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Review

Molecular targets of glioma invasion

M. Nakada[a,b], S. Nakada[a], T. Demuth[a], N. L. Tran[a], D. B. Hoelzinger[a], and M. E. Berens[a,*]

[a] Cancer and Cell Biology Division, The Translational Genomics Research Institute, 445 North Fifth Street, Phoenix, Arizona 85004 (USA), Fax: +1 602 343 8844, e-mail: mberens@tgen.org
[b] Department of Neurosurgery, Division of Neuroscience, Graduate School of Medical Science, Kanazawa University, Ishikawa 920-0934 (Japan)

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Abstract. Glioblastoma multiforme is the most common and lethal primary malignant brain tumor. Although considerable progress has been made in technical proficiencies of surgical and radiation treatment for brain tumor patients, the impact of these advances on clinical outcome has been disappointing, with median survival time not exceeding 15 months. Over the last 30 years, no significant increase in survival of patients suffering from this disease has been achieved. A fundamental source of the management challenge presented in glioma patients is the insidious propensity of tumor invasion into distant brain tissue. Invasive tumor cells escape surgical removal and geographically dodge lethal radiation exposure and chemotherapy. Recent improved understanding of biochemical and molecular determinants of glioma cell invasion provide valuable insight into the underlying biological features of the disease, as well as illuminating possible new therapeutic targets. These findings are moving forward to translational research and clinical trials as novel antiglioma therapies.

Keywords. Glioma, invasion.

Introduction

One of the insidious biological features of gliomas is the potential of single cells to invade normal brain tissue, blurring tumor margins and establishing numerous 'micro-tumors' at a distance from the primary tumor, which make surgical resection palliative and not curative (Fig. 1). Detailed mechanisms of glioma invasion are only beginning to be known, holding promise of new therapeutic approaches. In order to reveal molecular biological drivers of the malignant phenotype of glioma it is crucial to identify the major molecules and genes that contribute to glioma invasion. There have been many recent reviews on glioma invasion [1–9]. This review focuses on putative mechanisms and new knowledge concerning glioma invasion with the intent to foster clinical applications with the particular emphasis on new targets for anti-invasion therapy.

Clinical and pathological features of glioma

Gliomas are the most common brain tumors of the adult central nervous system. Gliomas are tumors of neuroepithelial tissue and are currently classified on the basis of morphological appearance: astrocytic, oligodendroglial, ependymal and choroid plexus tumors. Astrocytomas, which are tumors composed predominantly of neoplastic astrocytes, amount to 80–85% of all gliomas and will be the focus of this perspective. World Health Organization grading is performed using a scale, from low (grade I) to high

* Corresponding author.

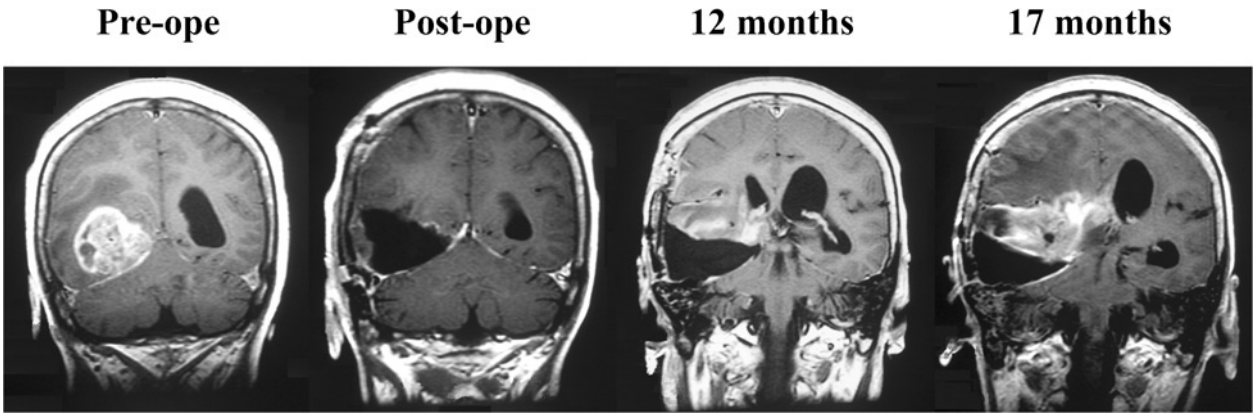

Figure 1. Local recurrence of glioblastoma. A right occipital glioblastoma (left) was operated upon, and a postoperative magnetic resonance imaging (MRI) scan confirmed complete removal (middle left). Chemotherapy and Sixty-Gy radiation therapy were administered. Twelve months later, a routine MRI showed a recurrent tumor immediately adjacent to the resection cavity (middle right). Seventeen months after the operation, the patient died (right).

(grade IV), according to hallmarks of the tumor histological aberrations: nuclear atypia, mitotic activity, endothelial hyperplasia and necrosis [10]. Grade IV astrocytomas [also referred to as glioblastoma multiforme (GBM)] manifest three or four of the morphological abnormalities (Fig. 2). GBM is a very aggressive, invasive, destructive malignancy, and its proliferation rates are two to five times higher than grade III tumors. Patients with GBM have a poor prognosis, with a median survival of 1 year despite aggressive therapy; fewer than 5% will survive 5 years. A characteristic of GBM is their ability to infiltrate and invade the surrounding normal brain tissue. The mainstays of treatment include surgical resection, radiation and chemotherapy, which address the bulk of the tumor mass, whereas recurrence occurs most often within 2 cm of the resection margin, and accounts for the fatal outcome of the disease. Although GBM shows a highly successful pattern of invasion into normal brain, it rarely metastasizes outside the brain. Thus, understanding the mechanisms which modulate glioma cell invasion will help to determine targets for the modification of existing therapies and lead to the development of novel therapeutic strategies in the management of gliomas. Therapies that effectively target invasive glioma cells may significantly improve clinical outcome. The infiltrative path of gliomas into the normal brain presents as a non-random process, often following white matter tracts, extending along perivascular spaces and the subependyma [7, 11]. These preferred anatomical routes for invasion suggest the importance of interactions between migrating cells and their microenvironment.

Putative mechanisms

The underlying molecular mechanisms of brain tumor invasion are complex and involve integrated biochemical processes requiring a coordinated effort managing a number of intracellular and extracellular interactions (Fig. 3). Active invasion necessitates cell detachment from its original site. Subsequently, the tumor cell will modify receptor-mediated adhesion to extracellular matrix (ECM) proteins, followed by matrix degradation achieved by tumor secreted proteases. Soluble motility factors function as autocrine or paracrine signaling, leading to changes in cell morphology: the cell becomes polarized and membrane protrusions including pseudopodia, lamellipodia, filopodia and invadopodia are extended from the leading edge of the cells. These extensions contain filamentous actin and various structural and signaling proteins. The formation of membrane anchors allows cytoskeletal contraction, which finally moves the cell forward.

Detachment from original site

To effect invasion, tumor cells must first detach from the nascent tumor mass and invade the surrounding stroma, composed of parenchymal cells and ECM. Cell surface adhesion molecules play an important role in the interaction between the cells and their immediate insoluble microenvironment.

CD44. CD44 is a transmembrane glycoprotein in the immunoglobulin receptor superfamily, which functions as an adhesion molecule which interacts with hyaluronic acid as its ligand. Hyalurnoic acid comprises a substantial fraction of brain ECM, and is implicated in a wide variety of physiological and pathological processes [12]. Overexpression of CD44

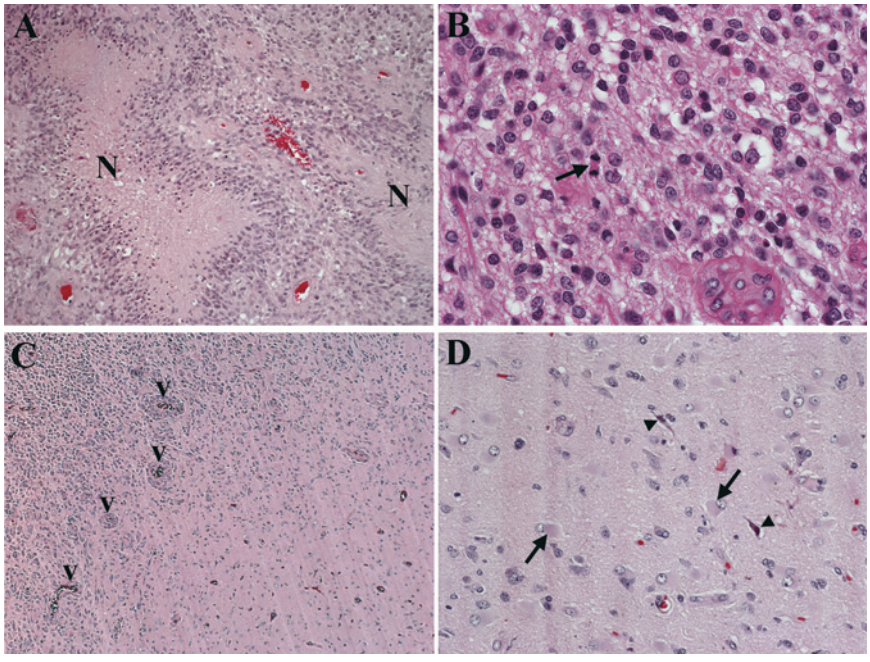

Figure 2. Histology of glioblastoma. (A) Specimen from the tumor core. Tumor necrosis (N) with pseudopalisading glioma cells and microvascular proliferation are characteristic in glioblastoma. Original magnification, ×100. (B) High magnification of tumor core. Glioma cells have pleomorphism and nuclear atypia. Mitotic cell was seen (arrow). Original magnification, ×400. (C) Specimen from border area between tumor mass and non-tumor brain. Left side is tumor core and right side is non-tumor area. The finding of microvascular proliferation (v) was seen in the border area. Glioma cells invade into the brain diffusely and the border line was unclear. Original magnification, ×100. (D) Specimen from rim of tumor. Invading glioma cells (arrowhead), which are elongated with atypical nuclei, are surrounded with non-tumor cells including reactive astrocytes (arrows). Original magnification, ×400.

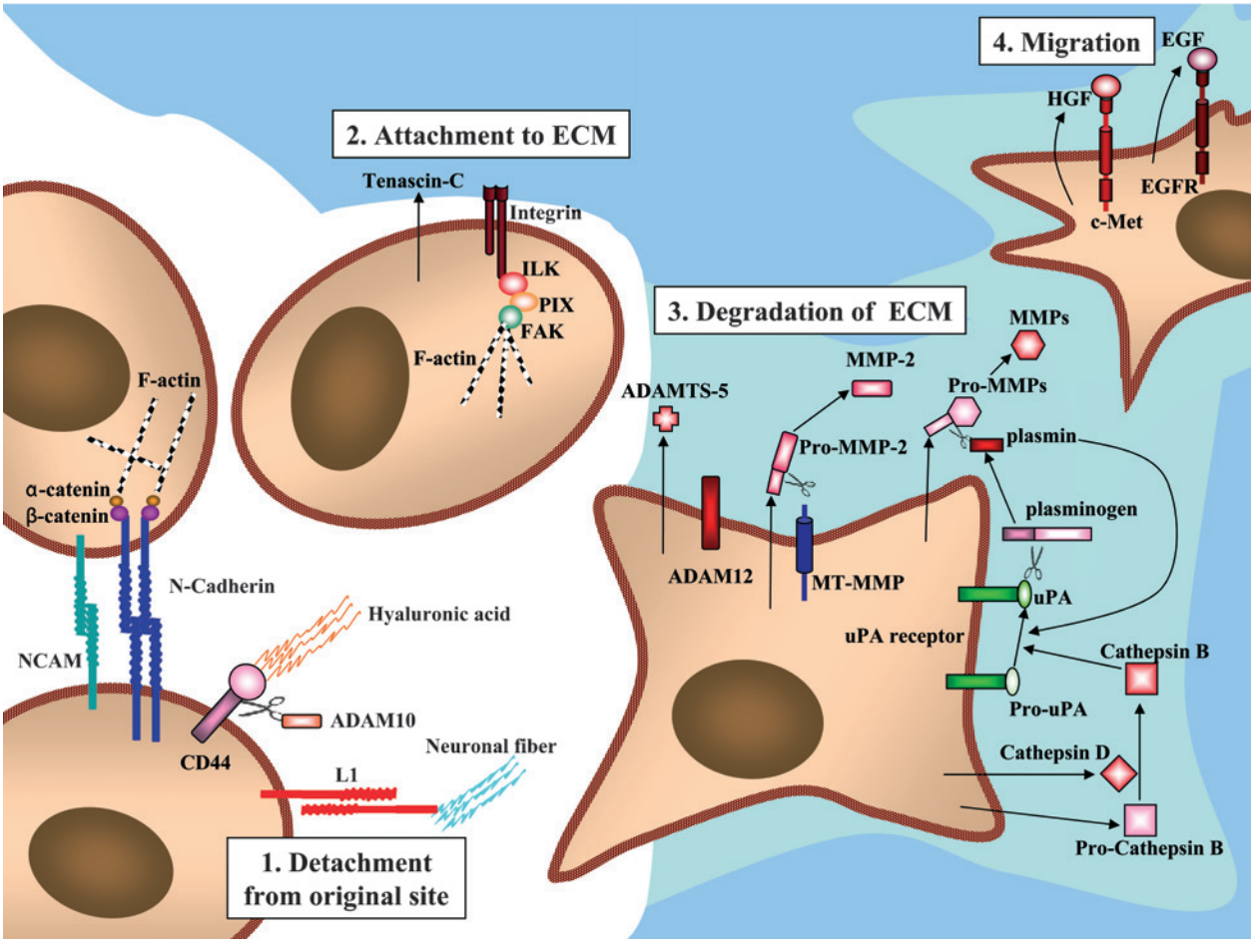

Figure 3. Putative mechanism of glioma invasion

in glioma may be related to invasion but has not proven instructive as a prognostic factor [13]. The CD44 gene product exists in standard (whole-length) and splice-variant forms. Standard CD44 is found in gliomas as well as in brain white matter, whereas variant CD44 is detected in metastatic tumors from other organs [13], suggesting that the detection of the variants could be useful for differentiating primary tumors from metastases. Monoclonal antibodies directed against CD44 decrease intracerebral invasion of glioma cells *in vivo* and through matrigel matrices *in vitro* [14, 15]. CD44 can be cleaved by ADAM (a disintegrin and metalloproteinase) 10 and 17, and both the extracellular and intracellular cleaved components of CD44 promote cell migration [16, 17]. The CD44 cleavage product can be detected in 60% of gliomas but not in normal brain [16].

Neural cell adhesion molecule. Neural cell adhesion molecule (NCAM) is a member of the glycoprotein immunoglobulin receptor superfamily and mediates homophilic binding. NCAM expression manifests in four isoforms (180, 170, 140 and 120 kDa) in the central nervous system, including both neurons and astrocytes. The expression of each NCAM isoform in astrocytic tumors is reported to decrease in proportion to progression of histological malignant grade [18]. Expression of the transmembrane 140 and 180 kDa isoforms of NCAM caused a significant reduction in cellular motility and proliferation *in vitro* as well as *in vivo* [18–20]. NCAM can act as a paracrine inhibitor of glioma cell locomotion through not only homophilic binding between the NCAM-expressing cells but also heterophilic interaction with a cell surface receptor [20]. NCAM signaling participates in ECM remodeling via its triggering downregulation of matrix metalloproteinase (MMP)-9 and MMP-1 expression [21].

Cadherin. Cadherins are calcium-dependent transmembrane cell adhesion molecules that mediate homophilic cell-cell adhesion and play an important role in tissue construction and morphogenesis in multicellular organisms. During embryogenesis, cadherins mediate tissue formation, including the assembly of neuronal structures. Cadherins are encoded by 13 different genes, the expression of which suggests mutual exclusivity [22]. Tissue-specific expression of the different cadherins – E-cadherin, N-cadherin, T-(or H-)cadherin and VE-cadherin – should be noted. E- and N-cadherins, which are the most thoroughly studied cadherins to date, bind directly to cytosolic β-catenins. β-catenin binds directly to α-catenin linking the cadherin complex to the actin cytoskeleton by direct interaction with actin stress fibers via α-actinin, an actin-binding protein. A loss of E-cadherin expression in epithelial tumors is associated with a more invasive phenotype and metastasis [23]. N-cadherin has been shown to promote cell motility and migration – an opposite effect to that of E-cadherin [23]. The cadherin switch may occur during the transition from a benign to an invasive, malignant tumor phenotype.

All gliomas regardless of grade lack E-cadherin expression, whereas conflicting results have been obtained concerning the involvement of N-cadherin in the acquisition of invasive properties. In GBM cell lines, no correlations have been found between N-cadherin expression and the invasive behavior or cell migration [24]; however, the invasion activity of U87 glioma cells is suppressed by N-cadherin inhibitor [25]. In GBM biopsy material from patients, N-cadherin expression is reported to be upregulated compared to normal brain but inversely correlated with invasion [26].

T- (or H-)cadherin, which is expressed in normal astrocytes but not in high-grade glioma, has been shown to decrease cell motility when it is stably transfected into glioma cells [27]. A working hypothesis is that the instability and disorganization of cadherin-mediated junctions, rather than reduced expression of the cadherin-catenin system components, promote migration and invasiveness in GBM cell lines [28].

L1. Neural cell adhesion molecule L1 is a member of the immunoglobulin superfamily, and is normally expressed in the nervous system. A short type of L1, L1cs is expressed in GBM and induced coincident with cell invasion. L1cs participates in tumor invasion along neuronal fibers which express L1 by their homophilic binding [29].

Attachment to ECM

A requisite for cell migration is careful coordination between adhesion and de-adhesion of the cell to the adjoining matrix. In the brain, the ECM can be viewed as the physical structure that acts as the lattice of the brain and also engages in a functional crosstalk with invading glioma cells. The ECM may promote or inhibit active locomotion of cells where various proteins mediate different effects. The true extracellular space represents 20% of total brain volume, and the majority of ECM proteins in the human brain are localized to the compartment where active movement of glioma cells occurs, the perivascular space (fibronectin, laminin, collagen and vitronectin) and brain parenchyma (hyaluron and glycosaminoglycan) [11, 30]. A number of *in vitro* studies have embarked on the importance of ECM proteins to induce the migratory phenotype in glioma cells [31]. While no

single, most-permissive substrate for all glioma cell lines has been documented, a clear correlation between cell-matrix adhesion and migration has been identified [32]. It seems noteworthy that the reaction of a glioma cell to specific ECM proteins might be highly dependent on the binding epitopes of the respective proteins.

Tenascin-C. While glioma cells in the early phases of invasion react to the surrounding native matrix, it is certain that the invasive cells also actively remodel [33] and secret their own matrix, which was found to be permissive for many glioma cell lines *in vitro* [32]. Among various ECM components, tenascin-C has been most intensely investigated. Tenascin-C has been related to angiogenesis in astrocytomas [34, 35] since it has been identified in hyperplastic vessels and was found to promote migration of endothelial cells [36]. Tenascin-C was identified to be transcriptionally upregulated in invasive glioma cells *in vivo* and in rat brain [37]. In glioma cells, the RhoA pathway seems to be important for the 'inside' effects of tenascin, which also increases formation of actin-rich filopodia while decreasing formation of stress fibers *in vitro* [38].

Integrins. To migrate and invade, a glioma cell must establish transient adhesive interactions with the ECM where a biphasic relationship between strength of adhesion and migration speed has been proposed [39]. The most important group of adhesion molecules are the integrins, transmembrane proteins that form dimers between 14 different α and 8 β subunits. Integrins interact with two major classes of ligands: (i) ECM proteins and (ii) immunoglobulin supergene family members such as intracellular adhesion molecules (ICAM-1, ICAM-2) and vascular cell adhesion molecule (VCAM-1). Integrins act as a cell's mechanical anchor to the ECM and as signal mediators via integrin-associated proteins such as CD47, tetraspanin proteins and growth factor receptors [40]. Local enrichment of these adaptor proteins leads to the formation of focal complexes and focal contacts linking cytoskeleton and extracellular binding sites [41]. 'Inside-out' signaling allows for adjustment of ligand–binding affinity through interaction of intracellular proteins, such as H-ras with the cytoplasmic tail of integrins.

Adhesion complexes of migrating cells consist of aggregated integrins, cytoskeletal proteins such as vinculin and focal adhesion kinase (FAK) and other phospho-proteins [39]. Different patterns of integrin-driven migration have been observed, as the composition of focal contacts depends on ECM-protein and cell type. Integrin α5β1 binds to fibronectin [42], α6β1 and α6β4 binds to laminin [43], αvβ3 binds to fibronectin, vitronectin and tenascin-C [44] and α2β1 bind to fibrillar collagen [45]. The β1 subunit plays an important role in glioma biology and its expression has been correlated with the invasive behavior of glioma [46] and is supported by the observation that inhibition of β1 integrin leads to decreased motility while inhibition of αv integrin results in increased motility. Taken together, these findings suggest a positive correlation between glioma cell adhesion and migration *in vitro* [11]. Integrins αvβ3 and αvβ6 interacting with tenascin have been shown to mediate adhesion rather than migration [34] and integrin β4 mediates resistance to induction of apoptosis in breast cancer cells [47]. Integrin β5 expression was found to correlate with *in vitro* invasiveness and migration of glioma cells [48]. Based on the findings described, targeted therapies with RGD inhibitors of αv integrins are currently under clinical development [49].

Remodeling of the ECM

ECM in the normal brain functions as a barrier to glioma cell invasion. Protease degradation of theECM creates intracellular space into which invading cells can migrate by an active mechanism that requires membrane synthesis, receptor turnover and rearrangement of cytoskeletal elements. Advancing cells express proteinases and/or proteinase activators at their leading edge where complex proteolysis can direct cell migration. In fact, previous studies have reported the expression of many ECM-degrading proteinases in glioma tissues, which include MMPs, ADAM, serine proteinases (urokinase-type plasminogen activator; uPA), cysteine proteinases (cathepsin B, L and S) and aspartic proteinases (cathepsin D). These proteases have different proteolytic functions and may be interdependent for their activation. On the other hand, glioma cells ingeniously reconstruct the ECM microenvironment as they can move easily. Glioma cells elaborate specific components of the ECM which induce cell migration (such as hyaruronan, tenascin-C, brevican and osteonectin) and decrease the expression of ECM which suppresses cell migration (such as testican) [7, 50].

Matrix metalloproteinase. MMPs are a gene family of Zn^{2+}-dependent enzymes that are essential for ECM turnover in normal and pathological conditions. Accumulated evidence has suggested that MMPs contribute to glioma cell invasion of the surrounding normal tissues through the cellsurface ECM degradation [3, 51–57]. Strong correlations have been reported between elevated MMP levels and tumor cell invasiveness in human gliomas. Many authors have demonstrated the expression of MMPs in gliomas and

much attention has been drawn to gelatinases (MMP-2 and MMP-9) and membrane-type MMPs (MT-MMPs). It is generally accepted that MMP activity is important for human glioma invasion for the following four reasons: (i) MMP-2, -9 and MT-MMPs can cooperatively degrade almost all types of ECM with their specific enzymatic activity for certain ECM components, (ii) they are activated rather specifically in tumor tissues and (iii) their activation correlates with tumor spread and poor prognosis; (iv) their inhibition by antisense or small interfering RNA (siRNA) oligonucleotides suppresses cell invasion. Although MMP-2 and -9 are secreted in latent form by glioma cells and activated in the extracellular space, MT-MMPs are membrane proteins and act not only as activators of proMMP-2 but also as ECM degradation enzymes. The MMP-2/MT-MMPs system is opportunistic to degrade pericellular ECM for glioma cells because proMMP-2 is activated by MT-MMPs on the tumor cell surface (Fig. 3). Integrin $\alpha v\beta 3$ is found to be implicated in this process in glioma cells [58]. Activated MMPs are inhibited by a family of tissue inhibitors of metalloproteinases (TIMPs). MT-MMPs are also inhibited by components of brain ECM such as testican-1, -3 and N-Tes [59]. However, the expression of testican-1, -3 and N-Tes in GBM is low compared with normal brain, suggesting that these ECM components are insufficient to inhibit MT-MMPs [50]. A biochemical 'balance' between the levels of activated MMPs and free inhibitors determines the overall MMP activity. This critical equilibrium is disturbed in glioma tissue [56].

ADAM. ADAMs are a gene family of multidomain membrane-anchored proteins comprising more than 30 members in various animal species, and are implicated in pathophysiological conditions which include neuronal development, cancer development and progression, and inflammatory responses through proteolysis, cell adhesion, and cell-matrix interaction. They contain the metalloproteinase domains, which are highly homologous to that of the MMPs. Among ADAM family members, membrane-type ADAM12 (ADAM12m) has been shown to be overexpressed in GBM and to contribute not only towards cell invasion but also proliferation through the shedding of heparin-binding epidermal growth factor (HB-EGF) [60]. Like ADAM12m, ADAMTS-5 (ADAM with thrombospondin motifs-5) expression levels in GBM are significantly higher than in the normal brain [61, 62]. ADAMTS-5 is capable of degrading brevican, which is a specific ECM component overexpressed in GBM; the degradation fragments of brevican are implicated in glioma cell invasion [63, 64].

uPA. uPA is a serine protease that catalyzes the conversion of inactive plasminogen into plasmin, a broadly acting enzyme able to degrade a variety of ECM proteins and activate MMPs, growth factors and pro-uPA. uPA binds to its specific receptor (uPAR) directing plasmin activity to the migrating tumor cell surface. This activation cascade forms a positive feedback accelerating the degradation of ECM at the cell surface. uPA and uPAR have been shown to be overexpressed in GBM [65, 66]. Ets-1 transcription factor, which is overexpressed in GBM, may regulate the expression of uPA as well as MMP and integrin $\alpha 5$ [67, 68]. The inhibition of uPA and uPAR by antisense oligonucleotides or siRNAs are shown to suppress glioma invasion [69–71].

Cathepsin. Cathepsin is a lysosomal acid hydrolase with a broad range of endopeptidase activity against substrates and has been found in association with the plasma membrane fraction of tumor cells and in the tumor cell media. Cathepsin B, D, L and S are known to be overexpressed in GBM [72–76]. Cathepsin B is activated by cathepsin D and activates MMPs and uPA (Fig. 3). The inhibition of cathepsin B has a potential to suppress invasion [70, 77, 78]. Cathepsin D may be a serum marker for poor prognosis in glioma patients [74].

Migration

The motility of glioma cells is stimulated by motility factors that may operate through autocrine and/or paracrine signaling.

Scatter factor/hepatocyte growth factor. (SF/HGF) is a pleiotropic factor that induces tumor and endothelial cell migration and proliferation, as well as angiogenesis *in vivo*. SF/HGF exerts its effects through its only known receptor, the c-Met proto-oncogene product, a transmembrane receptor that possesses tyrosine kinase activity. The expression levels of SF/HGF and c-Met frequently correlate with glioma grade and poor prognosis. Functionally, SF/HGF expression promotes glioma cell motility and proliferation [79]. SF/HGF inhibitor can inhibit GBM growth by antagonizing tumor cell proliferation, migration, promotion of apoptosis and inhibition of tumor angiogenesis *in vivo* [80].

Epidermal growth factor receptor (EGFR). Amplification and overexpression are observed in 50% of GBM and are associated with a worse prognosis especially among younger patients [81, 82]. EGFR signaling enhances proliferation, migration and invasion and inhibits apoptosis of glioma cells [83]. In the majority of these cases, amplification of the EGFR

gene is associated with structural rearrangements of the gene. The most common rearrangement is EGFRvIII [84]. Since the resulting mutant protein is ligand independent, constitutively phosphorylated, and localizes to the cell surface resulting, EGFRvIII is a more potent tumorigenic receptor than the wild-type receptor [85]. EGFRvIII-positive tumors have also been associated with worse prognosis and shorter life expectancies [82]. There have been a number of reports on the potential use of EGF as a targeted therapy [86–90].

New molecules involved in glioma invasion

In the era of the technologies supporting the human genome project, new or known genes have been identified to be associated with glioma progression. Complementary DNA and oligonucleotide microarrays, tissue microarrays and proteome profiling have provided high throughput and potentially comprehensive approaches for the molecular characterization of human gliomas. These innovative techniques have proven to be very useful in the molecular classification of astrocytic tumor grades, generating evidence of a molecular evolution driving progressive stages of astrocytoma malignancy. Gene expression analysis enhances histopathological diagnosis, especially for non-classic tumor histologies, providing a more accurate prognosis [91, 92]. Proteome analysis is useful to identify novel biomarkers for survival prediction and rational targets for antiglioma therapy [93]. Gliomas can be said to consist of two major cell populations, the proliferative cells at the tumor core, and cells invading the brain parenchyma. cDNA microarray technology has been used to investigate the spectrum of genes differentially expressed during glioma migration [94, 95]. This led to the identification of genes overexpressed in invasive glioma cells. These molecules may represent a promising target for the development of novel anti-invasive therapeutic strategies.

RNA interference is now widely accepted as the *in vitro* method of choice for rapidly assessing biological mechanisms and pathways through loss-of-function analysis. It has rapidly become a major tool for *in vitro* analysis of protein function. Gene silencing by siRNAs plays an important role for target validation in invading glioma cells. Additionally, innovative cell functional assays which are being developed help validation of target gene [8, 96].

Extracellular secreted proteins (Fig. 4)

Insulin-like-growth-factor-binding protein (IGFBP) family. Members of the IGFBP family function as IGF-stabilizing carriers and functional modulators of IGFs. These insulin-binding proteins extend the half-life of IGF-I and IGF-II, linking them to control of cellular proliferation. Transcriptional profiling of a progressive series of glial tumors has shown IGFBP2 to be a hallmark of advancing malignancy [97, 98]. Recent proteomic profiling of low- and high-grade gliomas confirm IGFBP2 as a key discriminator between tumor grades [99]. It is frequently co-expressed with IGFBP3 or IGFBP5 in GBM [100, 101], and within this category of astrocytomas, it is one of the genes that discriminate GBMs with EGFR overexpression/amplification (primary GBMs) from non-EGFR-expressing GBMs [102]. In addition to EGFR, phosphatase and tensin homology deleted on chromosome 10 (PTEN), a tumor suppressor linked to malignant glioma, may also play a role in IGFBP regulation. IGFBP2 expression is repressed by PTEN in U251 glioma cells [103], and therefore, the frequent loss of PTEN in GBM (40–50%) may be, in part, responsible for IGFBP2 upregulation in these tumors. Initially identified as an antimitotic agent expressed in the developing fetal brain, it also has pro-invasion effects. Whereas SNB19 GBM cells stably transfected with IGFBP2 show no increase in proliferation, they are significantly more invasive than control cells *in vitro* [104]. Enhanced invasion through a matrigel-coated transwell system is likely due to a panel of genes transcriptionally upregulated by IGFBP2-dependent signaling, including MMP-2 [104]. Histochemical analysis of GBM tumor samples shows a high correlation between IGFBP2 and MMP-2 expression. The role of IGFBP2 in *in vivo* invasion, however, remains to be clarified, since both IGFBP2 and IGFBP5 were found to be transcriptionally downregulated in invasive glioma cells [95].

Cysteine-rich 61/connective tissue growth factor/nephroblastoma overexpressed (CCN) family: Cyr61 (CNN1, IGFBP10). These cysteine-rich extracellular proteins with structural homology to IGFBPs are involved in cell proliferation, chemotaxis, adhesion and extracellular matrix formation in various tissues. Cyr61 is a heparin-binding protein, which can also interact with integrin $\alpha v\beta 3$ and $\alpha 6\beta 1$. It is secreted by various tumors and is associated with angiogenesis, proliferation, adhesion and migration. It is highly expressed by primary gliomas and invasive GBM cell lines, and significantly increases migration of U343 cells on vitronectin [105]. Overexpression of Cyr61 results in transcriptional upregulation of various

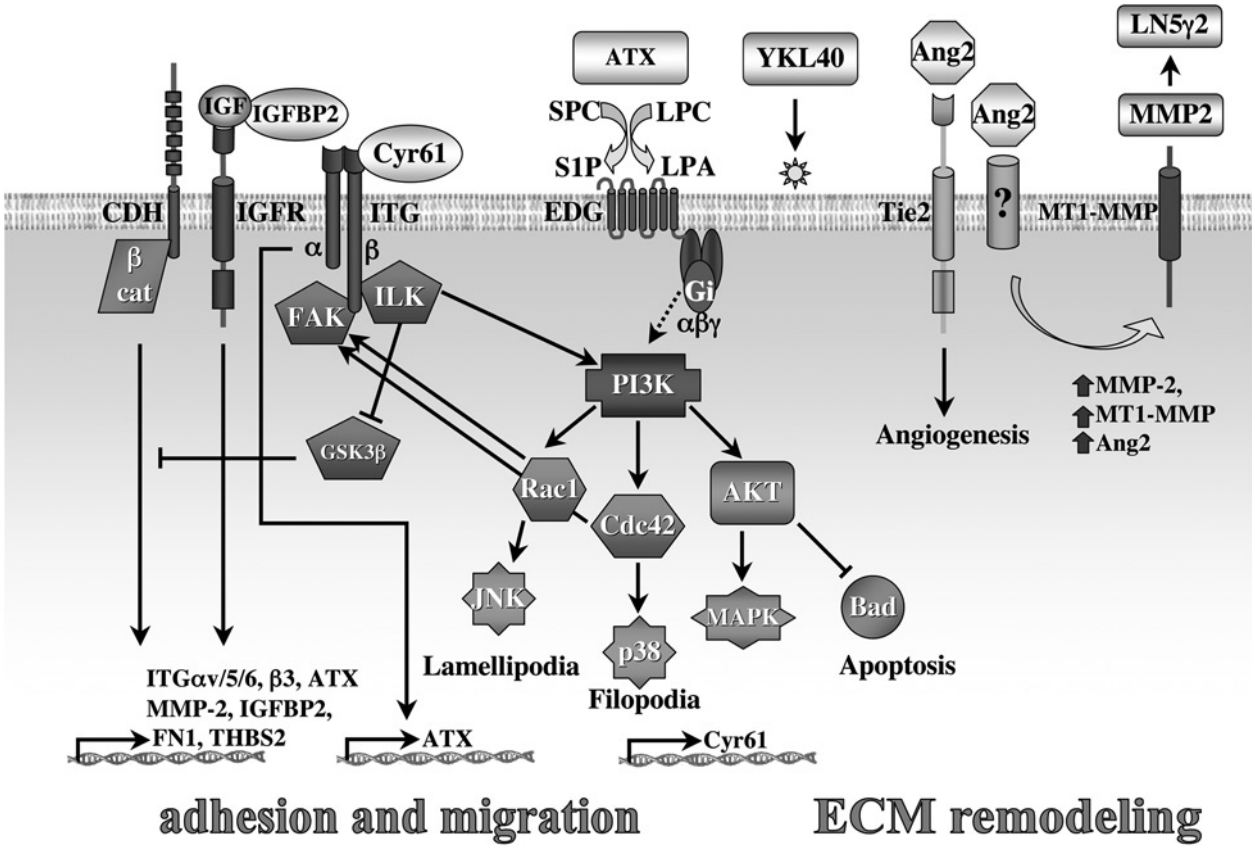

Figure 4. Novel secreted proteins that influence glioma invasion. Modular proteins of the IGFBP and CCN family have various functions in cancer biology, to which the promotion of invasion has recently been added. Cyr61 contributes to invasion by binding to integrins and eliciting a signaling cascade that involves ILK, PI3-K and AKT, to activate invasion and also activated transcription of invasion-related genes through β-catenin and TCF/LEF. The role IGFBP2 as an IGF-stabilizing protein is mainly linked to modulation of proliferation; however, IGF1 signaling through IGFR can also affect invasion, in part through transcriptional activation of invasion-related genes. It is not known if IGFBP2 signals in an IGFR-dependent or -independent manner in invasive glioma, but like Cyr61 it can bind to integrins. The effect of LPA as a stimulant of glioma invasion is well known, but the source of this bioactive lipid for cells invading the brain parenchyma, has not been defined. ATX is a phospholipase D that converts phospholipids such as lysophosphatidyl choline (LPC) into LPA and shingosylphosphorylcholine (SPC) into S1P. These bioactive phospholipids signal through the EDG family of G-protein-coupled receptors to elicit changes in the actin cytoskeleton that are conducive to invasion. Less is known about the function of Ang2 and YKL40, but these proteins play a direct (YKL40) and indirect (Ang2) role in remodeling the extracellular environment. Proteolytic degradation of the ECM, in part through MMP-2, results in a more permissive environment for invasion.

integrins, expression of αvβ3 and integrin-linked kinase (ILK), resulting in heightened ILK kinase activity [105]. Furthermore, expression of Cyr61 has been shown to be a prognostic marker for glioma tumor progression, as well as a strong predictor of survival [105].

Angiopoietin 2 (Ang2). Ang2 is a secreted glycosylated homodimer [106] that competes with Ang1 for binding of the Tie2 tyrosine kinase receptor, preventing receptor activation. Ang1 is angiogenic and induces stabilization of the vasculature, presumably through promotion of endothelial cell-to-support cell adhesion. Ang2 is thought to promote vessel plasticity; in glioma its overexpression results in vessel disorganization and inadequate angiogenesis [107]. Ang2 is expressed in endothelial cells, but is also implicated in tumor progression of various tumors, its function usually linked to angiogenesis [108]. In glioma it has been found to be expressed at low levels in the GBM tumor core, whereas invasive tumor cells showed an increase in staining intensity [109, 110]. In the invasive edge of human GBM, Ang2 expression coincides with MMP-2 expression [109, 110], and is capable of stimulating invasion of U87 glioma cells in a mouse orthotopic xenograft [110]. This same orthotopic model using Ang2-expressing U87 suggests a mechanism for its pro-invasive effect; invading cells also overexpress and activate MT1-MMP, MMP-2 and LN5γ2, facilitating tumor invasion [110]. Interestingly, it is not Tie2, but αvβ1 integrin that transduces the pro-invasive Ang2 signal in glioma. The direct interaction of Ang2 with αvβ1 integrin results in FAK activation, which ultimately leads to ERK1/2 and JNK

activation and transcriptional upregulation of MMP-2 [111].

YKL40. This secreted glycoprotein was previously described as a carbohydrate-binding chitinase involved in connective tissue degradation. YKL40 was first linked to glioma biology as a potential serum marker that correlates with astrocytic tumor grade [112]. High expression of YKL40 is linked to reduced overall survival and increased resistance to radiotherapy [113]. Interestingly, as with IGFBP2, higher YKL40 expression was observed in tumors with chromosome 10 (i.e. PTEN) loss [113, 114]. Recently, this secreted protein has also been linked to glioma invasion: a subtractive screen of genes expressed by non-invasive pilocytic astrocytomas against genes expressed by invasive GBM tumors, designed to find novel invasion gene candidates, revealed YKL40 as a potential candidate [115]. YKL40 transfection of immortalized astrocytes resulted in increased radiation resistance, invasion (with MMP activation) and protection from serum starvation-induced apoptosis [114].

Autotaxin (ATX)/lysophospholipase D. ATX was first identified as a secreted motogenic protein in melanoma [116] and is also expressed by additional tumors with an invasive phenotype, such as breast cancer, non-small cell lung cancer [117] and GBM [95]. Within GBM, it is one of the genes that segregate a novel sub-type of tumors characterized by over-expression of genes on 12q13–15 (the locus that includes cdk4) [102]. ATX is expressed by GBM cells invading into the brain parenchyma but not by normal astrocytes, neurons or mature oligodendrocytes [95]. In its function as a lysophospholipase-D, it catalyzes the production of lysophosphatidic acid (LPA) and sphingosine-1-phospate (S1P). These powerful bio-active phospholipids are linked with motility and invasion in GBM, signaling predominantly through LPA1 [118] and S1P [119] receptors expressed by glioma.

Membrane-type protein

Fn14/TWEAK. Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) is a member of the tumor necrosis factor (TNF) superfamily of cytokines regulates cellular responses such as proliferation, survival, apoptosis and migration [120] (Fig. 5). TWEAK is expressed as a type II transmembrane protein but can also be proteolytically cleaved to generate a soluble, trimeric, N-glycosylated cytokine [121]. TWEAK exerts its biological responses via binding to a small surface receptor known as fibroblast-growth-factor-inducible 14 (Fn14) [120]. In endothelial cells and astrocytes, TWEAK promotes cell survival and not death [122]. TWEAK has also been implicated in promoting angiogenesis [123]. Fn14 is a member of the tumor necrosis factor (TNF) superfamily of receptors and is characterized as a type Ia transmembrane receptor lacking a cytoplasmic death domain [124]. However, the cytoplasmic domain of Fn14 contains a single TNF-receptor-associated factor (TRAF)-binding site flanked by two conserved threonine residues [124]. TRAF functions to link transmembrane receptors to nuclear factor-κB (NF-κB) signaling pathways [125]. In fact, Fn14 signals via NF-κB regulate glioma cell survival [126]. Fn14 expression is high in a variety of normal tissues including heart, kidney, placenta, lung and pancreas and relatively low in brain and liver [127]. In cancer, high expression of Fn14 is reported in hepatocellular carcinoma [127], breast carcinomas, pancreatic cancer [128] and GBM [129]. Elevated expression of Fn14 has been correlated with glioma cell migration *in vitro* and in invasive GBM clinical specimens [126]. Activation of Fn14 by TWEAK has been shown to promote glioma cell migration [129].

Fn14 activation also fosters glioma cell survival. TWEAK treatment of glioma cells resulted in the activation of NF-κB and subsequently the translocation of NF-κB from the cytoplasm to the nucleus [126]. In addition, Fn14 activation results in the induction of pro-survival genes Bcl-X_L and Bcl-W, the expression of which is dependent upon NF-κB transcriptional activity [126]. Additionally, siRNA-mediated depletion of either Bcl-X_L or Bcl-W antagonized the TWEAK-Fn14 protective effect on glioma cells [126]. Thus, NF-κB-mediated upregulation of Bcl-X_L and Bcl-W expression in glioma cells increases cellular resistance to cytotoxic-therapy-induced apoptosis.

EphB2/ephrin-B3. The Eph receptor:ligand system represents the largest family of receptor protein tyrosine kinases, consisting of 14 receptors and 9 interacting ligands, the ephrins. Eph/ephrin signaling mediates repulsive mechanisms that guide migrating cells during embryonic neural development upon cell-cell contact. The Eph receptors and ephrins are divided into two subclasses, A and B, on the basis of their sequence, homologies, structures and binding affinities. Altogether, nine EphA, five EphB, five ephrin-A, and three ephrin-B members are currently known in humans. The Eph receptors are involved in critical processes during development of the nervous system, such as axon guidance, axon fasciculation, tissue border formation, vasculogenesis and cell migration [130]. When ligands bind to Eph receptors, the receptor kinase domain is phosphorylated causing

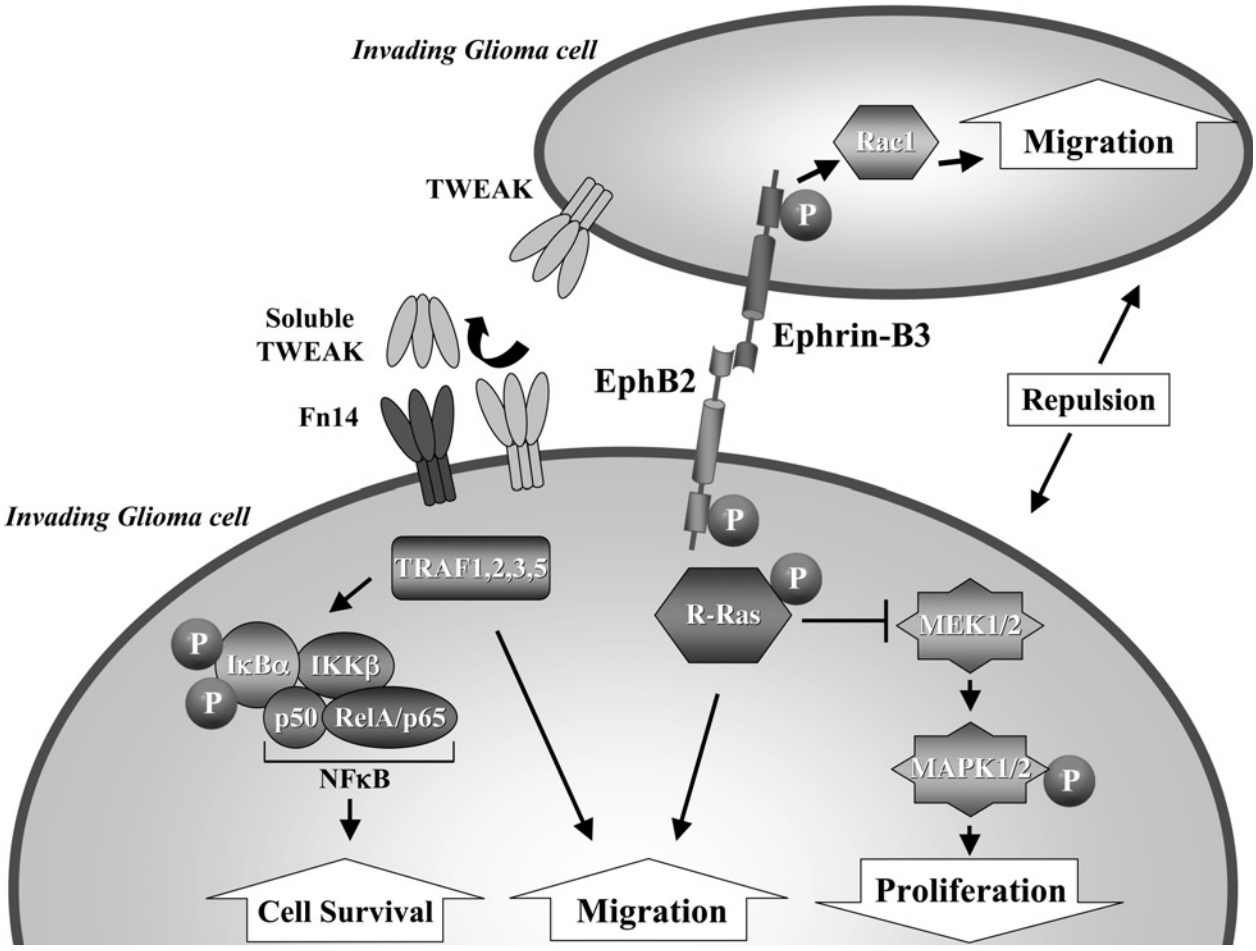

Figure 5. Schematic representation of Fn14/TWEAK and EphB2/ephrin-B3 receptor/ligand signaling pathways in invading glioma cells. TWEAK-Fn14 signaling promotes glioma cell survival via NF-κB activation. Additionally, activated EphB2 phosphorylates R-Ras, diminishes cell proliferation by inhibition of the MEK/MAPK pathway and increases migration via R-Ras signaling. Activated ephrin-B3 promotes cell invasion via Rac1 activation. P, tyrosine phosphorylation.

the phosphorylation of a number of cytoplasmic substrate proteins. The ephrin-B proteins, which are also transmembrane proteins, can function as reciprocal receptors for EphB molecules and transduce signals into cells. Recently, EphB2 receptor was reported to induce glioma cell migration *in vitro* and was associated with invasive glioma cells *in vivo* [131]. EphB2 receptor expression and activation are minimal in normal brain but increase in GBM. EphB2 activation increases cell invasion via R-Ras, a member of the intracellular small GTPase family, and reduces cell proliferation by inhibition of the mitogen-activated protein kinase (MAPK) pathway, also through R-Ras signaling [132]. Thus, the cellular responses induced by EphB2 signaling are consistent with those of invading glioma cells. As seen for its ligand EphB2, ephrin-B3 expression was increased in migrating glioma cells *in vitro* and *in vivo*, as well as EphB2, suggesting that ephrin-B3 on the membrane of GBM cells induces positive signaling for glioma invasion [95, 133]. Ephrin-B3 was overexpressed and phosphorylated in GBM, and Rac1 activation is involved in the downstream signaling of ephrin-B3 in invasive glioma cells [133]. This involvement of EphB2/ephrin-B3 in glioma invasion suggests the paradigm that cell-cell contact promotes glioma invasion.

CD155. CD155 is a member of the immunoglobulin family of cell adhesion molecules, first identified on the basis of its ability to mediate the binding of poliovirus to host cells. CD155 interacts with vitronectin which is reported to promote glioma cell invasion [30]. The role of CD155 in regulating glioma dispersion was recently revealed [134]. CD155 could be recruited to focal complexes through its interaction with vitronectin, resulting in a tripartite complex including an integrin pair. CD155 is not normally expressed in the adult brain but has been detected at high levels in glioma patient tissue [135]. Given its accessibility as a surface protein and its tumor-specific

expression, CD155 may have potential as a therapeutic target for drugs.

Intracellular protein

Bcl-2 family. Cells normally require survival signals to prevent apoptosis. Apoptosis resistance, however, leads to perturbations of cell growth involving cell accumulation, cell persistence and altered growth factor and hormone sensitivities in various cancer systems, including the brain [136]. Cell survival is mediated by signaling through surface receptors and by cell-cell and cell-matrix interactions. Members of the Bcl-2 family appear to function at a pivotal point in the decision process where cells become irreversibly committed to death. Some of the family members, including Bcl-x, Bcl-2, Bcl-w and Mcl-1, promote cell survival, whereas others, such as Bax, Bak, Bad, Bik, Bid, Bok and Bim, promote apoptosis [137]. These molecules form both homo- and heterodimers, and one putative mechanism for their control of apoptosis is that the relative proportions of their interactions regulate the balance between apoptosis and survival [138]. Bcl-2 and Bcl-X_L have been previously implicated as modulators of chemosensitivity and radiosensitivity in human malignant glioma cells [139]. In addition, Bcl-W has been shown to be highly expressed in invasive cells *in vivo* [95]. siRNA-mediated depletion of Bcl-X_L or Bcl-W sensitizes invasive glioma cells to cytotoxic-therapy-induced apoptosis [126].

A central mediator affecting the balance of pro- to antiapoptotic proteins is the Akt/protein kinase B (PKB) signaling pathway. Akt/PKB is a serine/threonine protein kinase that has been implicated in mediating a variety of biological responses including apoptosis inhibition, metabolism and cellular growth stimulation [140]. Activity of Akt is dependent on upstream activation of phosphatidylinositide 3-OH kinase (PI3-K), which functions to recruit Akt to the membrane. During glioma cell migration, active Akt is localized predominantly at the leading edge of migrating cells [141]. Suppression of Akt activity by LY29004, a PI3-K inhibitor, sensitizes migrating glioma cells to cytotoxic therapy-induced apoptosis [141].

Rac GTPases (Rac1 and Rac3). The Rho family of small GTPases consists of 20 members that regulate a large variety of cellular functions including actin cytoskeleton organization, cell migration, mitogenesis and cell survival [142]. In particular, Rho family members RhoA, Rac1 and Cdc42 have been shown to be essential for cell transformation [143], tumor cell invasion [144] and metastasis [145]. In the process of cell migration, Rho mediates the formation of stress fibers and focal adhesions at the rear of the cell [146]. In contrast, Rac1 directs the actin assembly that results in the formation of lamellipodia at the leading edge of migrating cells and is thought to be a key player in cell movement [146]. Although not directly required for cell motility, Cdc42 plays a role in the regulation of cell polarity and filopodia formation, thereby promoting directional cell movement [146]. Rac proteins consist of three isoforms, Rac1, 2 and 3. These proteins are highly homologous and differ in their transcriptional regulation and tissue distribution. Rac1 is ubiquitously expressed among various tissues, Rac2 is hematopoietically specific and Rac3 is highly expressed in the brain [147]. Rac1 is essential for various aspects of malignant transformation including anchorage-independent growth, survival, invasion and metastasis [143, 145, 148]. In glioma cells, depletion of Rac1 expression by siRNA results in a decrease in cell migration and invasion, and strongly inhibits lamellipodia formation [145, 148]. Additionally, suppression of Rac1 activity via a dominant-negative form of Rac1 induces death in glioma cell lines and primary GBM but not normal human adult astrocytes [149]. However, depletion of Rac3 strongly inhibits glioma cell invasion but minimally affects cell migration. Interestingly, inhibition of Rac3 by siRNA does not affect lamellipodia formation [148]. Thus, functional characterization of Rac1 and Rac3 using siRNA reveals specific roles of these GTPases in glioma invasion. Interestingly, inhibition of Rho-kinase, downstream of RhoA, induces glioma cell migration through Rac1 activation, suggesting that the relative degree of activation among RhoA and Rac1 regulates glioma cell movement [150].

Synaptojanin2. Synaptojanins are a family of polyphosphoinositide phosphatases that metabolize a variety of phosphoinositides including PtdIns(4,5)P_2. There are two synaptojanin proteins, synaptojanin 1 (SJ-1) [151], the major splice variant, which is most abundant in the brain, and synaptojanin 2 (SJ-2), which is expressed in a wide range of tissues [152].

Synaptojanins play an important role in clathrin-mediated endocytosis. SJ-1 functions in the decoating of synaptic vesicles [153], whereas SJ-2 plays a critical role in the formation of clathrin-coated pits [154]. Additionally, interaction of SJ-2 specifically with Rac1 suggests that SJ-2 mediates Rac1-regulated functions, whereas SJ-1 does not bind Rac1 [155]. In glioma, SJ-2 is important for Rac1-mediated glioma migration and invasion [155]. siRNA-mediated depletion of SJ-2 inhibits the formation of lamellipodia and invadopodia, specialized membrane structures that are speculated to be involved in extracellular matrix degrada-

tion. In addition, siRNA-mediated depletion of SJ-2 or Rac1 also decreases glioma migration and invasion, suggesting that SJ-2 signals through Rac-1 to foster glioma cell invasion and migration by regulating the formation of lamellipodia and invadopodia.

P311. P311 is an 8-kDa polypeptide that was initially identified in neurons and muscles [156]. Gene expression profiling using mRNA differential display identified P311 as a migration-associated gene that is highly expressed in invasive glioma cells [157]. The amino acid sequence of P311 contains a conserved PEST (Pro, Glu, Ser, Thr) domain which plays a role in targeting proteins for degradation by the ubiquitin/proteasome mechanism and a putative serine phosphorylation site at codon 59 (S59) [158]. P311 is rapidly degraded with a half-life less than 5 min [158]. Overexpression of P311 has been shown to promote glioma cell migration via Rac1 activation [159]. Site-directed mutagenesis of serine 59 to alanine (S59A) inhibits P311 degradation and reduces glioma cell migration, suggesting that P311 activity and stability are regulated by the phosphorylation of S59. In glioma cells, P311 interacts with filamin A and is regulated by the β1 integrin [159]. It appears that P311 functions in the reorganization of the actin cytoskeleton at the cell periphery necessary for glioma migration.

FAK and proline-rich tyrosine kinase 2. The FAK family of non-receptor tyrosine kinases consists of two proteins, FAK and proline-rich tyrosine kinase 2 (Pyk2). Both FAK and Pyk2 play important roles in linking integrins and growth factor receptor signaling to cell proliferation, migration, survival and apoptosis in many cell types. In glioma, overexpression of FAK has been shown to promote malignant cell proliferation [160]. Additionally, elevated FAK protein levels have been detected in glioma biopsy samples [160, 161], with elevated protein expression at the invasive edge of high-grade gliomas [161]. These results are consistent with reports in other types of malignant tumors [162]. Recently, Pyk2 has been implicated in promoting glioma cell migration [163] and invasion [164]. Pyk2 has a conserved domain structure and sequence similarity to FAK and can interact with many of the same proteins as FAK. Recent studies by Lipinski et al. [163] showed that Pyk2 overexpression promotes glioma cell migration, whereas FAK overexpression promotes glioma cell proliferation. The emerging role of Pyk2 and FAK in glioma biology suggests that the differential activity of these two kinases may be particularly relevant to the regulation of the proliferative or migratory behavior of these cells.

CrkI. Crk belongs to a group of adaptor proteins that are comprised of Src homology (SH)2 and SH3 domains, which interact with phosphotyrosine and proline-rich regions, respectively. The SH2 domain of Crk can bind to p130 Crk-associate substrate (p130cas), paxillin and growth factor receptors. The N-terminal SH3 domain of Crk can bind to C3G (a guanine nucleotide exchange factor/GEF for Rap1), Sos (GEF for Ras), DOCK180 (which activates Rac1 after binding to the CrkII/p130cas complex), c-Abl and PI3-K p85 regulatory subunit. The human crk gene is translated into two products, CrkI and CrkII, by alternative splicing. CrkII is a 42-kDa protein consisting of one SH2 and two SH3 domains. CrkI consists of one SH2 and only one SH3 domain, and it lacks a tyrosine phosphorylation site. Specific expression of CrkI was shown in GBM tissues and contributes to malignancy of GBM, while activating p130cas [25]. CrkI is also involved in promoting the invasive phenotype by activating PI3-K/Akt signaling [25].

Myosin II. Myosin II is a cytoskeletal component essential for the intracellular process of force generation required for motile behavior. Both pharmacological and molecular biological methods have demonstrated the importance of myosin II in powering a variety of motile behaviors, including growth cone motility, fibroblast locomotion and astrocyte process outgrowth and contractile responses. The activity of myosin II is controlled by phosphorylation of a serine residue on its regulatory light chain (RLC). Phosphorylation of the RLC by myosin light chain kinase (MLCK) activates myosin II. This action is opposed by dephosphorylation of the RLC, which is mediated by a specific myosin light chain phosphatase. Thus, the degree of activity of myosin II in a cell is controlled by the relative balance of activities of MLCK and myosin light chain phosphatase. Small GTPases, such as RhoA, Rac and cdc42, and RLC-interacting protein are involved in this process in glioma cells [150, 165]. MLCK inhibitors were shown to be potent and specific inhibitors of motility in glioma *in vitro* [166].

Clinical applications

A clear benefit of chemotherapy to glioma patients was not demonstrated until 2005 Stupp and co-workers [167] reported a 2.5-month increase in overall survival after temozolomide (TMZ) chemotherapy combined with radiation. While Wick et al. [168] recently suggested that TMZ reduces glioma invasion induced by irradiation *in vitro*, this effect is clinically negligible. Elucidation of the signaling pathways responsible for glioma invasion is now being trans-

lated into glioma treatment strategies. Currently, many efforts are dedicated to the exploitation of synthetic, low-molecular-weight inhibitors as anti-invasion drugs. Initial efforts have focused on the use of single agents, directed at specific molecular targets. Although there is precedence in cancer therapy for this single-agent approach, most other efforts have been disappointing. It is obvious that the complexity and crosstalk between signal transduction pathways limits the potential efficacy of targeting a single molecule. Therefore, future therapies will likely need to consider a combination of treatment approaches with multiple inhibitors.

Much experimental evidence indicates that there may be an inherent and inverse correlation of cell motility and proliferation of a cell population both *in vitro* and *in vivo* [94, 169, 170]. If invasive glioma cells activate a predominantly migratory cellular phenotype with a temporarily lowered proliferation rate, then these cells may also be relatively resistant to conventional cytotoxic treatments, which are frequently directed against proliferating cells. In fact, a variety of different studies have indicated that apoptosis is suppressed when cells adopt a migratory phenotype. For example, invasive glioma cells display a shift in expression of several apoptosis regulatory genes favoring decreased ability to undergo apoptosis [94]. Suppression of Rac activity induces apoptosis of human glioma cells [149]. Overexpression of the survival enhancer Bcl-2 in glioma cells promotes invasion into brain aggregates [171]. SF/HGF expression inhibits apoptosis of migrating GBM cells and confers resistance to cell death induced by chemotherapy and radiation [172]. EGFR signaling has antiapoptotic properties [173]. The tight linkage between migration activation and decreased susceptibility to apoptosis indicates that migration may activate survival signaling. The PI3-K/Akt survival pathway was shown to be activated in migrating glioma cells [141]. This relationship of migration/proliferation and apoptosis may present an example of a novel entry point for modification of the cellular behavior of invasive cells. Anti-invasive therapies based on this concept may not only inhibit further spread of an invading glial tumor but also provide therapeutic targets specifically sensitizing invasive cells to conventional cytotoxic treatments, inducing apoptosis. In fact, migration-inhibiting drugs increased susceptibility of migrating glioma cells to chemotherapy *in vitro* [141]. Therefore, the anti-invasion therapy should be combined with chemotherapy or radiotherapy.

Angiogenesis and tumor invasion can both be considered as an invasive process which requires adhesion to the ECM, and degradation and remodeling of the ECM in order to allow cells to migrate and invade into the surrounding tissues. The up-regulation of ECM degradation enzymes such as MMP, uPA/uPAR and cathepsin B has been linked not only to tumor invasion but also tumor-associated angiogenesis [174]. c-Met/HGF, Fn14/TWEAK and Eph/ephrin signaling also enhances glioma-associated angiogenesis [123, 175, 176]. Both processes are under the tight regulation of a balance between stimulating and inhibiting factors. This process requires a complex crosstalk between endothelial and tumor cells, ECM components, and cellular elements of the host microenvironment. The existence of common mechanisms of regulation and the presence of naturally occurring factors that inhibit angiogenesis and invasion makes the inhibition of both processes possible. Thus, control of common activity in these two different contexts is of considerable interest as a possible therapeutic target in GBM.

Anti-invasion clinical trials

To date there has been to our knowledge not a single clinical trial in gliomas with an agent specifically designed to target the invasive glioma cells. While the phenomenon of the highly invasive behavior of human gliomas is widely accepted, most compounds are aimed at targeting 'the tumor cells' rather than taking advantage of a 'target' specifically overexpressed or lost in invasive glioma cells. The development and proper application of anti-invasive agents that will inhibit tumor invasion, but not necessarily be directly cytotoxic, holds promise. Diffusion tensor imaging and molecular imaging such as PET and SPECT have the potential to allow for early detection of responses to a given treatment. In conjunction with the advent of novel biomarkers, clinical studies can be carried out more efficiently and will yield a higher chance of success.

Tenascin-C immunotherapy. Based on the identification of tenascin as a 'glioma-specific' antigen, a tenascin-specific antibody labeled with I131 [177] was tested on patients with high-grade gliomas and delivered intra-cystically or into the resection cavity. Phase II studies with this construct in malignant glioma were reported to yield slight increases in survival time, which could be due to bias in patient selection and the lack of an adequate control population [178, 179]. Targeting tenascin with an antibody might sound biologically reasonable; however, delivery by diffusion only might raise concerns about the ability of the antibody to find its most important target, i.e. invasive glioma cells that can be dispersed several centimetres away from the site of application.

Furthermore, inhibiting tenascin, a mediator of angiogenesis, may increase tumor invasiveness [180].

Integrin antagonist. The RGD motif of integrins is targeted by a very specific drug EMD121974, designed to block the interaction between αvβ3 and αvβ5 integrins on endothelial cells and ECM, thereby interfering with a key process in angiogenesis. On the basis of an *in vivo* study where significant tumor regression was observed in a xenograft model treated with EMD121974 [181], clinical trials with this compound in combination with radiation therapy are currently being conducted.

Thalidomide. Thalidomide, a drug initially designed as sleeping medication that was withdrawn from the market due to extreme embryotoxicity resulting in dysmelia has been found to exhibit strong antiangiogenic effects [182]. While the complex mode of thalidomide action is not understood, it has been suggested to interfere with the expression of integrin receptors αvβ3 and αvβ5 as well as to inhibit vascularization induced by basic fibroblastic growth factor (bFGF) and vascular endothelial growth factor (VEGF). This inhibition is due to intercalation of thalidomide into guanine-rich DNA promoter sites of bFGF and the αv and β3 integrin subunits. It has further been suggested that thalidomide increases the production of free radicals which reduces NF-κB-mediated gene transcription [183]. While thalidomide proved to be unsuccessful as a monotherapy in GBM [184], it has moderate effects on median survival in combination with the cytotoxic agent TMZ [185]. This result creates hope for a better response to more specific antiangiogenic drugs such as bevacizumab in combination with cytotoxic therapy.

MMP inhibitors. *In vitro*, several MMP-inhibitors (MPIs) have been shown to effectively downregulate glioma invasion [186]. The clinical development of MPIs was influenced by poor bioavailability of first-generation MPIs and significant musculoskeletal and inflammatory side effects caused by second-generation MPIs. Phase II trials struggled with the challenge to demonstrate efficacy in a new class of anticancer agents that does not exhibit cytotoxicity and therefore mandated establishment of novel endpoints. Phase III trials of combination therapy of MPIs with cytotoxic agents failed to demonstrate survival benefit [187] and some of these trials in patients with lung and pancreatic cancer showed poorer survival than patients treated with placebo [188].

EGFR inhibitors. In the 1980s, monoclonal antibodies (mAbs) against EGFR were introduced [189]. While these first-generation mAbs were of murine origin and thus immunogenic in humans, later generations were humanized. A prospective phase I/II trial in patients with recurrent malignant gliomas was not able to demonstrate therapeutic benefit [190] and a trial with intratumoral delivery of this mAb had to be terminated early due to a severe intra-cranial inflammatory reaction [191].

Tyrosine kinase inhibitors. A broad spectrum of inhibitors to receptor tyrosine kinases has been identified that prevents phosphorylation and activation of downstream signaling. Gefitinib is the best described receptor tyrosine kinase inhibitor and has been investigated in a phase II study in malignant glioma where no response was found [87].
Recently, co-expression of EGFRvIII and PTEN was retrospectively associated with clinical response to gefitinib or erlotinib treatment [192], underscoring the importance of biomarkers that have predictive value for patients' response to treatment.

Antiangiogenic treatments. Antiangiogenic therapies, designed to decrease blood supply to a tumor, have proven to be significantly successful in some untreatable cancers. Sorafenib (BAY 43-9006), a broad tyrosine kinase inhibitor that targets VEGF receptor/platelet-derived growth factor receptor in tumor vasculature has recently been shown to have an impact on renal cell carcinoma [193]. Avastin (bevacizumab) has been found to be a promising agent alone or in combination with chemotherapy in a variety of solid tumors [194]. Based on positive results from a preliminary study by Stark-Vance, bevacizumab is currently being tested in combination with irinotecan in a phase II study (NCT00268359) aimed at determining safety and progression-free survival under this regimen. Combination therapy of antiangiogenic agents with anti-invasive agents seems to be hopeful. However, we must be prudent, as *in vitro* work has suggested that VEGF antibodies increase invasiveness of human gliomas [180].

Perspectives

Despite better knowledge of the molecular changes underlying the tumor phenotype, the absence of improved survival for patients with malignant gliomas highlights the necessity to increase our understanding of the mechanism of glioma progression. A better understanding of the molecular biology of glioma invasion offers the hope of targeted therapy.
Currently, emerging techniques such as gene profiling by microarray and proteome analysis provide power-

ful approaches to identify new critical genes and to define a molecular classification based on homogenous clusters of tumors. The signaling events in motility and invasion of glioma cells are currently under intense investigation. Experimental evidence indicates that glioma invasion may be regulated by distinct and possibly independent molecular trigger mechanisms. Various candidate genes have been discussed in this review and many molecules have already proven to be associated with glioma invasion. New candidates must continue to appear. The next step should be identification of the main molecules which play a role in invasion and are promising as therapeutic targets on the candidate gene list. The answer to this question will advance the field of neuro-oncology. Downstream effector molecules of the invasion process may represent the most attractive treatment targets. Additionally, it has already become clear that motility shares common signaling pathways with proliferative and apoptotic events. A better understanding of these interdependent cellular programs will provide entry points that may allow modification of a cell's preference to invade versus a pro-apoptotic state. These approaches could specifically use mechanisms of invasion to sensitize cells to induction of cell death.

The target therapies have proven their efficacy in other cancers, such as leukemia, lung and breast cancer. This fact suggests a possibility that target therapies against invasion-related molecules may be efficacious for the glioma and prolong survival. However, such therapies should probably be used in synergistic association with either conventional treatments or other biologic therapies targeted to different critical molecular pathways, because malignant gliomas contain numerous molecular alterations.

Translational research is critical for the future of neuro-oncology. Investigators need to integrate translational research into future clinical trial design for gliomas whose treatment remains a huge challenge.

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