, 80).

### 3.7. Type IV collagen

Interstitial collagens are located in the leptomeninges and the fibromuscular layer of large blood vessels in the brain. Type IV collagen, mainly present in capillaries and large blood vessels, is the principal collagenous constituent of most basement membranes. The most common molecular form of type IV collagen is a heteropolymeric molecule, [a1(IV)]2 a2 (IV); other types of homopolymeric forms, [a1(IV)]3 and [a2(IV)]3, occur as well, and three additional type IV collagen chains are known to exist in kidney (81). Type IV collagen is secreted and assembled as a procollagen molecule, in which, each chain has an apparent molecular weight of 160,000-180,000. In rotary shadowed electron microscope preparations, type IV collagen appears as a rod with a knob like non-helical domain at the COOH- terminal end. Two to four monomers can be associated to form network like appearance in most basement membranes. Recent studies have shown that the glioblastoma cells are also capable of synthesizing type IV collagen *in vitro* (33). In an immunohistochemical study, Bjerkvig *et al.*, (82) showed that type IV collagen was strongly expressed in tumor spheroids from rat glioma cell line BT4C but was negative in monolayers, and fibronectin was strongly expressed in BT4C and BT4Cn cell lines. In an immunofluorescence study, Bellon *et al*., (83) demonstrated that type IV collagen was localized to the subendothelial basement membrane of blood vessels in gliomas. Similar results were reported earlier by Rutka *et al*., (84).

## 4. GLIOMAS

Gliomas, the most common primary brain tumors, account for more than 40% of all CNS neoplasms. Human astrocytic brain tumors have been divided into pilocytic (juvenile) astrocytoma, low-grade astrocytoma, anaplastic astrocytoma, and glioblastoma. Astrocytomas are defined as tumors comprised predominantly neoplastic astrocytes. Pilocytic astrocytoma (World Health Organization [WHO] grade I) is the most common brain tumor in children. These tumors are typically located in midline structures, e.g., the optic nerves, third ventricle, thalamus, medial temporal lobe, brain stem, and cerebellum. Pilocytic astrocytoma very rarely progresses to anaplasia. Low-grade astrocytoma (WHO grade II) shows a consistent tendency to diffusely infiltrate the surrounding brain parenchyma. These neoplasms typically occur in young adults; they may be localized in any CNS region, including the spinal cord, but they tend to localize in cerebral hemispheres. Anaplastic astrocytoma (WHO grade III) is characterized by neoplastic fibrillary or gemistocytic astrocytes. This astrocytoma has an inherent and often rapid tendency to progress to glioblastoma. Glioblastoma multiforme (WHO grade IV) is the most frequently occurring and most malignant brain tumor that typically affects adults. The most common location of glioblastoma is the frontotemporal region, but the parietal lobes are also often affected. Glioblastomas usually infiltrate through the corpus callosum, with extensions into the contralateral hemisphere.

### 4.1. Invasion

Human gliomas are characterized by highly diffuse infiltrative growth into the surrounding normal brain tissue, which makes surgical resection difficult (28, 85). In contrast to other tumor types, gliomas rarely metastasize outside the central nervous system (28). In an experimental model system using C6 glioma cells, laminin, was shown to be the principal constituent of the basement membrane of the blood vessels (86, 87), and it appeared to be responsible, in part, for the exclusion of glioblastoma cells from blood vessels (88). The role of laminin is, however, still controversial. During invasion, the tumor cells break up their cell-cell and cell-matrix

interactions, become motile and subsequently forge a path through the digested ECM. This is accomplished by changes in the expression of cytoskeletal proteins, cell adhesion molecules and matrix-degrading proteases. Whether the tumor cells invade in response to existing ECM components or themselves synthesize autologous ECM and then migrate to the newly laid matrix is still a debated issue. Tumor cell migration is also influenced by migrating signals, such as ECM components, either by chemo-and/or haptotactic mechanisms (89). Liotta (90) proposed a three-step hypothesis for tumor cell migration, suggesting that cellular invasion is the result of highly coordinated and complex mechanism that include a series of cell-matrix interactions: (1) modification of cell-cell and cell-matrix attachments, (2) proteolytic modification of the ECM, and (3) invasion through proteolytically modified ECM.

These events must be coordinated and integrated so that the leading edge of the invasive cells makes new matrix contacts while the trailing edge breaks previously formed ones. ECM proteolysis and cell migration are not mutually independent events. Cell attachment may influence protease production, and protease activity can alter cell attachment and spreading. Understanding how these events are coordinated will bring a better appreciation of the underlying mechanisms of cell invasion and help to identify new targets for therapeutic interventions. In most cases, degradation of the basement membrane results in the solubilization of ECM fragments that may have chemotactic effects.

In many pathological conditions, ECM remodeling is accompanied by a cellular invasion that can compromise matrix organization and disrupt tissue. Under normal conditions, a negative feedback mechanism may limit the behavior of normal cells, such as endothelial cells, and lymphocytes. Although tumor cells use the same mechanism as normal cells, they seem to lack the appropriate feed back mechanism during invasion (91).

## 5. ECM RECEPTORS AND CELL ADHESION MOLECULES

As they invade the tissue, tumor cells must adhere to a variety of ECM components and cell surface molecules. These interactions have a profound effect on the tumor cells invasive phenotypic behavior. Over the last several years, extensive progress has been made in identifying ECM components and particular sites on the components that mediate cellular adhesion / invasion. Tumor cells interact with the extracellular matrix by adhesion molecules or matrix receptors. Several classes of matrix receptors have been described, including integrins, gangliosides, CD44, and some members of the immunoglobulin superfamily such as the neural cell adhesion molecule (NCAM) (92).

### 5. 1. Gangliosides

Gangliosides are a class of sialic acid-containing glycosphingolipids that are selectively localized in the cell membrane (93, 94). Although their functional role is not well documented, the observation that cells that had high migratory capacity concomitantly express more gangliosides gave rise to the speculation that gangliosides may be involved in migratory and invasive behavior of glial tumor cells (22). In this context, Merzak *et al*. (95) showed for the first time that cell-membrane gangliosides are involved in glioma cell invasion *in vitro*. Moreover, the same group showed that exogenous addition of gangliosides enhanced the adhesion of human glioma cell lines to fibronectin, laminin, vitronectin and collagen I (96), thus consolidating the role of gangliosides in adhesion. The identification of a ganglioside binding site on fibronectin supported the role of gangliosides in fibronectin-mediated cell adhesion (97). Moreover, expression of GD3 ganglioside was found elevated in malignant astrocytomas (98) and gangliosides were shown to regulate the function of integrins (99-101).

### 5. 2. Cluster differentiation 44

Originally known as the lymphocyte homing receptor (also known as Hermes antigen, Pgp-1), cluster differentiation 44 (CD44) is a polymorphic family of glycoproteins that carries N- and O-linked sugars and glycosaminoglycan side chains found to be the major receptor for hyaluronic acid (HA) with a wide cellular distribution (102, 103). CD44 has two isoforms with a molecular mass of 80-90 kDa and 150 kDa respectively (104). CD44 is expressed on both normal and neoplastic astrocytes (105), and it has been shown to be upregulated in neoplastic cells (106). Recent studies have shown that CD44 mediates attachment of cells to various ECM proteins including HA, chondroitin sulfate, laminin, fibronectin, vitronectin, types I and IV collagen and modified basement membrane matrigel (107). Koochekpour *et al*. (19) recently reported that antibodies against CD44 inhibited the invasion of human glioma cells and they have showed that the factor responsible for the invasion is hyaluronic acid.

### 5. 3. Neural cell adhesion molecule (NCAM)

NCAMs are perhaps the most widely studied adhesion molecules that are expressed on developing neurons in normal brain (108-111). NCAMs are implicated in mediating adhesion to neural elements and have been shown to bind to several types of collagens (112). A glycoprotein, NCAM, is composed of three polypeptides, NCAM-A, NCAM-B, and NCAM-C, with a molecular mass of 180 kDa, 140 kDa, and 120 kDa, respectively. The A and B proteins are phosphorylated, integral membrane proteins, while C is a nonphosphorylated peripheral polypeptide linked to the plasma membrane by a phosphatidylinositol anchor (113, 114). During migratory events in embryogenesis,

NCAM is down-regulated, and it is re-expressed once the target organ has been reached and differention is initiated (21). Recent studies (115) showed that NCAM expression is correlated with different modes of invasion of rat glioma cell lines, both *in vivo* (116), and *in vitro* (117). NCAM expression has also been shown to correlate with downregulation of 92-kDa gelatinase. The finding that MMP-1 was downregulated by NCAM-B transfection but not in NCAM-C clones, suggested that the NCAM molecule's transmembrane domain, but not its extracellular domain, is involved in signal transduction (117).

**5. 4. Integrins**

Integrins are heterodimeric integral plasma membrane cell-surface receptors that modulate cell-cell and cell-ECM interactions (118). They consist of noncovalently linked α and β subunits. To date, 8β and 16α subunits have been identified; their combinations produce 20 distinct integrins (119-121). Integrins are proposed to be involved in signal transduction from the ECM to the nuclei of cells. The cytoplasmic domain interacts with the cytoskeletal proteins in the vicinity of the cell membrane and is also responsible for the cell adhesion, shape and migration. The binding of cells to different ECM proteins, fibronectin, laminin, thrombospondin, von Willebrand's factor, and tenascin depends on the specific combination of α and β subunits (120). The subunits (α, 120-180 kDa; β, 90-110 kDa) are noncovalently associated and expressed on a wide variety of cell types. Integrins recognize an (RGD) sequence in fibronectin and vitronectin, in addition to Lys-Gln-Ala-Gly-Asp-Val (KQAGDV) in fibrinogen, Glu-Ile-Leu-Asp-Val (EILDV) in alternatively spliced segment of fibronectin, and Gly-Pro-Arg-Pro (GPRP) in fibrinogen (122, 118).

The cytoplasmic domain of the integrin molecule interacts with cytoskeletal proteins such as actin and talin, and activates specific kinases and phosphatases. The extracellular domain of the integrin interacts with ECM components and with integral plasma membrane receptors. Divalent cations, such as calcium and magnesium, play an important role in the function of integrins; a distinct calcium binding site has been identified on an integrin subunit (118). Altered expression of integrins has been shown to correlate with the malignant phenotype of tumors (123, 124). Integrin subunits link the cell's extracellular environment with internal cytoskeletal elements via linking proteins, such as talin and vinculin (125).

**6. ECM TURNOVER AND DEGRADATION**

Matrix degradation and turnover are important processes in tissue remodeling during development, wound healing, tumor necrosis, and inflammation. Key components of ECM turnover and regulation are the matrix metalloproteases (MMP) and their inhibitors TIMPs (tissue inhibitors of metalloproteases). A recently characterized metalloprotease family consists of membrane-type matrix metalloproteases (MT-MMP) that activate progelatinase A and a cascade of matrix proteases that in turn degrade extracellular matrix.

**6.1. Matrix Metalloproteases**

Matrix metalloproteases (MMPs) are a group of zinc-dependent enzymes that degrade ECM molecules, proteoglycans, glycoproteins, and various types of collagens (126, 127). MMPs are secreted by cells in a prometalloprotease form. The activation of MMPs *in vivo* is poorly understood. The MMP family has nine members that can be divided into three distinct groups based on their substrate specificities (table 2).

Often, the activation of the enzyme is completed by an autocatalytic activation that results in the loss of an aminoterminal peptide (130). MMP may be activated *in vitro* by detergents, organomercurial compounds, and proteolytic enzymes such as plasmin, trypsin, kallikrein and stromelysin (127-131). All these agents induce a conformational change such that the cystein-residue in the propeptide and the zinc molecule in the enzyme is disrupted exposing the zinc molecule (131). This, in turn, results in a partially active intermediate enzyme that cleaves the propeptide by autocatalytic activation and results in an active enzyme. Local invasive growth is one of the key features of primary brain tumors. The most common and malignant brain tumors, glioblastoma multiforme, is characterized by aggressive invasion of the surrounding normal brain. The specific mechanisms for this invasive behavior is still obscure; however, tumor cell interaction with the immediate ECM and subsequent degradation of the extracellular matrix components plays a key role in this process. Studies have shown that both MMP-2 (132) and MMP-9 (133) were significantly higher in malignant brain tumors *in vivo*. Moreover, MMP-9 facilitates the invasion of glioblastoma cells *in vitro* (25) and MMP-9 over expression correlates with the malignant progression of gliomas *in vivo* (134). MMPs function is regulated by naturally occurring protease inhibitors, tissue inhibitors of metalloproteases (TIMPs). The TIMP family consists of three members, TIMP-1 (a 28-kDa protein), TIMP-2 (a 21-kDa protein), and TIMP-3 (a 21-kDa protein) all with broad specificity in inhibiting MMPs (Table 2). However, the ability of these inhibitors to form complexes with latent and proenzyme forms of various types of collagenases differs significantly. Decreased levels of TIMP-1 and TIMP-2 expression favors the invasion of glioblastoma cells (135).

Membrane-type matrix metalloproteases are, recently discovered, distinct metalloproteases that are expressed on the surface of tumor cells. MT-

**Table 2. Proteases and their inhibitors**

| Enzyme | Molecular weight | Substrate | Natural inhibitor |
|---|---|---|---|
| ***Matrix metalloproteases*** | | | |
| **Gelatinases** | 72 | Denatured Collagen | |
| 72 kDa gelatinase | | Collagen IV, V, VII,X | |
| (MMP-2; type IV collagenase A) | | Elastin, Fibronectin | |
| 92-kDa gelatinase | 92 | Gelatin | |
| (MMP-9; type IV collagenase B) | | Collagen IV, V | |
| | | Fibronectin | |
| **Collagenases** | 52 | Collagen I-III | |
| Interstitial collagenase | | VII, VIII, X | |
| (Fibroblast-type collagenase, MMP-1) | | | |
| Neutrophil collagenase | 75-85 | Collagen I-III | |
| (PMN-type collagenase, MMP-8) | | | |
| Collagenase-3 | 52 | Collagen I-III, VII VIII, X | |
| (MMP-13) | | | |
| **Stromelysins** | 57-60 | Proteoglycans | TIMP-1 |
| Stromelysin-1 | | Fibronectin | TIMP-2 |
| (MMP-3; Transin) | | Laminin | TIMP-3 |
| | | Denatured collagens | |
| | | Collagen III-IV | |
| Stromelysin-2 | 55 | Fibronectin | |
| (MMP-10, transin-2) | | Gelatin | |
| | | MMP precursors | |
| Stromelysin-3 | 61 | alpha-1-antitrypsin | |
| (MMP-11) | | | |
| Matrilysin | 28 | Fibronectin | |
| (MMP-7, Pump-1) | | Laminin Proteoglycans | |
| | | Gelatin | |
| | | Elastin, entactin | |
| Metalloelastase | 54 | Elastin | |
| (MMP-12) | | Fibronectin | |
| **Membrane MMP** | 66 | Progelatinase-A | |
| MT-MMP (MMP-14) | | | |
| MT-MMP-2 | | | |
| MT-MMP-3 | | | |
| ***Serine Proteinases*** | | | |
| Urokinase plasminogen activator | | Glycoprotein | PAI-1 |
| (uPA) | 55 | Amorphous collagens | PAI-2 |
| | | | PN-1 |
| Tissue plasminogen activator | | Glycoprotein | |
| (tPA) | 70 | Amorphous collagens | |

Abbreviations: TIMP, tissue inhibitor of metalloprotease; PA, plasminogen activator; MMP, matrix metalloprotease; pump-1, putative metalloproteinase; MT-MMP, membrane type matrix metalloprotease.

References: 115, 136, 128, 129, 138

MMPs activate gelatinase and also function as receptors for gelatinase A (136-138), inducing efficient pericellular proteolysis. So far, MT-MMP-1, MT-MMP-2, and MT-MMP-3 have been identified (139). MT-MMP expression has been found to correlate with the invasive behavior of HT-1080 and NIH3T3 cells (140). Our recent studies showed MT-MMP-1 to be highly upregulated in human glioblastomas (141).

### 6.2. Plasminogen activators

Plasminogen activators, (PAs) which belong to the serine protease family, are involved in matrix degradation. Two types of PA: urokinase-type plasminogen activator (u-PA) and tissue-type plasminogen activator (t-PA) convert plasminogen to plasmin, a broad-spectrum enzyme that cleaves fibrin, fibronectin, proteoglycans, and laminin (142, 143). uPA is involved in tissue remodeling during wound healing, inflammatory cellular migration, neo-vascularization and tumor cell invasion, while tPA, a key enzyme in thrombosis is involved in the dissolution of clots in blood vessels and the maintenance of hemostasis in the vasculature (144). tPA is expressed during brain development (145) and may be involved in neuronal migration and neurite outgrowth (146, 147). On the other hand, tPA is totally absent in glioblastoma, colon, lung and breast metastasis. However, anaplastic astrocytoma, low grade glioma and meningioma contain normal levels of tPA (148).

uPA is a highly specific serine protease that is secreted as zymogen, as a single chain (sc) polypeptide (scu-PA), and is readily converted into an active two-chain uPA (tcu-PA) by plasmin, kallikrein, cathepsin B, and nerve growth factor-γ (143, 149-152). Receptor-bound active tcu-PA increases the rate at which plasminogen converts to plasmin (142, 143). The major substrate for uPA is plasmin, which has trypsin-like broad-spectrum protease activity, and which activates MMP (153, 154). uPA is synthesized by various tissues and cells, including those of CNS. One of the plasminogen activator, uPA binds to a specific cell surface receptor, uPAR and provides a localized cell surface proteolysis for the degradation of ECM. Proteolytic activity of uPA is modulated by its cell surface receptor, as well as by plasminogen activator inhibitors (PAIs). Recent studies demonstrated that overexpression of uPA was associated with malignant progression of human gliomas (155) and high affinity binding uPARs significantly contributes to the invasive behavior of gliomas *in vitro* (156). *In situ* hybridization studies have shown that uPAR mRNA was significantly higher in glioblastomas than in low grade glioma and normal brain (157).

Plasminogen activators are regulated by plasminogen activator inhibitors (PAIs) and protease nexin-I (PN-1). PAI-1 is a 46-54 kDa glycoprotein with high specificity for both uPA and tPA (158). Recent immunohistochemical and biochemical studies (159) have shown that the amount of PAI-1 was higher in malignant brain tumors. Using northern blot and *in situ* hybridization techniques, Yamamoto *et al.* (160) have shown higher levels of PAI-1 mRNA in glioblastoma than normal brain tissue. Recent evidence also indicates a correlation between the presence of tumor necrosis and higher levels of PAI-1 in glioblastomas (161). PAI-2, originally found in placental tissue (162), has a stronger affinity for uPA than tPA; it also exists in plasma. PAI-3, a 50-kDa inhibitor, has stronger affinity for uPA than tPA. The protease nexin-1 is a 43-50 kDa secreted glycoprotein, that inhibits uPA and plasmin. In the ECM, nexin-1 binds to thrombin, urokinase and plasmin (163). PN-1 is present in normal human brain (164), is produced mainly by astrocytes (165) and by glioblastoma cells (166, 167). Higher PN-1 levels have been observed in an experimental rat 9L gliosarcoma tumor than in normal brain (167).

## 7. PERSPECTIVE

We briefly summarized the molecular repertoire of extracellular matrix components of the brain and the role of ECM receptors and proteases involved in glioma cell invasion. Although different ECM components have been identified as having a role in glioma cell invasion, so far no single molecule has been identified as being responsible for the invasion. Understanding the significance of the ECM molecules will have a profound effect on anti-invasion strategies. One target would be the inhibition of proteolytic enzymes by an antisense method. Our recent results showed that antisense oligonucleotide transfection might be a suitable therapeutic approach to the inhibition of tumor cell invasion (Y. Go *et al*., unpublished observations). Inhibiting the synthesis of ECM components does not seem to be effective because tumor cells can find ECM molecules in the normal brain. Another approach might be to target integrin signaling in cell-matrix interaction. Whether this strategy can be exploited remains to be seen.

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