Chapter 49
Glioma Invasion: Mechanisms and Therapeutic Challenges

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Abstract A hallmark of gliomas is the ability of individual tumor cells to infiltrate the neural tissue and extend beyond the visible borders of the tumor. Current treatments fail to remove these invasive cells, which almost invariably lead to tumor dissemination and therapy failure in the long term. The composition and properties of the extracellular matrix (ECM) in the adult central nervous system are notoriously inhibitory to cell motility and axonal extension. However, glioma cells are uniquely able to remodel this microenvironment by degradation of the neural ECM and production of a novel matrix that contains neural-specific and mesenchymal components. Structural signals from the ECM and soluble factors from the surrounding non-neoplastic cells regulate the molecular and cellular mechanisms of invasion, which include matrix remodeling, cytoskeletal reorganization, and phenotype transition from highly proliferative to migratory. Specific strategies against invading glioma cells are in their infancy due to the paucity of appropriate targets and the difficulty of predicting the effects of targeting this highly plastic cell population in vivo. Identification of the key molecular mechanisms necessary for cell invasion and the major switches that regulate the inter-conversion of migratory and proliferative phenotypes will provide a wealth of novel targets to direct therapies against brain tumor progression and improve long-term patient survival.

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49.1 Introduction

Malignant gliomas are the most common primary tumors of the central nervous system (CNS). A hallmark of these neoplasms is the ability of individual glioma cells to detach from the tumor mass and invade the neural tissue (Louis, 2006). This diffuse infiltration occurs with little distortion of the neural architecture and does not seem to trigger inflammatory or other immune responses, placing the migrating cells beyond the limits of current clinical detection (Claes et al., 2007). In addition, significant evidence has shown that migrating glioma cells divide more slowly than the cells at the core of the tumor (Demuth and Berens, 2004; Giese et al., 1996) and are consequently more resistant to cytotoxic therapies. Therefore, these cells remain in the CNS even after aggressive resection and treatment of the residual tumor, becoming a major factor for local recurrence and tumor dissemination throughout the CNS (Berens and Giese, 1999; Giese and Westphal, 2001).

Current therapeutic strategies after surgical resection, or directly applied to inoperable tumors, target proliferating cells through a combination of cytotoxic and anti-angiogenic drugs (Anderson et al., 2008; Salgaller and Liau, 2006). Few attempts have been made to specifically target the migratory malignant cells located far from the tumor center, as an additional strategy to prevent recurrence and improve long-term management of the disease. This approach is of major relevance because cell infiltration is a cause of rapid disease progression after initial response and therefore a major limitation against lasting success of current therapies (Lamszus et al., 2003). Understanding the cellular and molecular mechanisms underlying glioma invasion is thus a priority for developing effective therapeutic strategies against the spread and recurrence of these tumors (Claes et al., 2007; Louis, 2006). In this chapter we will focus on the composition and remodeling of the microenvironment that surrounds the motile glioma cells, briefly review the major signals and mechanisms involved in glioma cell invasion, and discuss potential anti-invasive strategies.

49.2 Overview of Glioma Cell Invasion in the CNS

In solid carcinomas, increased malignancy is characterized by the 'epithelial-to-mesenchymal' transition of the tumor cells (Guarino et al., 2007), a set of genotypic and phenotypic changes that enables individual cells to detach from

the primary tumor, disrupt the basal lamina of the epithelial tissue, invade the surrounding stroma, and eventually reach lymph and blood vessels that will allow dissemination of the tumor to other organs (Stetler-Stevenson et al., 1993). The dispersion of individual tumor cells from the primary lesion requires a number of sequential steps that include detachment from the tumor core, receptor-mediated adhesion to the immediate extracellular matrix (ECM), local degradation of the ECM to allow cell passage, and active motility mechanisms that drive the cells away from the tumor and/or toward attractants (Bellail et al., 2004; Giese and Westphal, 1996; Nakada et al., 2007). The dispersion of glioma cells within the CNS has been studied within this paradigm and in many ways reflects it. Accordingly, most assays to study glioma cell motility and invasion follow models of invasion and metastasis from other cancers (see Table 49.1 for the most common assays currently in use). However, there are important differences between the local dispersion of gliomas and the stromal infiltration and metastasis observed in non-neural carcinomas.

Compared to peripheral tissues, central nervous tissue is highly refractory to tumor infiltration and tumors that metastasize to the CNS almost never invade it but rather push the neural tissue aside, even when those tumors may aggressively infiltrate their tissues of origin (Subramanian et al., 2002). Conversely, glioma cells invade the brain parenchyma well, despite the presence of an inhibitory ECM and the absence of most basal-lamina elements and supporting stromal cells (Giese et al., 2003). At the same time, glioma cells do not intravasate into blood vessels (Bernstein and Woodard, 1995) nor metastasize to close peripheral tissues. Moreover, when implanted peripherally, gliomas grow as compact, encapsulated masses (Bolteus et al., 2001; Pilkington, 1997). The ability of gliomas to colonize neural tissue as an 'intra-parenchymal metastasis' (Bernstein, 1996) together with their metastatic failure outside the CNS indicates a high degree of specialization of these tumors to the neural environment and suggests that glioma cells may have unique mechanisms of invasion adapted for the particular composition and structure of the CNS (Bellail et al., 2004). Experimental models should therefore strive to reproduce the distinguishing properties of glioma cells, and their differences with other tumors should be kept in mind when analyzing glioma invasion in conditions that do not mimic the neural microenvironment (Claes et al., 2007) (see Table 49.1).

Histological evidence demonstrates that glioma cells follow typical dispersion routes and have preferential tropisms independently of the original localization of the tumor (Giese and Westphal, 1996; Giese, 2003) (Fig. 49.1). Most commonly, glioma cells disperse along white matter tracts, leading in many cases to the invasion of the tumor into the opposite hemisphere in the brain ('butterfly lesions' across the corpus callosum, see Fig. 49.1A). Glioma cells also migrate along the basal lamina of brain blood vessels or spread in the space between the glia limitans and the pia mater, forming perivascular and subpial foci of proliferation. Finally, glioma cells can also move through the network of unmyelinated cell processes that form the grey matter neuropil and proliferate

Table 49.1 Common models used to study glioma cell motility and invasion

	Assay	Timescale	Good to analyze	Advantages	Disadvantages
ADH	*Adhesion assay:* Cells plated on a substrate-coated surface	Minutes to hour long	Adhesive properties of the cells	Fast, simple, easy to reproduce and quantify.	Static assay does not inform about cell motility.
Motility	*'Scratch' assay:* Cell monolayer scratched with a tip and left to reform.	Overnight to 2 days	Overall population motility	Relatively fast, simple.	Gap-width is difficult to quantify. Not all cells form dense monolayers for this assay. Other: (1),(2)
	Radial migration assay: Cells are plated on the center of a pre-coated surface and migrate radially.	Overnight to a few days.	Random dispersion from a focal point	Simple, easy to reproduce.	Other: (1), (2), (3). Proper image analysis is required (see (Stein et al., 2007)).
	Boyden chamber assay: Cells move through a porous membrane toward chemo- or haptoattractants	Few hours to overnight	Motility under directional stimuli	Relatively fast, easy to reproduce and quantify. Can be used to test repellent molecules.	Pores are larger than inter-cellular spaces in the CNS. Cells that remain on the upper surface may skew quantification if not thoroughly removed. Other: (1)
	Scaffold-based migration: Cells plated on polymer scaffolds and allowed to move in 2D/3D	Usually 24–36 hours.	Topographic influences on motility	Scaffolds can be precisely engineered and functionalized with neural-specific biomolecules.	Nanofiber composition and engineering varies among laboratories. Time-lapse confocal cell tracking and complex image analysis required.
Invasion	*Collagen invasion assay:* Cell aggregates seeded inside a collagen matrix and allowed to disperse radially.	Several days	Random invasion through a matrix	3D version of the *Radial migration assay*. Cell morphologies approximate those observed in intracranial tumors	Changes in medium composition or hypoxia in the gel may develop over time. Degradation of basal-lamina matrix is uncommon to gliomas in the brain. Other: (2),(3),(4)

Table 49.1 (continued)

	Assay	Timescale	Good to analyze	Advantages	Disadvantages
	Matrigel invasion assay: Cells seeded onto a matrix inside a Boyden Chamber and stimulated to migrate toward an attractant	Usually 2–4 days	Directed invasion through a matrix.	A more complex version of the *Boyden Chamber assay*. Allows quantitative evaluation of pro-invasive factors, e.g., matrix proteases.	Degradation of basal-lamina matrix is uncommon to gliomas in the brain. Other: (2),(4).
	Slice invasion assay: Cell aggregates seeded on top of live brain slices	3 days to a week	Invasion through a realistic matrix	Substrate accurately mimics the neural architecture. Cell morphologies similar to those observed in intracranial tumors.	Long and time-consuming. Dying neural cells in the slice may affect glioma cells. Migrating cells may not show preferential attraction to white matter. Other: (2)
In vivo	*Tumor implantation:* Cells injected subcutaneously or intracranially and allowed to form a tumor	Several weeks	True invasion in vivo	Tumor growth and invasion under influence of neural, vascular, and immune factors. It is the most reliable assay to verify anti-tumoral strategies.	All subcutaneous gliomas and many intracranial xenografts do not show invasion. Brain invasion in syngeneic models is difficult to quantify and requires morphometric analysis.

Common disadvantages in several models: (1) Motility on a hard surface may not be representative of natural movement. (ADH: Adhesion) (2) Long-term assays must consider cell proliferation/viability as additional variables. (3) Radius and area of dispersion can be biased by outlier cells and image analysis algorithms. (4) Matrix proteins used in these assays are usually absent in the CNS.

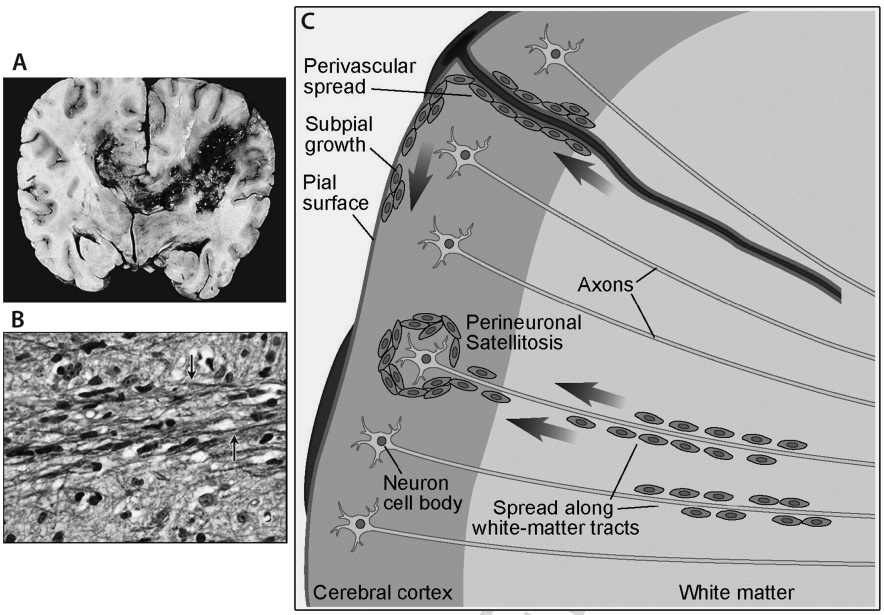

Fig. 49.1 Anatomical pathways for glioma invasion. (**A**) Dramatic infiltration of a "butterfly" glioblastoma multiforme across the *corpus callosum* (unfixed, gross specimen). (**B**) Microphotography of elongated, hyperchromatic tumor nuclei oriented along myelinated axons (Luxol fast blue and H&E stain, 400X, myelinated fibers indicated with arrows). (**C**) Glioma cells show preferential dispersion along myelinated tracts, blood vessels, and the basal lamina of the subpial surface. Tumor cells can also move throughout the neuropil of the brain parenchyma. Periaxonal and perineuronal migration often results in perineuronal satellitosis. Image in *A* reproduced from the collection of Dr. John J. Kepes, with permission from the University of Kansas, Department of Pathology and Laboratory Medicine; images in *B* and *C* reproduced from Louis, D., *Annual Review of Pathology: Mechanisms of Disease,* Volume 1 © 2006, with permission from Annual Reviews (www.annualreviews.org)

around individual neurons. Detailed illustrations of these routes of dispersion can be found in reviews by Claes et al. (2007) and Louis (2006).

The patterns of periaxonal, perivascular, and perineuronal accumulations, or satellitosis, known as *secondary structures of Scherer,* are typical of clinical specimens and can be replicated experimentally (Guillamo et al., 2001), suggesting that glioma cells may have a stereotyped set of substrate-dependent migratory behaviors. The routes of migration likely follow both appropriate biochemical cues as well as favorable anatomical structures that provide pathways of least resistance to cell dispersion (Giese, 2003).

Regardless of the biochemical or structural influences on the migrating cells, this invasive behavior is common to the overwhelming majority of gliomas. It has been suggested that this reflects the acquisition of motility, a 'mesenchymal' property (Wolf et al., 2003), early after transformation (Louis, 2006). Alternatively, given the mounting evidence (see Chapter 44) suggesting that gliomas

may arise from transformed neural stem cells (Barami, 2007; Gilbertson and Rich, 2007), the migratory phenotype could be a reflection of the motile nature of the committed neural progenitors that derive from those stem cells (Claes et al., 2007). Evidence from experimental models indicates that glioma cells move through the neural architecture in a manner that largely resembles the migration of neural progenitors and that is quite different from glioma cell motility in vitro (Beadle et al., 2008). The possibility that glioma-initiating cells derive from adult neural stem cells has raised interesting questions about the possibility of predicting pathways of invasion radiating from neural stem cell-rich zones. For example, recent results have shown that the depth of the tumor in the brain and its association with the subventricular zone may correlate with invasive and multifocal properties of glioblastomas (Lim et al., 2007).

49.3 Glioma Cell Microenvironment: Extracellular Matrix

Both grey and white matter in the adult neural parenchyma form an inhibitory environment for cellular motility and axonal extension (Busch and Silver, 2007). To disseminate in this tissue, migrating glioma cells must interact with a variety of substrates with different topography and molecular composition, such as the amorphous ECM of the grey matter neuropil, the surface of white matter fibers, and the basal lamina of the neural vasculature and the subpial surface (Giese, 2003). The ability of glioma cells to interact with a wide variety of substrates suggests the existence of multiple migratory mechanisms that overcome the inhibitory elements and respond to 'motogenic' signals to promote cell dispersion (Bellail et al., 2004).

49.3.1 Neural ECM

The major barrier opposing glioma cell movement through intercellular spaces is the ubiquitous and distinct neural ECM that comprises as much as 20% of the adult brain and spinal cord volume (Novak and Kaye, 2000). This matrix lacks the typical proteins found in the fibrillar ECM of other tissues, such as collagens, fibronectin and type-I laminin (Novak and Kaye, 2000), and is instead composed of the polysaccharide hyaluronic acid (HA), with associated glycoproteins and proteoglycans. The major structural constituents of this matrix, which in most cases are also expressed in gliomas, are listed in Table 49.2 and illustrated in Fig. 49.2. Of these, HA and its HA-binding proteins are the key molecules that form the neural ECM scaffold (Yamaguchi, 2000); other illustrations of these ECM components can be found in reviews by Viapiano and Matthews (2006) and Galtrey and Fawcett (2007).

HA is a very large (~500–1000 kDa) hygroscopic glycosaminoglycan that can retain large amounts of water, thus creating hydrated spaces used by cells to

Table 49.2 Major structural components of the neural extracellular matrix[a]

Molecules (Mw)	Properties	Neural tissue: Expression[b]	Neural tissue: Functions	Gliomas: Expression[c]	Gliomas: Functions	References
Hyaluronic acid (HA) ($>10^6$ Da)	Occupies large hydrated spaces and forms a compressible mesh at high concentrations.	↑ in early development ↓ and less soluble in the adult	Compression resistance. Facilitates cell migration and proliferation during development	↑↑ (~fourfold higher than normal adult CNS)	Cell proliferation and migration; HA fragments induce synthesis of ECM molecules and MMPs	Delpech (1993), Laurent (1996) and Novak (2000)
Lecticans (150–600 kDa) Aggrecan Neurocan Versican Brevican	Bind HA and cell-surface receptors. Many isoforms produced by alternative splicing, proteolysis, and differential glycosylation	Variable profiles for each lectican. ↑ neurocan during development. ↑ brevican and versican in adult CNS. Total levels of CSPGs are higher in adult than developing CNS	Restrict cell migration and axonal extension in adult CNS. May guide axonal extension and neuroblast migration in the developing CNS	Variable profiles. Versican and brevican are highly expressed in gliomas	Cell proliferation and resistance to apoptosis (versican); enhanced adhesion and migration (brevican); binding to fibronectin; EGFR activation	Crespo (2007), Novak (2000), Viapiano (2006) and Yamaguchi (2000)
Link proteins (HAPLN1 to 4) (50–55 kDa)	Link HA to the lecticans; condense the adult ECM	↓ during early development. ↑↑ HAPLN2 and HAPLN4 in adult CNS	Formation of perineuronal nets; might modulate synaptic plasticity.	↓↓ in glioma tissue ↑ HAPLN4 in GSCs (MSV, unpublished)	Unknown	Oohashi (2005)
RPTP-β/ζ Phosphacan (250–450 kDa)	Membrane-bound K/CSPG; does not bind HA.	↑ in early development.	Matrix remodeling, neurite outgrowth and	↑ RPTP-β/ζ in gliomas grade II–III	Pleiotropin signaling to stimulate cell	Margolis (1996), Rauch

Table 49.2 (continued)

Molecules (Mw)	Properties	Neural tissue: Expression[b]	Neural tissue: Functions	Gliomas: Expression[c]	Gliomas: Functions	References
	Phosphacan is the soluble form of RPTP-β/ζ. Major ligand: pleiotropin	↓↓ in the adult (restricted to neurons)	synaptic plasticity (both positive and negative effects reported)	↑ Phosphacan in gliomas grade IV	migration and proliferation	(2004) and Ulbricht (2003)
Tenascins (Tn) (180–250 kDa) Tn-C / Tn-R Tn-X / Tn-Y	Multimeric proteins; bind to the lecticans, to RPTP-β/ζ, and to several cell-surface receptors	↑ in early development. ↓ in the adult (mostly expressed in the white matter)	Proliferation and migration of neural precursors from the SVZ; axonal guidance; adult ECM condensation	↑↑ Tenascin-C (expressed in the tumor vasculature)	Tn-C combined with PDGF/LPA signaling triggers cell migration. Tenascins also increase glioma cell proliferation	Garwood (2001), Joester (2001), Lange (2008) and Rauch (2004)
SPARC family (40–50 kDa)	Small glycoproteins; bind to growth factors and basal lamina ECM proteins	↑ in early development. Expressed by astrocytes in adult CNS	May be involved in neurite extension and synapse formation	SPARC member of the family is highly expressed by glioma cells	HSP27 activation; MMP upregulation; cell migration on basal lamina matrix	Au (2007), Brekken (2000) and Vincent (2008)

[a]This table includes secreted matrix molecules known to have a structural role in the neural ECM and to be also expressed in the glioma matrix. Therefore, we have omitted the cleaved extracellular domains of membrane-bound proteins (heparan sulfate proteoglycans, NG2, and myelin-associated inhibitors), small soluble factors, and cell/axon guidance factors that are not structurally associated to the ECM (semaphorins, Wnts, slits).
[b](↑), high expression; (↓) low expression.
[c]Expression levels compared to normal adult CNS.
GSCs: Glioma stem cells; *HAPLN*: hyaluronan and proteoglycan link protein; *HSP27*: heat shock protein-27; *K/CSPG*: proteoglycan with keratan and chondroitin sulfate; *MMP:* matrix metalloprotease; *PDGDF/LPA*: plateled-derived growth factor and lysophosphatidic acid; *RPTP-β/ζ*: receptor-type protein tyrosine-phosphatase β/ζ; *SPARC*: secreted protein acidic and rich in cystein.

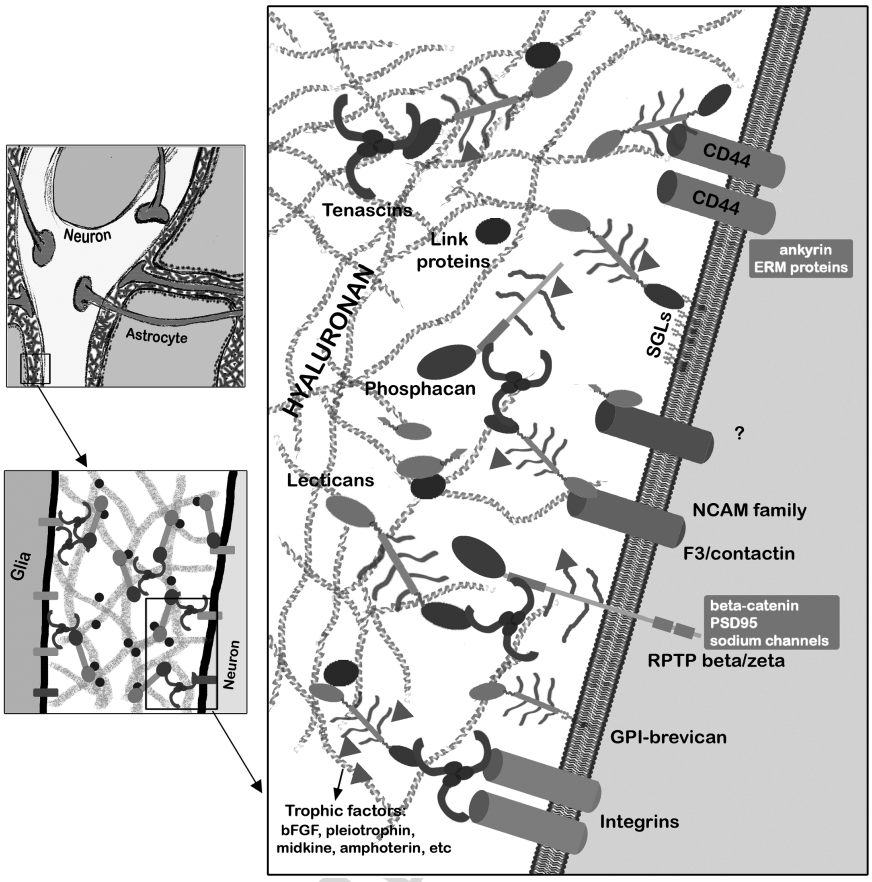

Fig. 49.2 Simplified model of the neural ECM. The cartoon depicts the predominant molecules that compose the ECM close to the surface of the neuronal and glial cells. The chondroitin sulfate proteoglycans of the lectican family can bind hyaluronic acid, secreted ECM proteins, and cell-surface receptors, thus acting as extracellular 'anchors'. *ERM proteins*: proteins of the Ezrin/Radixin/Moesin family; *SGLs*: sulfoglucuronyl-glycolipids. Figure and text reproduced from Viapiano M.S. and Matthews R. T., *Trends in Molecular Medicine*, Volume 12 © 2006, with permission from Elsevier

proliferate and migrate during development (Laurent et al., 1996). HA associates with secreted and membrane-bound HA-binding proteins, which act as organizers of the matrix scaffold around neural cells (Yamaguchi, 2000). Accumulation of these HA-binding proteins in the adult CNS reduces the interstitial spaces and renders the neural ECM largely insoluble, forming a restrictive environment for axonal navigation and cell motility (Rauch, 2004; Viapiano and Matthews, 2006).

The major group of HA-binding proteins in the adult CNS is formed by the secreted chondroitin sulfate proteoglycans (CSPGs) of the lectican family: aggrecan, versican, neurocan, and brevican (Yamaguchi, 2000). These large,

heavily glycosylated proteins exhibit a remarkable heterogeneity of isoforms, expression patterns and molecular partners, but in general predominate in the adult CNS and connect the matrix scaffold to receptors on the surface of neurons and glial cells (Viapiano and Matthews, 2006; Yamaguchi, 2000). The lecticans have been recognized as a major group of inhibitory molecules for axonal extension, cellular adhesion, and motility (Rhodes and Fawcett, 2004), a property attributed in part to a chemorepellent effect of the side chains of chondroitin sulfate (Crespo et al., 2007). Surprisingly, some members of this family are highly expressed in gliomas and promote glioma cell migration (see below) (Viapiano and Matthews, 2006).

The composition of the neural ECM is very similar in the grey and white matter, with some differences in the local expression of ECM molecules in subsets of neurons and grey matter neuropil (e.g., aggrecan, neurocan) or around myelinated axons (e.g., versican and the tenascins). In the white matter, the inhibitory effect of the secreted CSPGs on cell and neurite motility is potentiated by the effect of ECM-associated molecules normally involved in neuronal and axonal repulsion (most notably the netrins and slits (Barallobre et al., 2005; Wong et al., 2002)), as well as the well-known myelin-associated inhibitors (Nogo, the myelin-associated glycoprotein MAG, and the myelin oligodendrocyte glycoprotein (Xie and Zheng, 2008)). The latter are not matricellular proteins but membrane-bound 'collapse signals' that prevent extension of glioma cell processes and motility in vitro (Hensel et al., 1998; Liao et al., 2004), although at least one of these myelin inhibitors (MAG) exhibits ECM-associated, highly inhibitory soluble forms in vivo (Tang et al., 2001).

In addition to having strong inhibitory signals for cell migration and neurite extension, the scaffold of the neural ECM also presents structural constraints for cell motility. This scaffold is essentially a highly compressible mesh of HA and proteoglycan filaments that does not favor cell traction because it provides sparse, randomly organized anchorage points for the formation of intracellular stress fibers (Georges et al., 2006; Peyton et al., 2007). This structural limitation may be partially attenuated in the white matter due to the high density of parallel axons that may form guiding rails for motile cells. In vitro scaffolds composed of aligned fibers have been shown to provide topographic guidance to neural cells and neurites and promote cell adhesion and motility better than randomly oriented fibers (Yang et al., 2005) (also SEL and MSV, manuscript in preparation), independently of other cues in the surrounding environment.

49.3.2 Basal Lamina

In stark contrast with the HA- and proteoglycan-based matrix that fills the extracellular space around neurons and glial cells, the ECM that covers the abluminal surface of the brain–blood vessels and the subpial surface resembles the typical basal lamina that separates epithelial cells from their underlying

stroma in peripheral tissues. This matrix contains some of the proteoglycans and glycoproteins described in the neural ECM but is predominantly abundant in matricellular proteins that form networks of fibrillar aggregates, such as laminins, fibronectin, and vitronectin, and thus promote the adhesion of motile cells (Bellail et al., 2004; Gladson, 1999). The basal lamina ECM also contains several types of collagens, particularly collagen IV and VI, with lower amounts of the interstitial collagens I and III (Gladson, 1999; Paulus et al., 1988). This dense, organized matrix provides higher resistance to compression than the neural ECM and favors the formation of focal adhesions. Therefore, the basal lamina serves as a preferred substrate for integrin-mediated cell adhesion and motility along vascular and subpial surfaces (Goldbrunner et al., 1999). Interestingly, glioma cells accumulate along this basal lamina but do not degrade it in vivo (Paulus et al., 1988), although they can traverse the basal lamina of peripheral blood vessels when they are injected intravenously (Mandybur et al., 1984). The mechanisms that prevent degradation of the basal lamina of CNS blood vessels by glioma cells are unknown, and this phenomenon has been postulated as the underlying reason for lack of intravasation and extra-axial metastasis of these tumors (Bernstein and Woodard, 1995)

49.4 Extracellular Remodeling and Glioma Invasion

As described above, tumor cells attempting to disseminate in the CNS are challenged by a variety of molecular and structural inhibitory factors in the neural parenchyma that limit their adhesion and motility. Peripheral tumors facing these obstacles continue growing but individual cells rarely detach from the tumor mass and almost never infiltrate CNS structures. Conversely, glioma cells actively remodel the surrounding matrix to reach the anatomical structures along which they disseminate. This remodeling involves degradation of the pre-existing ECM, overproduction of neural ECM molecules, secretion of novel ECM molecules that are absent in the neural parenchyma, and expression of novel cell-surface receptors for ECM signals. These processes are likely potentiated by additional tissue remodeling produced by infiltrating immune cells and the proliferative endothelium of hyperplastic tumor blood vessels (Bellail et al., 2004; Gladson, 1999; Kaur et al., 2005). For the scope of this review we will focus only on the major molecular changes exerted by isolated glioma cells invading normal neural tissue.

49.4.1 ECM Degradation

Compared to adult normal neural tissue, glioma cells exhibit increased expression of lysosomal hyaluronidases and secreted proteases. These enzymes can degrade the pericellular matrix, opening spaces for cell motility and releasing protein and glycosaminoglycan fragments that act as mitogenic and motogenic

signals (Junker et al., 2003; Rao, 2003). A wealth of evidence has demonstrated that several families of proteases actively contribute to matrix remodeling in gliomas, including the plasminogen activators, the members of the matrix metalloprotease (MMP) family, and the lysosomal/secreted cathepsins (Levicar et al., 2003; Rao, 2003). Recent investigations have expanded the set of glioma-active proteases to include the disintegrin-and-metalloproteases (ADAMs) and the ADAMs with thrombospondin motifs (ADAMTSs) (Rocks et al., 2008). The major members of these families that are expressed in gliomas, their functional relevance, and their relationship with glioma progression are summarized in Table 49.3.

Secreted metalloproteases, particularly members of the MMP family such as MMP-2 and MMP-9, have been clearly demonstrated to promote brain tumor growth and progression, as well as cell proliferation and invasion in vitro (Levicar et al., 2003; Rao, 2003). Accordingly, upregulation of those MMPs in gliomas correlates with tumor grade and lower survival rates (Levicar et al., 2003; Rao, 2003). Secreted MMPs and membrane-bound ADAMs have been involved in several functions that promote tumor progression, including matrix degradation, release of trophic factors, regulation of cell proliferation, stimulation of angiogenesis, and control of the immune response in the tumor (Egeblad and Werb, 2002; Rocks et al., 2008).

Pericellular matrix degradation by infiltrating glioma cells in vivo, however, may be far more restricted than what is thought from in vitro observations or results from metastatic tumors. For example, the predominant CSPGs in the CNS, versican and brevican, are degraded by MMPs in vitro (Nakamura et al., 2000); however, MMP-dependent proteolysis of these proteins in intracranial gliomas is a very minor event (Viapiano et al., 2008), suggesting that the scaffold of the neural ECM could be less affected by degradation than by production of novel matrix components, as described below. Similarly, despite of their high MMP expression, glioma cells do not disrupt the basal lamina of the brain blood vessels in vivo, even though these enzymes degrade basal lamina components during in vitro invasion assays (Bernstein and Woodard, 1995; Paulus et al., 1988).

It is thus possible that, in addition to regulated pericellular proteolysis, proteases in glioma may promote cell infiltration through a variety of non-degradative mechanisms. For example, ADAMTS enzymes cleave the neural lecticans preferentially at a single site, producing fragments that remain associated to the ECM scaffold and act as pro-migratory signals (Ang et al., 1999; Hu et al., 2008). In another example, MT1-MMP can induce EGFR transactivation independently of its catalytic activity (Langlois et al., 2007). Moreover, it has been shown that inhibition of metalloproteases may drive metastatic tumor cells to adopt an amoeboid phenotype and infiltrate by 'squeezing' through the intercellular spaces (Wolf et al., 2003). This causes tumor cells to acquire elongated morphologies that strongly resemble those usually seen in migratory glioma cells in the brain (Beadle et al., 2008), suggesting that these cells may naturally adapt to the surrounding neuropil rather than degrade their way through it.

Table 49.3 Proteases involved in glioma cell invasion

Protease family	Members studied in gliomas	Activity and substrates in the CNS	Functional relevance	Relationship with tumor progression	Endogenous inhibitors	References
Plasminogen activators (PAs)	– Urokinase-type PA (expressed by glioma cells) – Tissue-type PA (expressed in the endothelium of glioma blood vessels)	Serine proteases. Convert plasminogen to activated plasmin	Plasmin degrades pericellular matrix and activates MMPs. uPA also signals for integrin- and vitronectin-mediated cell adhesion through its receptor, uPA-R. tPA is presumed to play a role in tumor angiogenesis rather than invasion	High levels of uPA and uPAR correlate with a more invasive phenotype and poor prognosis. Inhibition of uPA/uPAR reduces invasiveness and promotes apoptosis	Plasminogen activator inhibitors (PAI): PAI-1 to PAI-3	Gondi (2003, 2007), Rao (2003) and Tsatas (2003)
Matrix Metallo-proteases (MMPs) (28 members)	gelatinases (MMP2, MMP9), stromelysins (MMP3, MMP7, MMP10, MMP11), membrane-type MMPs (MT1-MMP)	Zinc-dependent endopeptidases. Degrade most proteins of the basal lamina and proteoglycans of the neural ECM	MMPs are secreted by glioma cells, endothelial cells and infiltrating immune cells; involved in perivascular invasion and tumor angiogenesis. Membrane-bound MT-MMPs implicated in white matter invasion.	MMP2, MMP9 and MT1-MMP strongly correlate with glioma progression. Inhibition of these MMPs sharply reduces invasion in vitro and tumor formation in vivo	Tissue inhibitors of metallo-proteases (TIMP): TIMP-1 to TIMP-4	Binder (2002), Chintala (1999), Lakka (2005) and Nakada (2003)

Table 49.3 (continued)

Protease family	Members studied in gliomas	Activity and substrates in the CNS	Functional relevance	Relationship with tumor progression	Endogenous inhibitors	References
ADAMS (40 members)	ADAM8, ADAM17 (TACE), ADAM19	Similar to MMPs.	Membrane-bound proteases upregulated in brain tumors. Specific targets in CNS and functional relevance yet to be determined.	ADAMS upregulation correlates with glioma invasiveness	TIMPs	Held-Feindt (2006) and Rocks (2008)
ADAMTS (20 members)	Aggrecanases (ADAMTS-4 and ADAMTS-5)	Similar to MMPs. Degrade CSPGs of the neural ECM	Lectican cleavage releases N-terminal fragments that promote cell proliferation, adhesion, and migration	ADAMTS upregulation correlates with glioma invasiveness	TIMP-1 and -3	Held-Feindt (2006), Rocks (2008) and Viapiano (2008)
Cathepsins	Cathepsin B and D	Cystein proteases. Degrade collagens, fibronectin, laminin	Lysosomal degradation of ECM molecules. Cathepsins are also secreted and may remain active in the ECM. Cathepsin B indirectly activates MMPs via uPA/plasmin	Cathepsin upregulation correlates with tumor grade and more invasive profile. Inhibition reduces tumor growth, invasion, and angiogenesis	Cystatins (extracellular inhibitors)	Gondi (2006), Levicar (2002) and Rao (2003)

49.4.2 ECM Synthesis

Matrix remodeling depends not only on controlled pericellular ECM degradation but also on the formation of a new pericellular scaffold that contains novel ECM molecules produced by glioma cells. These structural molecules can be roughly grouped into three categories: molecules that are highly expressed during early neural development (HA, SPARC, phosphacan), molecules that predominate in the adult neural ECM (lecticans), and molecules that are not present in the normal neural ECM (basal lamina proteins).

Most gliomas contain high levels of HA comparable to those in the early developing brain (Delpech et al., 1993). The overproduction of HA and subsequent turnover by upregulated hyaluronidases in gliomas creates a regionally disorganized scaffold that becomes very soluble and permissive for cell proliferation, detachment, and movement (Novak et al., 1999). In addition, soluble HA acts as a pro-invasive signal by increasing the expression of metalloproteases and ECM proteins (MMP9 and SPARC, Kim et al., 2005a; Kim et al., 2005b); it may also promote apoptosis of immune cells (Yang et al., 2002) and could activate tyrosine kinase signaling as demonstrated in other carcinomas (Misra et al., 2006). Moreover, glioma cells express HA receptors that are absent or expressed at very low levels in the normal CNS, such as CD168/RHAMM and CD44, the latter being one of the hallmark cell-surface markers in high-grade gliomas (Akiyama et al., 2001; Baltuch et al., 1995; Ranuncolo et al., 2002). These receptors have a demonstrated role in promoting tumor cell proliferation and migration through several signaling pathways in a variety of malignancies (Hall and Turley, 1995; Knudson, 1998) (Fig. 49.3), and their blockade or downregulation in gliomas inhibits tumor proliferation and invasion in vitro and in vivo (Akiyama et al., 2001; Ward et al., 2003).

Glioma cells secrete SPARC and phosphacan, proteins that abound in the permissive matrix of the developing CNS, but that are highly reduced in the adult ECM. SPARC, which is overexpressed in many solid tumors (Brekken and Sage, 2000), interacts with basal lamina proteins and promotes the proliferation and migration of glioma cells in vitro, as well as tumor dispersion, but not mass growth, in vivo (Schultz et al., 2002). At the molecular level, it has been shown that SPARC can modulate integrin-linked kinase activity (Barker et al., 2005), upregulate MMP expression (McClung et al., 2007), and induce cytoskeletal changes through the chaperone HSP-27 (Golembieski et al., 2008). These activities result in the reorganization of cell morphology, matrix remodeling, and a net increase in cell motility.

The membrane-bound receptor-type protein tyrosine-phosphatase RPTP-β/ζ is highly upregulated in grades I–III gliomas and its soluble isoform, phosphacan, predominates in grade IV glioblastomas (Norman et al., 1998). This complex proteoglycan activates NF-kappaB-dependent transcription, promotes the association of glioma cells to the ECM and increases cell migration (Lorente et al., 2005), although the underlying molecular mechanisms for

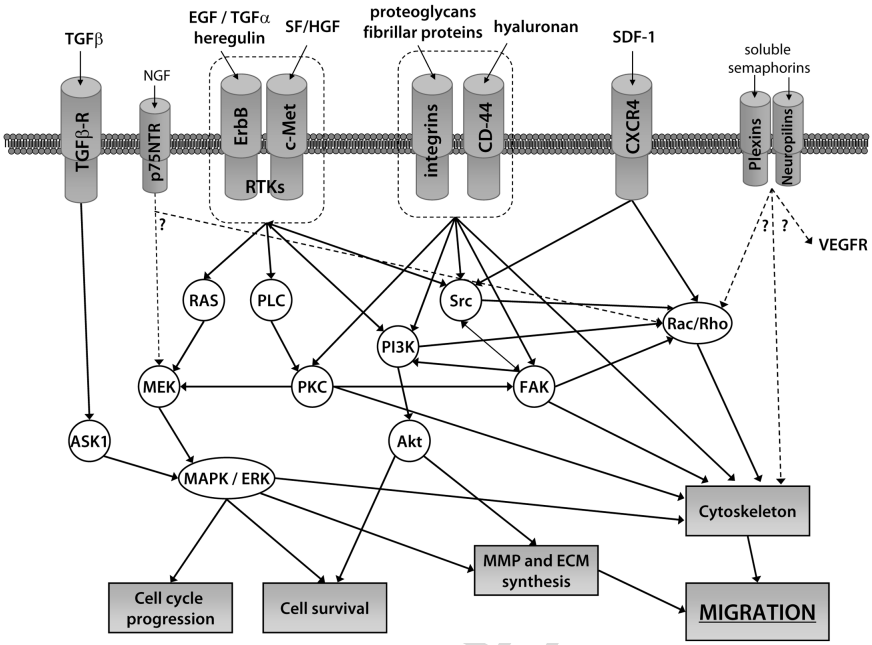

Fig. 49.3 Soluble factors and signaling pathways involved in glioma cell migration. The figure depicts some of the major intracellular transduction pathways that respond to the well-characterized extracellular signals that promote cell motility. The interactions in the figure were summarized from several bibliographical sources and verified individually using the Kyoto Encyclopedia of Genes and Genomes (*KEGG*) pathway database, stored at the Bioinformatics Center of Kyoto University and the Human Genome Center of the University of Tokyo (http://www.genome.ad.jp/kegg/pathway.html). Small-sized receptors indicated on both sides of the figure (p75NTR and Plexins/Neuropilins) represent novel, mostly unexplored signaling mechanisms recently implicated in glioma invasion

these processes are mostly unknown. Recent evidence suggests that the major ligand of phosphacan/RPTP-β/ζ, the soluble factor pleiotropin, is also upregulated in gliomas and may be part of an autocrine loop that promotes glioma cell motility (Ulbricht et al., 2003). Accordingly, targeting strategies against RPTP-β/ζ disrupt glioma cell motility in vitro and tumor growth in vivo (Foehr et al., 2006; Ulbricht et al., 2006).

Somewhat surprisingly, gliomas also overexpress two CSPGs of the lectican family that are well characterized as inhibitory molecules against cell motility in the adult CNS: versican and brevican (Viapiano and Matthews, 2006). These CSPGs have a clear role in promoting tumor growth and dispersion in neural tissue (Viapiano and Matthews, 2006), although their mechanisms of action are poorly understood. Results from in vitro assays suggest that both versican and brevican bind to fibronectin, which is present in the glioma ECM but not the neural ECM (Hu et al., 2008; Wu et al., 2004). Moreover, both lecticans promote

765 fibronectin-dependent cell adhesion and may activate EGFR signaling (Hu et al.,
766 2008; Wu et al., 2004), suggesting that they could act through pathways well
767 characterized in brain tumor invasion.

768 In addition, recent evidence suggests that the role of these lecticans as moto-
769 genic signals in gliomas could also be consequence of the expression of isoforms
770 that are minor or absent in the normal adult CNS (Viapiano and Matthews,
771 2006). It is possible that the functions of those isoforms may differ from the
772 predominant role of the lecticans as inhibitors of cell motility and axonal exten-
773 sion (Rauch, 2004; Viapiano and Matthews, 2006). For example, versican has
774 four known splice variants, V_0–V_3, of which V_0/V_1 predominates in the develop-
775 ing CNS while V_2 is the major adult isoform. Coincidentally, the isoforms V_0/V_1
776 are upregulated in glioma cells and have proliferative and anti-apoptotic func-
777 tions, while V_2 versican is highly reduced in gliomas and does not protect glioma
778 cells from apoptosis (Paulus et al., 1996; Rauch, 2004; Sheng et al., 2005).
779 Similarly, brevican exhibits novel glycoforms in gliomas that are absent in normal
780 adult human brain but appear during early neural development (Viapiano et al.,
781 2005). Moreover, full-length brevican does not have effects on glioma cell adhe-
782 sion or migration, but a fragment produced by ADAMTS-4/5 cleavage is suffi-
783 cient to act as a signal for cell dispersion in vitro and in vivo (Hu et al., 2008;
784 Viapiano et al., 2008). Consistent with this observation, cleavage of brevican is
785 increased in gliomas compared to normal CNS (Viapiano et al., 2005).

786 Finally, in stark contrast with normal neural cells, glioma cells secrete a group
787 of basal lamina components that are not expressed in the ECM of the neural
788 parenchyma, such as type-I laminin, fibronectin, and variable amounts of col-
789 lagens type I, III, IV, and VI (Gladson, 1999; Paulus et al., 1988). The expression
790 of these proteins in culture has been attributed to the loss of distinctive glial
791 features in cell lines and phenotype adaptation to culture conditions, a phenom-
792 enon called *mesenchymal drift* (Paulus et al., 1994). However, basal lamina
793 proteins can be detected in culture conditions that attempt to avoid this drift,
794 such as short-term primary cultures of gliomas and culture of glioma-derived
795 neurospheres in serum-free conditions (Paulus et al., 1994; Tso et al., 2006) (also
796 MSV and SEL, unpublished results). Similarly, these matrix proteins can be
797 detected in glioblastoma cells in vivo (Gladson, 1999; Paulus et al., 1994; Tso
798 et. al., 2006). Taken together, these observations suggest that subsets of glioma
799 cells naturally have the ability to secrete mesenchymal ECM molecules into the
800 surrounding matrix in the brain. These molecules are key elements in the engage-
801 ment of integrin receptors to promote cell adhesion and migration in all solid
802 tumors and have a demonstrated role in glioma invasion (D'Abaco and Kaye,
803 2007; Giese and Westphal, 1996). In addition, type-VI collagen and fibronectin
804 can interact directly with HA (Kielty et al., 1992; Yamada et al., 1980), which
805 could affect the structure of the HA-based matrix that surrounds infiltrating
806 glioma cells, thus favoring cell migration.

807 In sum, glioma cells are able to detach from the original surrounding matrix
808 and produce a novel ECM that contains a variety of neural-specific proteins
809 absent in other tissues (e.g., phosphacan, brevican) as well as mesenchymal

proteins absent from the brain parenchyma (fibronectin, collagens) (Bellail et al., 2004). Thus, this ECM differs from the original neural matrix and at the same time is distinct from the matrix secreted by peripheral tumors that metastasize to the CNS. The glioma-specific ECM may be a unique source of haptotactic cues and soluble signals that regulate and direct the migration of glioma cells and could thus underlie the distinct ability of these tumors to invade and disperse within the nervous tissue.

49.5 Soluble Signals and Transduction Mechanisms in Glioma Invasion

In addition to the haptotactic and mechanical effects of the ECM on tumor invasion, glioma cells have a complex paracrine interplay with neurons, glial, endothelial, and immune cells in the microenvironment of the tumor (Hoelzinger et al., 2007; Oliveira et al., 2005). These non-neoplastic cells secrete soluble factors and provide substrate molecules that may promote glioma proliferation, enhance cell adhesion/motility, and regulate the overall process of invasion (Giese and Westphal, 1996; Hoelzinger et al., 2007). There is a multitude of potential signals and transduction mechanisms implicated or proposed to be involved in glioma invasion (Nakada et al., 2007)(see Chapters 37–40); here we will consider only the major soluble factors that have been shown to consistently influence glioma cell migration and the key signaling pathways that respond to those factors by promoting cytoskeletal reorganization or transcription of pro-migratory genes.

49.5.1 Chemoattractants

The best characterized chemoattractants in gliomas are the ligands of the ErbB receptors (EGF, HB-EGF, TGF-α, heregulin, etc.), the scatter factor/hepatocyte growth factor (SF/HGF), transforming growth factor beta (TGF-β), and more recently the stromal-cell-derived factor (SDF)-1 (Mueller et al., 2003). Most of these factors are produced by the neural and endothelial cells surrounding the tumor (paracrine stimulation) or the glioma cells themselves (autocrine/paracrine loop) (Hoelzinger et al., 2007; Mueller et al., 2003). The major signaling pathways that respond to these factors and activate a pro-migratory phenotype are summarized in Fig. 49.3. Other soluble molecules shown to be involved in regulating glioma cell migration on ECM substrates include additional growth factors (basic fibroblast growth factor, platelet-derived growth factor, and nerve growth factor), cytokines (IL12), molecules involved in neuronal guidance, and some bioactive lipids (lysophosphatidic acid and sphingosine-1-phosphate) (Hoelzinger et al., 2007; Lange et al., 2008; Young and Van Brocklyn, 2007).

EGF and related factors are prototypical pro-migratory signals for glioma cells in vitro (Pedersen et al., 1994). They act by activation of the receptor tyrosine kinases (RTKs) of the ErbB family, of which the EGF receptor (EGFR/ErbB1) is the most commonly amplified gene in malignant gliomas (Ohgaki and Kleihues, 2007). Interestingly, amplification of this receptor could have a direct relation to the process of invasion in high-grade gliomas; FISH analysis has shown a gradient of EGFR amplification in glioblastoma samples, with the highest amplification levels found in the cells at the invasive border of the tumor (Okada et al., 2003). Activation of a different RTK, c-Met, initiates the effect of another pro-migratory signal, the soluble factor SF/HGF (Moriyama et al., 1999). This receptor is also upregulated in gliomas, and more importantly, localizes predominantly to the invasive pseudopodiae of motile glioma cells (Abounader and Laterra, 2005; Beckner et al., 2005).

The key role of RTK signaling on glioma cell proliferation in vitro and tumor growth in vivo has been well established and makes these receptors one of the major current therapeutic targets (Furnari et al., 2007; Nakada et al., 2007) (see also Chapters 20 and 39) . Accordingly, pharmacological inhibition of EGFR and c-Met, as well as antibody-mediated receptor blocking, has been shown to powerfully inhibit both cell proliferation and migration in vitro, as well as tumor invasion in animal models (Abounader and Laterra, 2005; Furnari et al., 2007). The molecular mechanisms by which RTKs promote glioma cell motility are complex and involve multiple parallel and redundant pathways, indicated in Fig. 49.3. RTK signaling results, among other effects, in the upregulation of matrix metalloproteases, synthesis of ECM proteins, and cytoskeletal reorganization initiated by activation of actin-binding proteins (Lal et al., 2002; Van Meter et al., 2004).

The growth factors TGF-β and SDF-1 act through their cognate receptors, the serine/threonine-kinase TGF-β receptors I/II, and the G-protein-coupled receptor CXCR4, respectively. These factors have been implicated in a variety of trophic effects for glioma progression, including stimulation of cell proliferation, angiogenesis, resistance to apoptosis, and cell motility (Leivonen and Kahari, 2007; Savarin-Vuaillat and Ransohoff, 2007; Wick et al., 2006). Although these factors can be produced by glioma cells, another major source is found in the microglial cells that invade the tumor mass in the brain, which has led to the hypothesis that microglia may actually promote glioma cell invasion by paracrine stimulation (Wesolowska et al., 2008). The signaling pathways initiated by these growth factors in gliomas are not fully determined, but in other cancer models they have been shown to exhibit significant cross talk with RTK-mediated signaling (Bhola and Grandis, 2008; Uchiyama-Tanaka et al., 2002), resulting in MAPK activation, upregulation of extracellular MMPs and ECM proteins, and enhanced cell adhesion and motility (Leivonen and Kahari, 2007; Savarin-Vuaillat and Ransohoff, 2007). Accordingly, inhibition of TGF-β receptors and CXCR4 reduces glioma cell invasion in vitro and tumor growth in vivo (Ehtesham et al., 2006; Uhl et al., 2004) (see Chapter 34 for CXCR4 targeting). Interestingly, the expression patterns of SDF-1 in brain

tissue show good correlation with the dispersion pathways favored by glioma cells in vivo (Zagzag et al., 2008). Consistent with this observation, the receptor CXCR4 is highly expressed at the leading edge of the tumors (Ehtesham et al., 2006; Zagzag et al., 2008).

49.5.2 Chemorepellents

Most growth factors and cytokines that induce glioma cell motility have been identified as attractants of the cells in vitro. However, glioma cells could also respond to repellent factors that would stimulate dispersion outward from the tumor core. The existence of tumor-produced chemorepellents has been postulated from the observation of cell-avoidance patterns during migration in vitro (Mueller et al., 2003; Werbowetski et al., 2004).

Members of the Slit and semaphorin families are potential candidates for a role as chemorepellents in gliomas. The members of the Slit family act in the normal CNS as axon guidance molecules during development and promote the migration of neural progenitors away from the subventricular zone in the adult brain (Wong et al., 2002). Recent results have demonstrated that Slit-2 can effectively repel glioma and medulloblastoma cells in vitro through its receptor Robo-1 (Mertsch et al., 2008; Werbowetski-Ogilvie et al., 2006). However, the role of this protein as a repellent from the tumor core in vivo is uncertain, because the *Slit2* gene has been found frequently inactivated epigenetically in gliomas (Dallol et al., 2003).

The soluble class-III semaphorins constitute another group of well-known chemorepellents for extending axons and neural progenitors, and, in addition, have been implicated in tumor progression and angiogenesis (Neufeld and Kessler, 2008). Glioma cells express the soluble semaphorins sema-3A and -3C as well as their cognate receptors, neuropilins and plexins, and can retain sema-3A on the cell surface (Rieger et al., 2003). However, sema-3A does not seem to affect glioma cell morphology or motility in vitro (Rieger et al., 2003), making it an unlikely repellent from the tumor core.

It has been proposed that the gradually increasing hypoxic status of the growing tumor could act as a major repellent condition through exhaustion of metabolic substrates and local decrease of pH (Werbowetski et al., 2004). Indeed, glioma cells under hypoxia migrate at a faster rate in vitro, which has been postulated as a possible explanation for the formation of hypercellular zones (pseudopalisades) around necrotic foci (Brat et al., 2004) (see Chapter 22). Reduced available oxygen is known to cause the stabilization and transcriptional activation of the hypoxia-inducible factor HIF-1α, a master transcriptional regulator with a well-established role in promoting glioma angiogenesis and invasion (Kaur et al., 2005).

HIF-1α is upregulated in the invasive borders of the tumor and its activity is increased by integrin- and RTK-initiated signaling (Zagzag et al., 2003),

suggesting that this transcription factor may act as a downstream regulator of the pathways that promote glioma cell motility. Furthermore, HIF-1-dependent transcription results in the upregulation of MMPs and the membrane receptors c-Met and CXCR4 (Eckerich et al., 2007; Zagzag et al., 2006), likely resulting in positive feedback mechanisms that mobilize glioma cells in response to both hypoxia-induced and growth factor-induced signaling. This complex signaling produces local tissue remodeling that favors the formation of new blood vessels (Kaur et al., 2005) and at the same time promotes glioma cell dispersion (Zagzag et al., 2006).

The use of RNA interference against HIF-1α reduces hypoxia-induced migration and invasion of several glioma cell lines in vitro (Fujiwara et al., 2007). However, direct targeting of HIF-1α has shown some contradictory results in different gflioma models. For example, immortalized astrocytes where HIF-1α has been deleted by recombination form small tumors when injected subcutaneously in nude mice (Blouw et al., 2003); however, the same cells show enhanced growth and invasion when implanted in the well-vascularized brain parenchyma. These results suggest that the HIF-1-dependent response of these tumors is strongly dependent on their microenvironment (Blouw et al., 2003). Further work is thus needed to define conditions where HIF-1α could be an effective target of strategies against migratory tumor cells.

49.6 Targeting Strategies Against Glioma Cell Invasion

The current standard of care for malignant gliomas after surgery is based on the combination of irradiation and novel alkylating agents (temozolomide) to promote apoptosis in the residual tumor. Novel stand-alone and adjuvant approaches under clinical investigation are being directed against specific molecular targets in the tumor cells or blood vessels, to prevent cell proliferation, angiogenesis, and local re-growth. These approaches have been extensively reviewed elsewhere in this book and include, among others, small-molecule inhibitors against RTK signaling (EGFR, c-Met), signal transduction pathways (PKC, RAS, mTOR), integrin-mediated adhesion, and matrix degrading enzymes (MMPs), as well as antibody-based therapies against major molecular targets in the tumor cells (EGFR and EGFRvIII) and blood vessels (VEGF, VEGF receptors) (Salgaller and Liau, 2006; Stupp et al., 2007). The points of contact between these anti-tumoral strategies and the mechanisms underlying the migratory phenotype of glioma cells are summarized in Fig. 49.4.

Several of these strategies could have a direct impact on glioma cell dispersion, such as protease inhibitors and integrin blockers that could inhibit late steps in the process of cell adhesion and migration. However, current therapies essentially target the local re-growth of residual tumor and their effects on the slowly proliferating, far-reaching migratory cells are at present difficult to

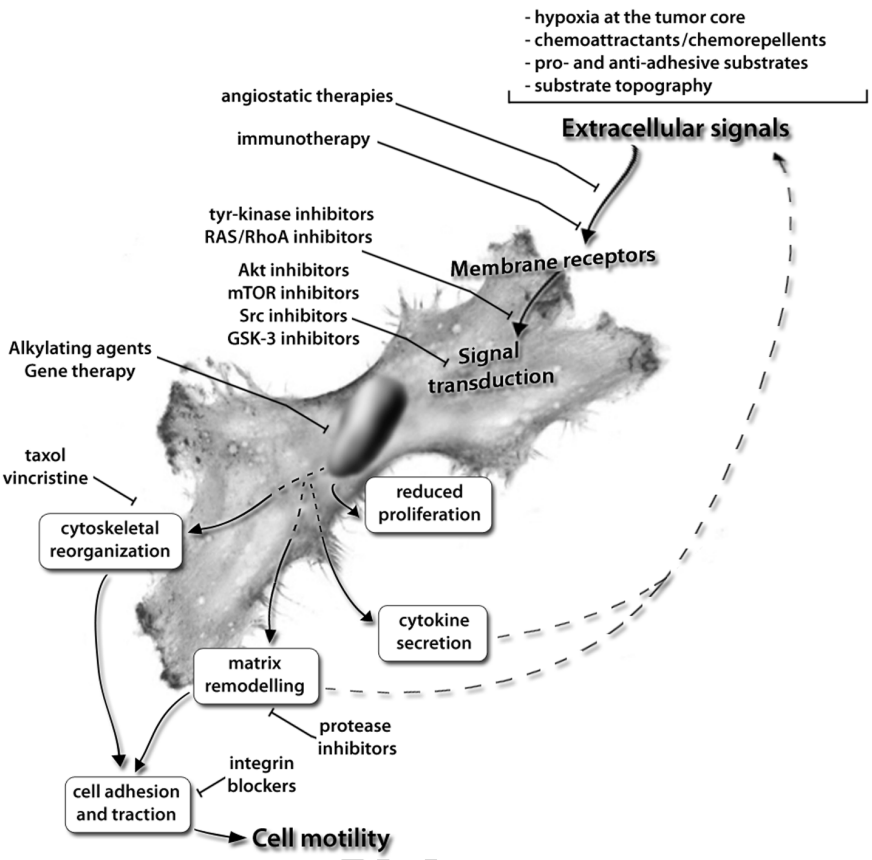

Fig. 49.4 Targeting strategies against invasive glioma cells. The figure summarizes the microenvironmental factors that stimulate migration, the cellular processes that underlie the migratory phenotype (*white boxes*), and the autocrine/paracrine feedback during glioma cell dispersion (*dashed lines*). Glioma cell migration could be disrupted at several levels, including blocking of extracellular signals, inhibition of motogenic signal transduction, and interruption of cell adhesion and matrix remodeling. The figure indicates pharmacological strategies currently used against glioma cells and the steps where they could disrupt pro-migratory processes

gauge. Indeed, because migratory glioma cells have reduced expression of proliferative and pro-apoptotic genes (Mariani et al., 2001) they could be expected to resist most cytotoxic insults better than the residual proliferating cells (Lefranc et al., 2005) (see Chapter 28). In addition, there is evidence that both radiation (Zhai et al., 2006) and angiostatic treatments (Lamszus et al., 2003) can stimulate glioma cell migration. Thus, there is a risk that current cytotoxic treatments not only may spare actively dispersing tumor cells but could also exert a selective pressure toward an enhanced migratory phenotype that would cause tumor recurrence in the long term (Lamszus et al., 2003).

Therefore, there is a clear need for understanding the effect of current therapies on migrating glioma cells and designing novel therapeutic approaches with an explicitly formulated anti-invasive adjuvant component (Claes et al., 2007).

A first obstacle for the design of an effective anti-invasive approach resides in the 'invisibility' of infiltrating tumor cells to current detection methods and the anatomical difficulty of reaching them in the neural parenchyma, where they lie behind a functional blood–brain barrier (see Chapter 33) and too far from the tumor core to be affected by drugs delivered locally into the post-resection cavity (Bolteus et al., 2001). These difficulties argue for the need of better delivery systems, such as the currently employed convection enhanced delivery (Ferguson et al., 2007), as well as improved ECM dispersion of the anti-invasive agents, in the hope of reaching the tumor cells located farthest from the original lesion.

The second, and likely largest, difficulty for an anti-invasive strategy is the identification of effective pro-migratory targets. Glioma invasion is, at the molecular level, a highly redundant process that responds to multiple signals and depends on diverse, overlapping, signaling pathways. This molecular redundancy has made it difficult to envision and implement targeting strategies relying on individual ECM components, cell-surface receptors, or signaling molecules (Giese, 2003; Giese and Westphal, 1996). For example, inhibition of the catalytic activity of MMPs, which effectively reduces glioma cell invasion in animal models, has shown no efficacy in clinical trials (Salgaller and Liau, 2006), underscoring the limitations of current glioma models to identify or predict alternative mechanisms of glioma dispersion (Beadle et al., 2008; Wolf et al., 2003).

It is worth noting that extracellular targets, however, have proved useful to enhance the specificity and efficacy of adjuvant therapies (see Chapters 34–36). This has been observed, for example, in the modest but significant effect of radio-immunotherapy directed against the ECM protein tenascin-C that is highly upregulated in gliomas (Goetz et al., 2003). In addition, pericellular and cell-surface molecules represent the most accessible targets in the tumor cells and a significant proportion of these potential targets in gliomas are restricted to the CNS (tenascin-R, phosphacan, Margolis et al., 1996) or are tumor-specific (certain brevican isoforms, Viapiano et al., 2005), which could facilitate directed therapies with reduced non-specific and systemic responses (Viapiano and Matthews, 2006).

To avoid the potentially limited efficacy of targeting individual signals and receptors, a significant bulk of research on pro-invasive molecular targets is currently focused on downstream transduction pathways that integrate extracellular signals and intracellular mechanisms and may contain molecular 'bottlenecks' appropriate for intervention (Giese, 2003; Giese and Westphal, 1996). The non-receptor tyrosine kinases of the Src family and the small GTPases of the Rho family have been highlighted as major examples of such molecular integrators. These molecules are key cross-signaling factors in pathways initiated by a variety of extracellular signals and mediated by RTKs,

integrins, CD44, and G-protein-coupled receptors (Fig. 49.3). Src kinases and the RhoA/ROCK pathway are key transduction mechanisms of glioma cell motility (Angers-Loustau et al., 2004; Goldberg and Kloog, 2006) and are required for the convergence of multiple signals on cytoskeletal reorganization and upregulation of pro-invasive proteins. The RhoA/ROCK pathway has been shown as a major target for radiosensitization and adjuvant therapy in solid cancers (Rattan et al., 2006), which makes it worthy of attention for future anti-invasive approaches in gliomas. On the other hand, recent evidence has shown that the kinase Lyn constitutes more than 90% of the total Src kinase activity in glioblastomas (Stettner et al., 2005), making this single enzyme a potential major target of anti-Src strategies.

Another interesting integrative target is the enzyme glycogen synthase kinase-3 (isozymes GSK-3α and β), which can be regulated by both Rho and Src members in glioma cells (Kleber et al., 2008; Skuli et al., 2006). This multitasking enzyme is capable of interacting directly with more than 50 different substrates (Jope et al., 2007) and is a key point of convergence of many pathways that regulate expression of pro-invasive genes, cellular structure, apoptosis, and motility (Jope et al., 2007; Meijer et al., 2004). Indeed, specific inhibition of GSK-3 can potently and specifically block glioma cell migration without causing other significant changes in the cell phenotype (Nowicki et al., 2008). Many small-molecule inhibitors, including, for example, lithium salts that are used in current psychiatric practice, are already available against this enzyme (Meijer et al., 2004).

Interestingly, strategies that were designed to prevent neo-vascularization, such as VEGF targeting and integrin competition, may also impact the migratory ability of glioma cells. Anti-VEGF antibodies inhibit the association of this growth factor not only to VEGF receptors in endothelial cells but also to receptors in glioma cells, which are thought to regulate cell migration (Herold-Mende et al., 1999). In a similar manner, soluble RGD-based peptides that compete with integrin binding are well known to affect glioma cell adhesion and motility in vitro (D'Abaco and Kaye, 2007). Accordingly, the integrin competitor peptide cilengitide is currently being tested as adjuvant therapy of chemoradiotherapy for gliomas, with positive preliminary results (Stupp et al., 2007).

A last major concern for the design of anti-invasive strategies is the difficulty of predicting the effects of anti-migratory compounds on the residual population(s) of tumor cells in vivo. Evidence from cultured glioblastoma cells suggests that proliferation and migration are highly plastic processes under control of poorly understood molecular switches that respond to microenvironmental signals (Gao et al., 2005). Indeed, actively migrating glioma cells cultured within brain slices may revert to a phenotype of local proliferation if they become stalled on blood vessel branches (Farin et al., 2006), which has been suggested as a mechanism for the formation of the distant perivascular tumor foci observed in clinical specimens. Thus, sub-lethal doses of anti-invasive drugs unable to cause apoptosis in motile cells could instead have the risk of promoting the growth of secondary tumors (Giese and Westphal, 1996).

Therefore, in addition to their inherent genetic instability, the ability of glioma cells to convert phenotype (proliferative <=> migratory) under microenvironmental influences (Giese et al., 2003) could significantly contribute to the appearance of resistant populations against therapies that act through a single type of selection pressure. Interestingly, seminal work by Goldie and Coldman (1979) suggested the use of rapidly alternating cytotoxic treatments to avoid single-pressure bias and deal more effectively with resistant clones during adjuvant chemotherapy. It is tempting to speculate whether a similar strategy, alternating anti-proliferative and anti-migratory compounds acting through highly different mechanisms, could prove efficient against invasive glioma cells. Future research on the characterization of the major switches that regulate the conversion of migratory and proliferative phenotypes in vivo (Gao et al., 2005) will be critical to better understand the influence of the microenvironment on residual tumor cells, and the evolution and weak points of these cell populations.

In sum, the cellular mechanisms that make glioma cells invade efficiently and selectively within central nervous tissue, as well as the specific molecular mediators underlying these mechanisms, are still largely unexplored. Novel in vitro and in vivo models are required to reproduce more accurately the multiple influences that stimulate glioma cell migration in the CNS and to identify the molecules that control this process. This information must be combined with the wealth of data from molecular profiling of patients and seminal bioinformatic analysis (Phillips et al., 2006) (see Chapters 23–24), to identify glioma-specific, pro-invasive targets amenable to pharmacological intervention. Together with current cytostatic and angiostatic strategies, inclusion of a 'motostatic' strategy is likely to maximize the efficacy of adjuvant therapies and improve progression-free survival and long-term management of these devastating tumors.

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References

Abounader, R. and Laterra, J. (2005) Scatter factor/hepatocyte growth factor in brain tumor growth and angiogenesis. Neuro. Oncol., 7: 436–451

Akiyama, Y., Jung, S., Salhia, B., Lee, S., Hubbard, S., Taylor, M., Mainprize, T., Akaishi, K., van Furth, W., and Rutka, J. T. (2001) Hyaluronate receptors mediating glioma cell migration and proliferation. J Neurooncol, 53: 115–127

Anderson, E., Grant, R., Lewis, S. C., and Whittle, I. R. (2008) Randomized Phase III controlled trials of therapy in malignant glioma: where are we after 40 years? Br. J. Neurosurg., 22: 339–349

Ang, L. C., Zhang, Y., Cao, L., Yang, B. L., Young, B., Kiani, C., Lee, V., Allan, K., and Yang, B. B. (1999) Versican enhances locomotion of astrocytoma cells and reduces cell adhesion through its G1 domain. J Neuropathol Exp Neurol, 58: 597–605

Angers-Loustau, A., Hering, R., Werbowetski, T. E., Kaplan, D. R., and Del Maestro, R. F. (2004) SRC regulates actin dynamics and invasion of malignant glial cells in three dimensions. Mol. Cancer Res., 2: 595–605

Baltuch, G. H., de, T. N., and Van Meir, E. G. (1995) Expression of the CD44 adhesion molecule in tumours of the central and peripheral nervous system. J. Neurooncol., 26: 191–198

Barallobre, M. J., Pascual, M., Del Rio, J. A., and Soriano, E. (2005) The Netrin family of guidance factors: emphasis on Netrin-1 signalling. Brain Res. Brain Res. Rev., 49: 22–47

Barami, K. (2007) Biology of the subventricular zone in relation to gliomagenesis. J. Clin. Neurosci., 14: 1143–1149

Barker, T. H., Baneyx, G., Cardo-Vila, M., Workman, G. A., Weaver, M., Menon, P. M., Dedhar, S., Rempel, S. A., Arap, W., Pasqualini, R., Vogel, V., and Sage, E. H. (2005) SPARC regulates extracellular matrix organization through its modulation of integrin-linked kinase activity. J. Biol. Chem., 280: 36483–36493

Beadle, C., Assanah, M. C., Monzo, P., Vallee, R., Rosenfeld, S. S., and Canoll, P. (2008) The Role of Myosin II in Glioma Invasion of the Brain. Mol. Biol. Cell, 19: 3357–3368

Beckner, M. E., Chen, X., An, J., Day, B. W., and Pollack, I. F. (2005) Proteomic characterization of harvested pseudopodia with differential gel electrophoresis and specific antibodies. Lab Invest, 85: 316–327

Bellail, A. C., Hunter, S. B., Brat, D. J., Tan, C., and Van Meir, E. G. (2004) Microregional extracellular matrix heterogeneity in brain modulates glioma cell invasion. Int J Biochem Cell Biol, 36: 1046–1069

Berens, M. E. and Giese, A. (1999) ". . .those left behind." Biology and oncology of invasive glioma cells. Neoplasia, 1: 208–219

Bernstein, J. J. (1996) Local invasion and intraparenchymal metastasis of astrocytomas. Neuropathol. Appl. Neurobiol., 22: 421–424

Bernstein, J. J. and Woodard, C. A. (1995) Glioblastoma cells do not intravasate into blood vessels. Neurosurgery, 36: 124–132

Bhola, N. E. and Grandis, J. R. (2008) Crosstalk between G-protein-coupled receptors and epidermal growth factor receptor in cancer. Front Biosci., 13: 1857–1865

Blouw, B., Song, H., Tihan, T., Bosze, J., Ferrara, N., Gerber, H. P., Johnson, R. S., and Bergers, G. (2003) The hypoxic response of tumors is dependent on their microenvironment. Cancer Cell, 4: 133–146

Bolteus, A. J., Berens, M. E., and Pilkington, G. J. (2001) Migration and invasion in brain neoplasms. Curr. Neurol. Neurosci. Rep., 1: 225–232

Brat, D. J., Castellano-Sanchez, A. A., Hunter, S. B., Pecot, M., Cohen, C., Hammond, E. H., Devi, S. N., Kaur, B., and Van Meir, E. G. (2004) Pseudopalisades in glioblastoma are hypoxic, express extracellular matrix proteases, and are formed by an actively migrating cell population. Cancer Res., 64: 920–927

Brekken, R. A. and Sage, E. H. (2000) SPARC, a matricellular protein: at the crossroads of cell-matrix. Matrix Biol., 19: 569–580

Busch, S. A. and Silver, J. (2007) The role of extracellular matrix in CNS regeneration. Curr. Opin. Neurobiol., 17: 120–127

Claes, A., Idema, A. J., and Wesseling, P. (2007) Diffuse glioma growth: a guerilla war. Acta Neuropathol., 114: 443–458

Crespo, D., Asher, R. A., Lin, R., Rhodes, K. E., and Fawcett, J. W. (2007) How does chondroitinase promote functional recovery in the damaged CNS? Exp. Neurol., 206: 159–171

D'Abaco, G. M. and Kaye, A. H. (2007) Integrins: molecular determinants of glioma invasion. J. Clin. Neurosci., 14: 1041–1048

Dallol, A., Krex, D., Hesson, L., Eng, C., Maher, E. R., and Latif, F. (2003) Frequent epigenetic inactivation of the SLIT2 gene in gliomas. Oncogene, 22: 4611–4616

Delpech, B., Maingonnat, C., Girard, N., Chauzy, C., Maunoury, R., Olivier, A., Tayot, J., and Creissard, P. (1993) Hyaluronan and hyaluronectin in the extracellular matrix of human brain tumour stroma. Eur J Cancer, 29A: 1012–1017

Demuth, T. and Berens, M. E. (2004) Molecular mechanisms of glioma cell migration and invasion. J. Neurooncol., 70: 217–228

Eckerich, C., Zapf, S., Fillbrandt, R., Loges, S., Westphal, M., and Lamszus, K. (2007) Hypoxia can induce c-Met expression in glioma cells and enhance SF/HGF-induced cell migration. Int. J. Cancer, 121: 276–283

Egeblad, M. and Werb, Z. (2002) New functions for the matrix metalloproteinases in cancer progression. Nat. Rev. Cancer, 2: 161–174

Ehtesham, M., Winston, J. A., Kabos, P., and Thompson, R. C. (2006) CXCR4 expression mediates glioma cell invasiveness. Oncogene, 25: 2801–2806

Farin, A., Suzuki, S. O., Weiker, M., Goldman, J. E., Bruce, J. N., and Canoll, P. (2006) Transplanted glioma cells migrate and proliferate on host brain vasculature: a dynamic analysis. Glia, 53: 799–808

Ferguson, S. D., Foster, K., and Yamini, B. (2007) Convection-enhanced delivery for treatment of brain tumors. Expert. Rev. Anticancer Ther., 7: S79-S85

Foehr, E. D., Lorente, G., Kuo, J., Ram, R., Nikolich, K., and Urfer, R. (2006) Targeting of the receptor protein tyrosine phosphatase beta with a monoclonal antibody delays tumor growth in a glioblastoma model. Cancer Res., 66: 2271–2278

Fujiwara, S., Nakagawa, K., Harada, H., Nagato, S., Furukawa, K., Teraoka, M., Seno, T., Oka, K., Iwata, S., and Ohnishi, T. (2007) Silencing hypoxia-inducible factor-1alpha inhibits cell migration and invasion under hypoxic environment in malignant gliomas. Int. J. Oncol., 30: 793–802

Furnari, F. B., Fenton, T., Bachoo, R. M., Mukasa, A., Stommel, J. M., Stegh, A., Hahn, W. C., Ligon, K. L., Louis, D. N., Brennan, C., Chin, L., DePinho, R. A., and Cavenee, W. K. (2007) Malignant astrocytic glioma: genetics, biology, and paths to treatment. Genes Dev., 21: 2683–2710

Galtrey C. M. and Fawcett, J. W. (2007) The role of chondroitin sulfate proteoglycans in regeneration and plasticity in the central nervous system. Brain Res. Rev. 54: 1–18.

Gao, C. F., Xie, Q., Su, Y. L., Koeman, J., Khoo, S. K., Gustafson, M., Knudsen, B. S., Hay, R., Shinomiya, N., and Vande Woude, G. F. (2005) Proliferation and invasion: plasticity in tumor cells. Proc. Natl. Acad. Sci. U.S.A, 102: 10528–10533

Georges, P. C., Miller, W. J., Meaney, D. F., Sawyer, E. S., and Janmey, P. A. (2006) Matrices with compliance comparable to that of brain tissue select neuronal over glial growth in mixed cortical cultures. Biophys. J., 90: 3012–3018

Giese, A. (2003) Glioma invasion – pattern of dissemination by mechanisms of invasion and surgical intervention, pattern of gene expression and its regulatory control by tumorsuppressor p53 and proto-oncogene ETS-1. Acta Neurochir. Suppl, 88: 153–162

Giese, A., Bjerkvig, R., Berens, M. E., and Westphal, M. (2003) Cost of migration: invasion of malignant gliomas and implications for treatment. J. Clin. Oncol., 21: 1624–1636

Giese, A., Loo, M. A., Tran, N., Haskett, D., Coons, S. W., and Berens, M. E. (1996) Dichotomy of astrocytoma migration and proliferation. Int. J. Cancer, 67: 275–282

Giese, A. and Westphal, M. (2001) Treatment of malignant glioma: a problem beyond the margins of resection. J. Cancer Res. Clin. Oncol., 127: 217–225

Giese, A. and Westphal, M. (1996) Glioma invasion in the central nervous system. Neurosurgery, 39: 235–250

Gilbertson, R. J. and Rich, J. N. (2007) Making a tumour's bed: glioblastoma stem cells and the vascular niche. Nat. Rev. Cancer, 7: 733–736

Gladson, C. L. (1999) The extracellular matrix of gliomas: modulation of cell function. J Neuropathol Exp Neurol, 58: 1029–40

Goetz, C., Riva, P., Poepperl, G., Gildehaus, F. J., Hischa, A., Tatsch, K., and Reulen, H. J. (2003) Locoregional radioimmunotherapy in selected patients with malignant glioma: experiences, side effects and survival times. J Neurooncol, 62: 321–8

Goldberg, L. and Kloog, Y. (2006) A Ras inhibitor tilts the balance between Rac and Rho and blocks phosphatidylinositol 3-kinase-dependent glioblastoma cell migration. Cancer Res., 66: 11709–11717

Goldbrunner, R. H., Bernstein, J. J., and Tonn, J. C. (1999) Cell-extracellular matrix interaction in glioma invasion. Acta Neurochir (Wien), 141: 295–305

Goldie, J. H. and Coldman, A. J. (1979) A mathematic model for relating the drug sensitivity of tumors to their spontaneous mutation rate. Cancer Treat. Rep., 63: 1727–1733

Golembieski, W. A., Thomas, S. L., Schultz, C. R., Yunker, C. K., McClung, H. M., Lemke, N., Cazacu, S., Barker, T., Sage, E. H., Brodie, C., and Rempel, S. A. (2008) HSP27 mediates SPARC-induced changes in glioma morphology, migration, and invasion. Glia, 56: 1061–1075

Guarino, M., Rubino, B., and Ballabio, G. (2007) The role of epithelial-mesenchymal transition in cancer pathology. Pathology, 39: 305–318

Guillamo, J. S., Lisovoski, F., Christov, C., Le, G. C., Defer, G. L., Peschanski, M., and Lefrancois, T. (2001) Migration pathways of human glioblastoma cells xenografted into the immunosuppressed rat brain. J. Neurooncol., 52: 205–215

Hall, C. L. and Turley, E. A. (1995) Hyaluronan: RHAMM mediated cell locomotion and signaling in tumorigenesis. J. Neurooncol., 26: 221–229

Hensel, T., Amberger, V. R., and Schwab, M. E. (1998) A metalloprotease activity from C6 glioma cells inactivates the myelin-associated neurite growth inhibitors and can be neutralized by antibodies. Br. J. Cancer, 78: 1564–1572

Herold-Mende, C., Steiner, H. H., Andl, T., Riede, D., Buttler, A., Reisser, C., Fusenig, N. E., and Mueller, M. M. (1999) Expression and functional significance of vascular endothelial growth factor receptors in human tumor cells. Lab Invest, 79: 1573–1582

Hoelzinger, D. B., Demuth, T., and Berens, M. E. (2007) Autocrine factors that sustain glioma invasion and paracrine biology in the brain microenvironment. J. Natl. Cancer Inst., 99: 1583–1593

Hu, B., Kong, L., Matthews, R. T., and Viapiano, M. S. (2008) The proteoglycan brevican binds to fibronectin after proteolytic cleavage and promotes glioma cell adhesion and motility. J. Biol. Chem., 283: 24848–24859

Jope, R. S., Yuskaitis, C. J., and Beurel, E. (2007) Glycogen synthase kinase-3 (GSK3): inflammation, diseases, and therapeutics. Neurochem. Res., 32: 577–595

Junker, N., Latini, S., Petersen, L. N., and Kristjansen, P. E. (2003) Expression and regulation patterns of hyaluronidases in small cell lung cancer and glioma lines. Oncol. Rep., 10: 609–616

Kaur, B., Khwaja, F. W., Severson, E. A., Matheny, S. L., Brat, D. J., and Van Meir, E. G. (2005) Hypoxia and the hypoxia-inducible-factor pathway in glioma growth and angiogenesis. Neuro. Oncol., 7: 134–153

Kielty, C. M., Whittaker, S. P., Grant, M. E., and Shuttleworth, C. A. (1992) Type VI collagen microfibrils: evidence for a structural association with hyaluronan. J. Cell Biol., 118: 979–990

Kim, M. S., Park, M. J., Kim, S. J., Lee, C. H., Yoo, H., Shin, S. H., Song, E. S., and Lee, S. H. (2005a) Emodin suppresses hyaluronic acid-induced MMP-9 secretion and invasion of glioma cells. Int. J. Oncol., 27: 839–846

Kim, M. S., Park, M. J., Moon, E. J., Kim, S. J., Lee, C. H., Yoo, H., Shin, S. H., Song, E. S., and Lee, S. H. (2005b) Hyaluronic acid induces osteopontin via the phosphatidylinositol 3-kinase/Akt pathway to enhance the motility of human glioma cells. Cancer Res., 65: 686–691

Kleber, S., Sancho-Martinez, I., Wiestler, B., Beisel, A., Gieffers, C., Hill, O., Thiemann, M., Mueller, W., Sykora, J., Kuhn, A., Schreglmann, N., Letellier, E., Zuliani, C., Klussmann, S., Teodorczyk, M., Grone, H. J., Ganten, T. M., Sultmann, H., Tuttenberg, J., von, D. A., Regnier-Vigouroux, A., Herold-Mende, C., and Martin-Villalba, A. (2008) Yes and PI3K bind CD95 to signal invasion of glioblastoma. Cancer Cell, 13: 235–248

Knudson, W. (1998) The role of CD44 as a cell surface hyaluronan receptor during tumor invasion of connective tissue. Front Biosci., 3: d604-d615

Lal, A., Glazer, C. A., Martinson, H. M., Friedman, H. S., Archer, G. E., Sampson, J. H., and Riggins, G. J. (2002) Mutant epidermal growth factor receptor up-regulates molecular effectors of tumor invasion. Cancer Res, 62: 3335–3339

Lamszus, K., Kunkel, P., and Westphal, M. (2003) Invasion as limitation to anti-angiogenic glioma therapy. Acta Neurochir. Suppl, 88: 169–177

Lange, K., Kammerer, M., Saupe, F., Grotegut, S., Hegi, M. E., Grotegut, S., Fluri, E., and Orend, G. (2008) Combined Lysophosphatidic acid/Platelet-Derived Growth Factor signaling Triggers Glioma Cell Migration in a Tenascin-C microenvironment. Cancer Res., 68: 6942–6952

Langlois, S., Nyalendo, C., Di, T. G., Labrecque, L., Roghi, C., Murphy, G., Gingras, D., and Beliveau, R. (2007) Membrane-type 1 matrix metalloproteinase stimulates cell migration through epidermal growth factor receptor transactivation. Mol. Cancer Res., 5: 569–583

Laurent, T. C., Laurent, U. B., and Fraser, J. R. (1996) The structure and function of hyaluronan: An overview. Immunol Cell Biol, 74: 1–7

Lefranc, F., Brotchi, J., and Kiss, R. (2005) Possible future issues in the treatment of glioblastomas: special emphasis on cell migration and the resistance of migrating glioblastoma cells to apoptosis. J. Clin. Oncol., 23: 2411–2422

Leivonen, S. K. and Kahari, V. M. (2007) Transforming growth factor-beta signaling in cancer invasion and metastasis. Int. J. Cancer, 121: 2119–2124

Levicar, N., Nuttall, R. K., and Lah, T. T. (2003) Proteases in brain tumour progression. Acta Neurochir. (Wien.), 145: 825–838

Liao, H., Duka, T., Teng, F. Y., Sun, L., Bu, W. Y., Ahmed, S., Tang, B. L., and Xiao, Z. C. (2004) Nogo-66 and myelin-associated glycoprotein (MAG) inhibit the adhesion and migration of Nogo-66 receptor expressing human glioma cells. J. Neurochem., 90: 1156–1162

Lim, D. A., Cha, S., Mayo, M. C., Chen, M. H., Keles, E., VandenBerg, S., and Berger, M. S. (2007) Relationship of glioblastoma multiforme to neural stem cell regions predicts invasive and multifocal tumor phenotype. Neuro. Oncol., 9: 424–429

Lorente, G., Nelson, A., Mueller, S., Kuo, J., Urfer, R., Nikolich, K., and Foehr, E. D. (2005) Functional comparison of long and short splice forms of RPTPbeta: implications for glioblastoma treatment. Neuro.-oncol., 7: 154–163

Louis, D. N. (2006) Molecular Pathology of Malignant Gliomas. Annual Review of Pathology: Mechanisms of Disease, 1: 97–117

Mandybur, T. I., Liwnicz, B. H., and Wechsler, W. (1984) Disseminated (metastatic) tumors in nude mice produced by intravenous injection of cells of human and nonhuman neurogenic tumor lines. Acta Neuropathol., 63: 203–209

Margolis, R. K., Rauch, U., Maurel, P., and Margolis, R. U. (1996) Neurocan and phosphacan: two major nervous tissue-specific chondroitin sulfate proteoglycans. Perspect. Dev. Neurobiol., 3: 273–290

Mariani, L., Beaudry, C., McDonough, W. S., Hoelzinger, D. B., Demuth, T., Ross, K. R., Berens, T., Coons, S. W., Watts, G., Trent, J. M., Wei, J. S., Giese, A., and Berens, M. E. (2001) Glioma cell motility is associated with reduced transcription of proapoptotic and proliferation genes: a cDNA microarray analysis. J Neurooncol, 53: 161–176

McClung, H. M., Thomas, S. L., Osenkowski, P., Toth, M., Menon, P., Raz, A., Fridman, R., and Rempel, S. A. (2007) SPARC upregulates MT1-MMP expression, MMP-2 activation, and the secretion and cleavage of galectin-3 in U87MG glioma cells. Neurosci. Lett., 419: 172–177

Meijer, L., Flajolet, M., and Greengard, P. (2004) Pharmacological inhibitors of glycogen synthase kinase 3. Trends Pharmacol. Sci., 25: 471–480

Mertsch, S., Schmitz, N., Jeibmann, A., Geng, J. G., Paulus, W., and Senner, V. (2008) Slit2 involvement in glioma cell migration is mediated by Robo1 receptor. J. Neurooncol., 87: 1–7

Misra, S., Toole, B. P., and Ghatak, S. (2006) Hyaluronan constitutively regulates activation of multiple receptor tyrosine kinases in epithelial and carcinoma cells. J. Biol. Chem., 281: 34936–34941

Moriyama, T., Kataoka, H., Koono, M., and Wakisaka, S. (1999) Expression of hepatocyte growth factor/scatter factor and its receptor c-Met in brain tumors: evidence for a role in progression of astrocytic tumors (Review). Int. J. Mol. Med., 3: 531–536

Mueller, M. M., Werbowetski, T., and Del Maestro, R. F. (2003) Soluble factors involved in glioma invasion. Acta Neurochir.(Wien.), 145: 999–1008

Nakada, M., Nakada, S., Demuth, T., Tran, N. L., Hoelzinger, D. B., and Berens, M. E. (2007) Molecular targets of glioma invasion. Cell Mol. Life Sci., 64: 458–478

Nakamura, H., Fujii, Y., Inoki, I., Sugimoto, K., Tanzawa, K., Matsuki, H., Miura, R., Yamaguchi, Y., and Okada, Y. (2000) Brevican is degraded by matrix metalloproteinases and aggrecanase-1 (ADAMTS4) at different sites. J Biol Chem, 275: 38885–38890

Neufeld, G. and Kessler, O. (2008) The semaphorins: versatile regulators of tumour progression and tumour angiogenesis. Nat. Rev. Cancer, 8(8):632–645

Norman, S. A., Golfinos, J. G., and Scheck, A. C. (1998) Expression of a receptor protein tyrosine phosphatase in human glial tumors. J. Neurooncol., 36: 209–217

Novak, U. and Kaye, A. H. (2000) Extracellular matrix and the brain: components and function. J. Clin. Neurosci., 7: 280–290

Novak, U., Stylli, S. S., Kaye, A. H., and Lepperdinger, G. (1999) Hyaluronidase-2 overexpression accelerates intracerebral but not subcutaneous tumor formation of murine astrocytoma cells. Cancer Res., 59: 6246–6250

Nowicki, M. O., Dmitrieva, N., Stein, A. M., Cutter, J. L., Godlewski, J., Saeki, Y., Nita, M., Berens, M. E., Sanders, L. M., Newton, H. B., Chiocca, E. A., and Lawler, S. E. (2008) Lithium inhibits invasion of glioma cells; possible involvement of glycogen synthase kinase-3. Neuro-Oncology, 10: 690–699

Ohgaki, H. and Kleihues, P. (2007) Genetic pathways to primary and secondary glioblastoma. Am. J. Pathol., 170: 1445–1453

Okada, Y., Hurwitz, E. E., Esposito, J. M., Brower, M. A., Nutt, C. L., and Louis, D. N. (2003) Selection pressures of TP53 mutation and microenvironmental location influence epidermal growth factor receptor gene amplification in human glioblastomas. Cancer Res., 63: 413–416

Oliveira, R., Christov, C., Guillamo, J. S., De, B. S., Palfi, S., Venance, L., Tardy, M., and Peschanski, M. (2005) Contribution of gap junctional communication between tumor cells and astroglia to the invasion of the brain parenchyma by human glioblastomas. BMC. Cell Biol., 6: 7

Paulus, W., Baur, I., Dours-Zimmermann, M. T., and Zimmermann, D. R. (1996) Differential expression of versican isoforms in brain tumors. J. Neuropathol. Exp. Neurol., 55: 528–533

Paulus, W., Huettner, C., and Tonn, J. C. (1994) Collagens, integrins and the mesenchymal drift in glioblastomas: a comparison of biopsy specimens, spheroid and early monolayer cultures. Int. J. Cancer, 58: 841–846

Paulus, W., Roggendorf, W., and Schuppan, D. (1988) Immunohistochemical investigation of collagen subtypes in human glioblastomas. Virchows Arch. A Pathol. Anat. Histopathol., 413: 325–332

Pedersen, P. H., Ness, G. O., Engebraaten, O., Bjerkvig, R., Lillehaug, J. R., and Laerum, O. D. (1994) Heterogeneous response to the growth factors [EGF, PDGF (bb), TGF-alpha, bFGF, IL-2] on glioma spheroid growth, migration and invasion. Int. J. Cancer, 56: 255–261

Peyton, S. R., Ghajar, C. M., Khatiwala, C. B., and Putnam, A. J. (2007) The emergence of ECM mechanics and cytoskeletal tension as important regulators of cell function. Cell Biochem. Biophys., 47: 300–320

Phillips, H. S., Kharbanda, S., Chen, R., Forrest, W. F., Soriano, R. H., Wu, T. D., Misra, A., Nigro, J. M., Colman, H., Soroceanu, L., Williams, P. M., Modrusan, Z., Feuerstein, B. G., and Aldape, K. (2006) Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell, 9: 157–173

Pilkington, G. J. (1997) The paradox of neoplastic glial cell invasion of the brain and apparent metastatic failure. Anticancer Res, 17: 4103–4105

Ranuncolo, S. M., Ladeda, V., Specterman, S., Varela, M., Lastiri, J., Morandi, A., Matos, E., Bal de Kier Joffe, E., Puricelli, L., and Pallotta, M. G. (2002) CD44 expression in human gliomas. J Surg Oncol, 79: 30–35

Rao, J. S. (2003) Molecular mechanisms of glioma invasiveness: the role of proteases. Nat. Rev. Cancer, 3: 489–501

Rattan, R., Giri, S., Singh, A. K., and Singh, I. (2006) Rho/ROCK pathway as a target of tumor therapy. J. Neurosci. Res., 83: 243–255

Rauch, U. (2004) Extracellular matrix components associated with remodeling processes in brain. Cell Mol. Life Sci., 61: 2031–2045

Rhodes, K. E. and Fawcett, J. W. (2004) Chondroitin sulphate proteoglycans: preventing plasticity or protecting the CNS? J. Anat., 204: 33–48

Rieger, J., Wick, W., and Weller, M. (2003) Human malignant glioma cells express semaphorins and their receptors, neuropilins and plexins. Glia, 42: 379–389

Rocks, N., Paulissen, G., El, H. M., Quesada, F., Crahay, C., Gueders, M., Foidart, J. M., Noel, A., and Cataldo, D. (2008) Emerging roles of ADAM and ADAMTS metalloproteinases in cancer. Biochimie, 90: 369–379

Salgaller, M. L. and Liau, L. M. (2006) Current status of clinical trials for glioblastoma. Rev. Recent Clin. Trials, 1: 265–281

Savarin-Vuaillat, C. and Ransohoff, R. M. (2007) Chemokines and chemokine receptors in neurological disease: raise, retain, or reduce? Neurotherapeutics., 4: 590–601

Schultz, C., Lemke, N., Ge, S., Golembieski, W. A., and Rempel, S. A. (2002) Secreted protein acidic and rich in cysteine promotes glioma invasion and delays tumor growth in vivo. Cancer Res., 62: 6270–6277

Sheng, W., Wang, G., Wang, Y., Liang, J., Wen, J., Zheng, P. S., Wu, Y., Lee, V., Slingerland, J., Dumont, D., and Yang, B. B. (2005) The roles of versican V1 and V2 isoforms in cell proliferation and apoptosis. Mol. Biol. Cell, 16: 1330–1340

Skuli, N., Monferran, S., Delmas, C., Lajoie-Mazenc, I., Favre, G., Toulas, C., and Cohen-Jonathan-Moyal, E. (2006) Activation of RhoB by hypoxia controls hypoxia-inducible factor-1alpha stabilization through glycogen synthase kinase-3 in U87 glioblastoma cells. Cancer Res., 66: 482–489

Stein, A. M., Nowicki, M. O., Demuth, T., Berens, M. E., Lawler, S. E., Chiocca, E. A., and Sander, L. M. (2007) Estimating the cell density and invasive radius of three-dimensional glioblastoma tumor spheroids grown in vitro. Appl. Opt., 46: 5110–5118

Stetler-Stevenson, W. G., Aznavoorian, S., and Liotta, L. A. (1993) Tumor cell interactions with the extracellular matrix during invasion and metastasis. Annu. Rev. Cell Biol., 9: 541–573

Stettner, M. R., Wang, W., Nabors, L. B., Bharara, S., Flynn, D. C., Grammer, J. R., Gillespie, G. Y., and Gladson, C. L. (2005) Lyn kinase activity is the predominant cellular SRC kinase activity in glioblastoma tumor cells. Cancer Res., 65: 5535–5543

Stupp, R., Hegi, M. E., Gilbert, M. R., and Chakravarti, A. (2007) Chemoradiotherapy in malignant glioma: standard of care and future directions. J. Clin. Oncol., 25: 4127–4136

Subramanian, A., Harris, A., Piggott, K., Shieff, C., and Bradford, R. (2002) Metastasis to and from the central nervous system – the 'relatively protected site'. Lancet Oncol, 3: 498–507

Tang, S., Qiu, J., Nikulina, E., and Filbin, M. T. (2001) Soluble myelin-associated glycoprotein released from damaged white matter inhibits axonal regeneration. Mol. Cell Neurosci., 18: 259–269

Tso, C. L., Shintaku, P., Chen, J., Liu, Q., Liu, J., Chen, Z., Yoshimoto, K., Mischel, P. S., Cloughesy, T. F., Liau, L. M., and Nelson, S. F. (2006) Primary glioblastomas express mesenchymal stem-like properties. Mol. Cancer Res., 4: 607–619

Uchiyama-Tanaka, Y., Matsubara, H., Mori, Y., Kosaki, A., Kishimoto, N., Amano, K., Higashiyama, S., and Iwasaka, T. (2002) Involvement of HB-EGF and EGF receptor transactivation in TGF-beta-mediated fibronectin expression in mesangial cells. Kidney Int., 62: 799–808

Uhl, M., Aulwurm, S., Wischhusen, J., Weiler, M., Ma, J. Y., Almirez, R., Mangadu, R., Liu, Y. W., Platten, M., Herrlinger, U., Murphy, A., Wong, D. H., Wick, W., Higgins, L. S., and Weller, M. (2004) SD-208, a novel transforming growth factor beta receptor I kinase inhibitor, inhibits growth and invasiveness and enhances immunogenicity of murine and human glioma cells in vitro and in vivo. Cancer Res., 64: 7954–7961

Ulbricht, U., Brockmann, M. A., Aigner, A., Eckerich, C., Muller, S., Fillbrandt, R., Westphal, M., and Lamszus, K. (2003) Expression and function of the receptor protein tyrosine phosphatase zeta and its ligand pleiotrophin in human astrocytomas. J. Neuropathol. Exp. Neurol., 62: 1265–1275

Ulbricht, U., Eckerich, C., Fillbrandt, R., Westphal, M., and Lamszus, K. (2006) RNA interference targeting protein tyrosine phosphatase zeta/receptor-type protein tyrosine phosphatase beta suppresses glioblastoma growth in vitro and in vivo. J. Neurochem., 98: 1497–1506

Van Meter, T. E., Broaddus, W. C., Rooprai, H. K., Pilkington, G. J., and Fillmore, H. L. (2004) Induction of membrane-type-1 matrix metalloproteinase by epidermal growth factor-mediated signaling in gliomas. Neuro. Oncol., 6: 188–199

Viapiano, M. S., Bi, W. L., Piepmeier, J., Hockfield, S., and Matthews, R. T. (2005) Novel tumor-specific isoforms of BEHAB/brevican identified in human malignant gliomas. Cancer Res., 65: 6726–6733

Viapiano, M. S., Hockfield, S., and Matthews, R. T. (2008) BEHAB/brevican requires ADAMTS-mediated proteolytic cleavage to promote glioma invasion. J. Neurooncol., 88: 261–272

Viapiano, M. S. and Matthews, R. T. (2006) From barriers to bridges: chondroitin sulfate proteoglycans in neuropathology. Trends Mol. Med., 12: 488–496

Ward, J. A., Huang, L., Guo, H., Ghatak, S., and Toole, B. P. (2003) Perturbation of hyaluronan interactions inhibits malignant properties of glioma cells. Am. J. Pathol., 162: 1403–1409

Werbowetski, T., Bjerkvig, R., and Del Maestro, R. F. (2004) Evidence for a secreted chemorepellent that directs glioma cell invasion. J. Neurobiol., 60: 71–88

Werbowetski-Ogilvie, T. E., Seyed, S. M., Jabado, N., ngers-Loustau, A., Agar, N. Y., Wu, J., Bjerkvig, R., Antel, J. P., Faury, D., Rao, Y., and Del Maestro, R. F. (2006) Inhibition of medulloblastoma cell invasion by Slit. Oncogene, 25: 5103–5112

Wesolowska, A., Kwiatkowska, A., Slomnicki, L., Dembinski, M., Master, A., Sliwa, M., Franciszkiewicz, K., Chouaib, S., and Kaminska, B. (2008) Microglia-derived TGF-beta as an important regulator of glioblastoma invasion – an inhibition of TGF-beta-dependent effects by shRNA against human TGF-beta type II receptor. Oncogene, 27: 918–930

Wick, W., Naumann, U., and Weller, M. (2006) Transforming growth factor-beta: a molecular target for the future therapy of glioblastoma. Curr. Pharm. Des, 12: 341–349

Wolf, K., Mazo, I., Leung, H., Engelke, K., von Andrian, U. H., Deryugina, E. I., Strongin, A. Y., Brocker, E. B., and Friedl, P. (2003) Compensation mechanism in tumor cell migration: mesenchymal-amoeboid transition after blocking of pericellular proteolysis. J. Cell Biol., 160: 267–277

Wong, K., Park, H. T., Wu, J. Y., and Rao, Y. (2002) Slit proteins: molecular guidance cues for cells ranging from neurons to leukocytes. Curr. Opin. Genet. Dev., 12: 583–591

Wu, Y., Chen, L., Cao, L., Sheng, W., and Yang, B. B. (2004) Overexpression of the C-terminal PG-M/versican domain impairs growth of tumor cells by intervening in the interaction between epidermal growth factor receptor and beta1-integrin. J. Cell Sci., 117: 2227–2237

Xie, F. and Zheng, B. (2008) White matter inhibitors in CNS axon regeneration failure. Exp. Neurol., 209: 302–312

Yamada, K. M., Kennedy, D. W., Kimata, K., and Pratt, R. M. (1980) Characterization of fibronectin interactions with glycosaminoglycans and identification of active proteolytic fragments. J. Biol. Chem., 255: 6055–6063

Yamaguchi, Y. (2000) Lecticans: organizers of the brain extracellular matrix. Cell Mol Life Sci, 57: 276–89

Yang, F., Murugan, R., Wang, S., and Ramakrishna, S. (2005) Electrospinning of nano/micro scale poly(L-lactic acid) aligned fibers and their potential in neural tissue engineering. Biomaterials, 26: 2603–2610

Yang, T., Witham, T. F., Villa, L., Erff, M., Attanucci, J., Watkins, S., Kondziolka, D., Okada, H., Pollack, I. F., and Chambers, W. H. (2002) Glioma-associated hyaluronan induces apoptosis in dendritic cells via inducible nitric oxide synthase: implications for the use of dendritic cells for therapy of gliomas. Cancer Res., 62: 2583–2591

Young, N. and Van Brocklyn, J.R. (2007) Roles of sphingosine-1-phosphate (S1P) receptors in malignant behavior of glioma cells. Differential effects of S1P2 on cell migration and invasiveness. Exp. Cell Res., 313: 1615–1627

Zagzag, D., Esencay, M., Mendez, O., Yee, H., Smirnova, I., Huang, Y., Chiriboga, L., Lukyanov, E., Liu, M., and Newcomb, E. W. (2008) Hypoxia- and Vascular Endothelial Growth Factor-Induced Stromal Cell-Derived Factor-1{alpha}/CXCR4 Expression in Glioblastomas. One Plausible Explanation of Scherer's Structures. Am. J. Pathol., 4(4):291–303

Zagzag, D., Lukyanov, Y., Lan, L., Ali, M. A., Esencay, M., Mendez, O., Yee, H., Voura, E. B., and Newcomb, E. W. (2006) Hypoxia-inducible factor 1 and VEGF upregulate CXCR4 in glioblastoma: implications for angiogenesis and glioma cell invasion. Lab Invest, 86: 1221–1232

Zagzag, D., Nomura, M., Friedlander, D. R., Blanco, C. Y., Gagner, J. P., Nomura, N., and Newcomb, E. W. (2003) Geldanamycin inhibits migration of glioma cells in vitro: a potential role for hypoxia-inducible factor (HIF-1alpha) in glioma cell invasion. J. Cell Physiol, 196: 394–402

Zhai, G. G., Malhotra, R., Delaney, M., Latham, D., Nestler, U., Zhang, M., Mukherjee, N., Song, Q., Robe, P., and Chakravarti, A. (2006) Radiation enhances the invasive potential of primary glioblastoma cells via activation of the Rho signaling pathway. J. Neurooncol., 76: 227–237