Acta Neuropathol (2007) 114:443–458
DOI 10.1007/s00401-007-0293-7

REVIEW

Diffuse glioma growth: a guerilla war

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Received: 20 June 2007 / Revised: 23 August 2007 / Accepted: 23 August 2007 / Published online: 6 September 2007

Abstract In contrast to almost all other brain tumors, diffuse gliomas infiltrate extensively in the neuropil. This growth pattern is a major factor in therapeutic failure. Diffuse infiltrative glioma cells show some similarities with guerilla warriors. Histopathologically, the tumor cells tend to invade individually or in small groups in between the dense network of neuronal and glial cell processes. Meanwhile, in large areas of diffuse gliomas the tumor cells abuse pre-existent "supply lines" for oxygen and nutrients rather than constructing their own. Radiological visualization of the invasive front of diffuse gliomas is difficult. Although the knowledge about migration of (tumor)cells is rapidly increasing, the exact molecular mechanisms underlying infiltration of glioma cells in the neuropil have not yet been elucidated. As the efficacy of conventional methods to fight diffuse infiltrative glioma cells is limited, a more targeted ("search & destroy") tactic may be needed for these tumors. Hopefully, the study of original human glioma tissue and of genotypically and phenotypically relevant glioma models will soon provide information about the Achilles heel of diffuse infiltrative glioma cells that can be used for more effective therapeutic strategies.

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Abbreviations

Cdc	Cell division cycle
CNS	Central nervous system
CT	Computerized tomography
CXCR4	Chemokine (C-X-C motif) receptor 4
ECM	Extracellular matrix
EGF(R)	Epidermal growth factor (receptor)
FAK	Focal adhesion kinase
GBM	Glioblastoma multiforme
HGF	Hepatocyte growth factor
HIF	Hypoxia inducible factor
MMP	Matrix metalloproteinase
MRI	Magnetic resonance imaging
NF-κB	Nuclear factor kappa B
NSC	Neural stem cell
PI3K	Phosphatidylinositol 3-kinase
PTEN	Protein phosphatase and tensin homolog
RGD	Arginine-glycine-aspartic acid
SDF-1	Stromal cell-derived factor-1
SF	Scatter factor
SPARC	Secreted protein acidic and rich in cystein
uPA(R)	Urokinase-type plasminogen activator (receptor)
VEGF	Vascular endothelial growth factor
WHO	World Health Organization

Introduction

Diffuse infiltrative gliomas are by far the most common primary brain tumors in adults, esp. its most malignant form,

glioblastoma multiforme (GBM) [31]. In contrast to almost all other brain tumors, such diffuse gliomas are characterized by extensive, diffuse infiltration of tumor cells in the neuropil, i.e., the dense network of interwoven neuronal and glial cell processes. Based on the resemblance of the tumor cells with non-neoplastic glial cells, most diffuse gliomas are histopathologically typed as astrocytic, oligodendroglial, or oligoastrocytic [105]. Partly because of their growth pattern, curative treatment for diffuse gliomas is generally impossible. Although patients with low-grade [World Health Organization (WHO) grade II] diffuse gliomas may survive for multiple years, these tumors lead to death of the patient sooner or later, often after progression to high-grade (WHO grade III or IV) malignancy. Esp. in older age groups, diffuse gliomas frequently present as high-grade malignant lesions and carry a grim prognosis from the start. A subset of gliomas (e.g., ependymomas, pilocytic astrocytomas) shows a more circumscribed than diffuse infiltrative growth pattern, these latter gliomas will not be further discussed.

In the present review, we will first focus on the pathology of diffuse infiltrative glioma growth and its consequences for radiological diagnosis of these tumors. We will then systematically review the molecular mechanisms and factors that underlie this growth pattern (without trying to be complete) and discuss the implications for different therapeutic approaches. In the last part of this review we will touch on the limitations of in vitro and in vivo models for the study of diffuse infiltrative glioma growth. For other excellent recent reviews on (some of) these aspects we refer the reader to [40, 57, 69, 70, 99, 137, 148, 172]. As diffuse infiltrative glioma cells show some similarities with guerilla warriors [130], we will use guerilla war as a metaphor for diffuse glioma growth throughout this manuscript in order to enhance the understanding of these tumors.

Pathology

Like guerilla warriors, in large areas of diffuse gliomas the tumor cells tend to invade individually or in small groups in "foreign" territory and to abuse pre-existent supply lines.

Diffuse infiltrative growth of tumor cells in the neuropil is almost unique for gliomas. Only very few non-glial tumors (esp. small cell lung carcinoma, lymphoma) occasionally display "pseudo-gliomatous" growth in the neuropil [12, 144, 189]. One of the pioneers in the study of glioma growth patterns is Hans-Joachim Scherer [129]. Scherer designated the arrangement of glioma cells that does not seem to depend on pre-existing tissue but can be considered as an expression of the intrinsic architectural potential of the tumor (e.g., canalicular structures, papillary formations) as "proper structures". Furthermore, he defined "secondary structures" as different patterns of arrangements of glioma cells that are considered to be dependent on pre-existing tissue elements. Examples of secondary structures are perineuronal growth (perineuronal satellitosis), surface (subpial) growth, perivascular growth, and intrafascicular growth. "Tertiary structures" were defined by Scherer as formations brought about by the interaction of glioma cells with proliferating mesenchymal tissue of the tumor [157]. In diffuse gliomas, the cells preferentially invade along myelinated fibers in white matter tracts (intrafascicular growth), and subpial, perivascular, and perineuronal accumulation of tumor cells is frequently encountered [57] (Fig. 1). The most extreme example of diffuse infiltrative glioma growth is represented by gliomatosis cerebri. According to the WHO-2007 classification, this neoplasm involves at least three cerebral lobes, usually bilaterally, and even the entire neuraxis may be involved [36, 105, 111].

Recognition of diffuse infiltrative versus other types of glial tumors has significant prognostic and therapeutic implications. While the diffuse infiltrative growth pattern is characteristic for both low- and high-grade diffuse gliomas, esp. high-grade gliomas frequently show marked phenotypical heterogeneity with spatial differences in cellular phenotype and malignancy grade. Since molecular genetic studies demonstrated a common origin in different components of such heterogeneous diffuse gliomas, these tumors are considered as clonal lesions [17, 105]. The exact growth pattern of gliomas can not always be assessed in biopsy specimens, but histopathological features like intrafascicular growth, perineuronal satellitosis, and subpial accumulation of tumor cells strongly favor a diffuse infiltrative nature of the glial neoplasm.

In gliomas of high-grade malignancy, florid (often glomeruloid) microvascular proliferation and necrosis emerge. These changes, which are in fact used as histopathological criteria to diagnose high-grade malignancy in these tumors [105], are often spatially and temporally related. While high-grade gliomas may focally show an extreme angiogenic response, quantitative studies revealed that the vascular density in many regions of both low- and high-grade diffuse gliomas and of gliomatosis cerebri is in the range of that for normal cerebral grey or white matter, indicating that in large areas of these tumors angiogenesis is lacking [19, 191, 192]. In these latter areas, the diffuse infiltrative glioma cells seem to behave like guerilla warriors that do not construct their own supply lines but incorporate ("co-opt") and abuse pre-existent ones. Around areas of necrosis in high-grade gliomas the tumor cells often show pseudopalisading. Such perinecrotic cells were demonstrated to be less proliferative and have a higher apoptosis rate

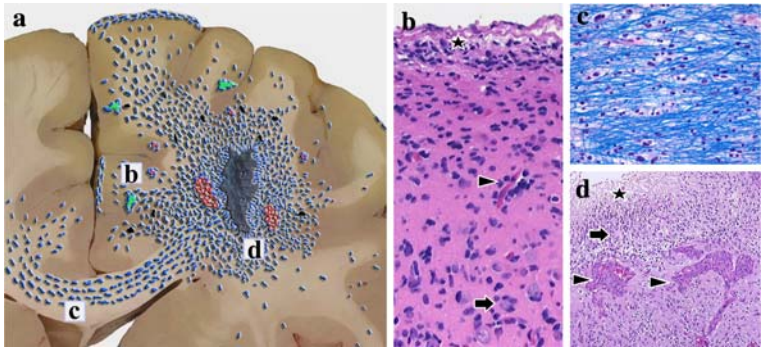

Fig. 1 Schematic representation of the growth pattern of a GBM (**a**), including the following secondary structures of Scherer: perivascular accumulation of tumor cells (example in area indicated by *b*; vessels in *red*, tumor cells in *blue*), perineuronal satellitosis (*b*; neurons in *green*), subpial growth of tumor cells (**b**), and intrafascicular growth in the corpus callosum (**c**). Mitotic tumor cells are depicted in *black*. Furthermore, in GBMs necrosis (*dark grey area*) surrounded by pseudopalisading tumor cells and adjacent florid/glomeruloid microvascular proliferation (**d**) are often present. Images **b–d** on the *right* represent the histology of these features: in **b** *asterisk* indicates subpial growth, *arrow* indicates perineuronal satellitosis, *arrowhead* indicates perivascular accumulation of tumor cells; image **c** shows increased cellularity with diffuse infiltration of tumor cells in the relatively well preserved myelinated tracts of the corpus callosum; in image **d** *asterisk* indicates area of necrosis, *arrow* indicates peri-necrotic pseudopalisading tumor cells, *arrowheads* indicate glomeruloid microvascular proliferation [**b**, **d**: H&E staining, **c**: combined Luxol Fast Blue and H&E staining; original magnification ×200 (**b**, **c**) and ×100 (**c**)]

than the tumor cells more distant from necrotic areas. The pseudopalisading cells also show increased expression of hypoxia inducible factor 1α (HIF-1α) and vascular endothelial growth factor (VEGF), two factors that play a crucial role in the induction of angiogenesis [22, 23, 49]. There is evidence that the accumulation of tumor cells in the pseudopalisading zone is the result of migration of tumor cells away from the necrotic area [23]. Furthermore, it has been hypothesized that in this context necrosis selects for tumor cells that are more aggressive and more resistant to different therapeutic modalities [138].

Glioma cells can disseminate via white matter tracts, cerebrospinal fluid pathways, or meninges and thus give rise to multifocal gliomas. It is important to note that glioma growth in the subarachnoid/leptomeningeal compartment in itself does not imply malignant progression [105]. Despite the different ways of spread inside the CNS, extraneural metastases of diffuse gliomas are extremely rare and generally occur only after craniotomy or shunting [172]. A post mortem study investigating whole brain sections underscored that multifocal GBMs can emerge in the background of a better differentiated astrocytic neoplasm [26]. Multiple gliomas can occur as synchronous (diagnosed at initial presentation) and metachronous (appearing some time after initial diagnosis) lesions [89]. Widely separated glioma lesions that can not be attributed to the pathways just mentioned are called multicentric gliomas [11, 149]. Only a small percentage of glioma patients (estimated by some authors as 2%) show multiple, seemingly independent lesions at initial presentation, most of these patients appear to have GBM [10, 11, 149].

Radiology

Like in a guerilla war, visualization of the invasive front of diffuse infiltrative gliomas is problematic.

Magnetic resonance imaging (MRI) is now the gold standard for defining brain tumor anatomy in a clinical setting [141]. Low-grade diffuse gliomas are typically hypointense lesions on T1-weighted MR images with limited edema and mass effect and lack of enhancement after the use of Gadolinium-DTPA [72]. On T2-weighted and FLAIR sequences low-grade diffuse gliomas are generally hyperintense. Discrimination of edema and infiltrating glioma is difficult using T1, T2, and FLAIR MR images. The lack of neovascularization and the apparently limited changes to the pre-existent, incorporated vessels explain the absence of contrast-enhancement in MRI examinations of these tumors [8]. As, according to the WHO-2007 classification [105], the main histopathological difference between WHO grade II and III diffuse astrocytic neoplasms is increased mitotic activity in the latter, it is not surprising that part of the non-enhancing diffuse gliomas are histopathologically diagnosed as high-grade lesions at the time of biopsy [8].

Compared to low-grade diffuse gliomas, high-grade tumors are often radiologically more heterogeneous and are accompanied by more severe edema. The occurrence of contrast-enhancement in diffuse gliomas generally signifies a more malignant biological behavior [8, 72, 141]. The extent of contrast-enhancement is influenced by the dosage of the contrast material [200]. The central area in "ring-enhancing" high-grade diffuse gliomas most often represents necrosis, while the enhancing rim contains vital

glioma tissue with microvascular changes including increased vascular permeability (Fig. 2). Some therapeutic interventions (e.g., surgical removal of glioma tissue, radiotherapy) may induce contrast-enhancement [166, 181]. Furthermore, it is important to note that contrast-enhancement in non-diffuse gliomas such as pilocytic astrocytomas does not implicate malignant progression.

Conventional radiological investigations tend to significantly underestimate the extent of diffuse infiltrative glioma growth. Correlation of whole brain histological sections of high-grade gliomas with computerized tomography (CT) scans revealed that tumor cells were present even outside the peritumoral areas of low density [27]. Compared with MRI, infiltrating glioma cells can be found beyond the hyperintensive region on T2-weighted images [43, 55]. As a consequence, radiological distinction between multifocal and multicentric gliomas can be challenging. Multifocal malignant progression in a diffuse glioma may radiologically result in multiple, seemingly independent, contrast-enhancing lesions (Fig. 2). One study reported that using MRI, CT, or both, only in 12 out of 26 patients with multiple foci of glioma at initial diagnosis various patterns of spread were evident or suggested (subarachnoid > intraventricular > direct brain penetration) [88].

New MR modalities may contribute to better radiological classification and delineation of glial brain tumors as well as assist in identification of the best spot for a biopsy [30]. With diffusion-weighted imaging (DWI) and a related approach called diffusion tensor imaging, differences in motility of water due to differences in cellularity, cell membrane permeability, intra- and extracellular diffusion, and tissue structure can be visualized. Theoretically, DWI can thus be used to image indirectly infiltration of glioma cells in normal brain tissue [30, 96]. Perfusion weighted imaging (PWI) is a technique which allows for quantitative assessment of the cerebral blood volume (CBV). With PWI vascularization and perfusion of gliomas can be measured [192]. The (relative) CBV correlates with both vascularization and malignancy grade as assessed by histology. As long as tumor infiltration is accompanied by changes in vascularization and perfusion, PWI may also indirectly visualize the presence of infiltrating glioma cells [3, 78, 173]. Proton MR Spectroscopy (MRS) allows for obtaining metabolic spectra from (brain) tissue. Such spectra can be obtained in a single voxel or in multiple voxels in two or three dimensions [9, 176]. These 2D and 3D approaches are also known as chemical shift imaging or MRS imaging (MRSI).

Several studies suggest that MRSI may be helpful for better delineation of diffuse gliomas [35, 55, 114, 132]. Combining different MR modalities (e.g., DWI, PWI, MRS) is expected to further improve these results [41, 135]. Up till now, a major drawback of most novel MR modalities is the limited spatial resolution: for conventional T1-weighted MRI at 3 T this resolution is about 0.5 mm × 0.5 mm × 0.5 mm, while DWI and PWI reach a

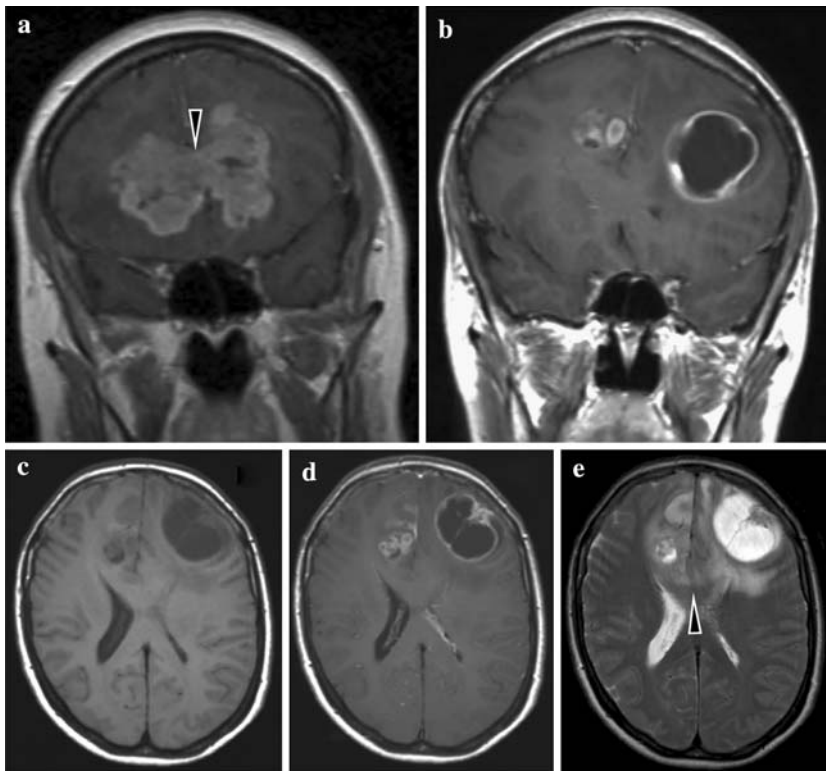

Fig. 2 Examples of MR images in two glioblastoma patients. In patient 1 (**a**), the T1-weighted image reveals bifrontal Gadolinium enhancement of a tumor that crosses the corpus callosum (*arrowhead*), resulting in a so called "butterfly glioma". In the second patient (**b**–**e**), the T1-weighted images with (**b**, **d**) and without Gadolinium (**c**) suggest multiple, independent lesions. In the T2-weighted image (**e**), however, these bifrontal lesions appear to be interconnected via the corpus callosum (*arrowhead*), indicating that in this latter area disruption of the blood-brain barrier by infiltrating glioma cells is (still) limited. **a**, **b**: coronal plane; **c**–**e**: axial plane

resolution of about 2 mm × 2 mm × 2 mm, and MRS of 10 mm × 10 mm × 10 mm. None of these new imaging techniques is expected to replace conventional MRI soon. Obviously, visualization of dispersed infiltrative glioma cells will improve when the technical development of these MR imaging modalities advances. Direct visualization of infiltrative glioma cells may also be performed by positron emission tomography (PET) and single photon emission CT (SPECT) imaging [16, 75, 106, 131]. Promising compounds for PET imaging of gliomas are O-(2-18F-fluoroethyl)-L-tyrosine [134] and 18F-Galacto-arginine-glycine-aspartic acid (RGD), an $\alpha v\beta 3$ binding molecule [71]. Furthermore, in the near future MR and PET imaging may be significantly improved by the application of nanoparticles [18, 82, 184] and labelled antibodies [61].

Molecular background

Like guerilla warriors, glioma cells posses specific qualities that allow for diffuse infiltration.

The diffuse infiltrative growth of glioma cells in the neuropil warrants specific, tightly regulated and converging interactions between these cells and their microenvironment. Up till now it is not known what exactly initiates this behavior of glioma cells. As the group of diffuse gliomas is genotypically heterogeneous, it is unlikely that one particular genetic aberration accounts for this growth pattern in all diffuse gliomas. Several studies suggest that gliomas are derived from neural stem cells (NSCs) or glial progenitor cells rather than from derailed mature glial cells [54, 162, 199]. CD133 (Prominin-1) is frequently used as a marker for identification of NSC features in glioma cells, but other markers such as nestin, CD90, CD44, CXCR4, musashi homolog 1 (Msi1), and maternal embryonic leucine zipper kinase are also used for this purpose [6, 102]. Interestingly, in in vivo and in vitro experiments CD133-positive glioma cells displayed a greater tumorigenic potential than CD133-negative cells, showed increased radio- and chemoresistance, and contributed in a major way to angiogenesis via VEGF production [6, 7, 102, 163].

During normal embryonic and fetal development of the CNS, extensive proliferation and migration of stem cells and progenitor cells is essential. In contrast, in the normal adult brain only in some locations (e.g., subventricular zone, hippocampus, dentate gyrus and sub-cortical white matter, rostral migratory system) some of these phenomena can still be present [104, 152]. Clues for elucidation of the molecular mechanisms enabling diffuse infiltrative glioma growth may thus be provided by the rapidly expanding research focussing on such stem cells and progenitor cells. Although the molecular biology underlying NSC migration is far from clear, molecules like nuclear factor kappa B (NF-κB), macrophage chemoattractant protein-1, stem cell factor, stromal cell-derived factor-1 (SDF-1), and platelet derived growth factor were demonstrated to play an important role in the regulation of this process (reviewed in [194]). For most of these factors, however, the role in glioma cell migration is not yet known.

For a more systematic discussion of the mechanisms and factors that are relevant for diffuse infiltration of glioma cells in the neuropil, a comparison with guerilla warriors may be helpful again. One would not only like to know what exactly initiates the migratory behavior of such warriors, but also which qualities and environmental factors enable them to successfully perform this behavior. With regard to these latter aspects, one could recognize (a) an internal system that coordinates input and output of signals, (b) a locomotor apparatus, (c) trails to travel on, (d) parts that directly interact with these trails, (e) tools to remove obstacles, (f) microenvironmental signals that guide the way, and (g) other stimulatory or permissive microenvironmental factors (Fig. 3). Before discussing these aspects in more detail it is important to realize that (the interactions of) these underlying mechanisms are complex, that the list of factors associated with glioma cell invasion/migration given below is not complete, and that information about the exact role of these factors is often obtained in glioma models that do not exactly mimic diffuse infiltration of glioma cells in the neuropil.

Intracellular integration of signals

Interactions of glioma cells with their microenvironment via membrane receptors (integrins, growth factor receptors) induces intracellular signals which are transmitted through effectors like the focal adhesion kinase (FAK) family of cytoplasmic, non-receptor tyrosine kinases and P311. The FAK family consists of two proteins, FAK and pyruvate kinase (Pyk) 2, which both play an important role in intracellular events such as proliferation, migration, survival, and apoptosis [117]. Glioma cells were reported to show increased expression of FAK, esp. at the invasive front [201]. FAK is activated by phosphorylation on critical tyrosine residues [161] and subsequently it phosphorylates cytoskeleton-associated substrates (e.g., Src, paxillin) [99]. While some studies suggest a role for FAK activation mainly in glioma cell proliferation [101], other studies show involvement in activation of Rac, which in turn leads to actin polymerization and formation of cell protrusions, focal adhesion, and subsequent motility [24, 142]. Pyk2 has a similar sequence and structure as FAK and can, upon activation by phosphorylation, interact with many of the same intracellular proteins as FAK [101]. P311 is a small polypeptide that was identified as migration-associated by

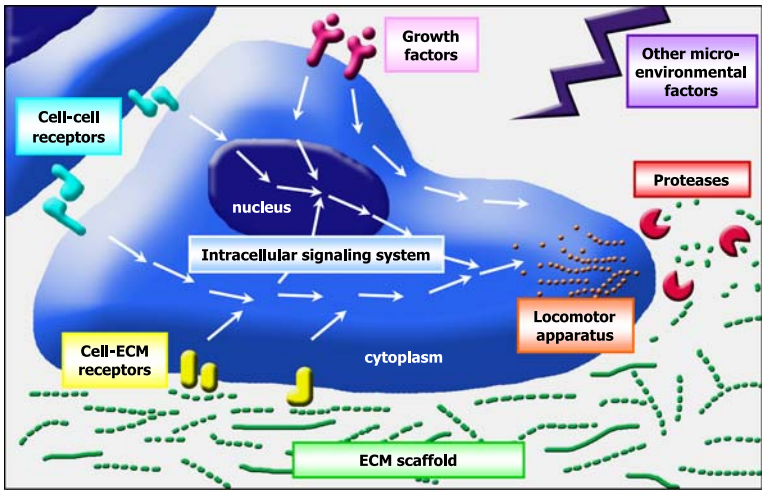

Fig. 3 Schematic overview of factors and mechanisms important for diffuse infiltration of glioma cells in the neuropil. As discussed in the section on the molecular background of diffuse infiltrative glioma growth, the following aspects relevant for this growth pattern can be recognized: (a) an intracellular system that coordinates all incoming and outgoing signals via a complex set of pathways, (b) a locomotor apparatus in which the actin cytoskeleton plays a crucial role, (c) a scaffold (ECM, surface of cells/cell processes) on which the glioma cells can travel, (d) cell–ECM and/or cell–cell receptors that allow direct interaction with the ECM and cellular microenvironment, (e) tools to remove obstacles like ECM degrading proteases, (f) growth factors that guide the way, and (g) other stimulatory or permissive microenvironmental factors (e.g., chemokines derived from inflammatory cells). In this scheme, the protrusion on the right side of the cell represents the lamellipodium at the front

comparing invasive human GBM cells with cells from the tumor core. The overexpressed P311 localizes to focal adhesions and promotes glioma cell migration via Rac1 activation [109, 113]. Transcription factors like FoxM1B and NF-κB also contribute to intracellular integration of signals [37, 103, 148]. FoxM1B was shown to be overexpressed in human GBMs, while in vitro and in vivo experiments revealed that this factor enhances glioma invasion by stimulation of matrix metalloproteinase 2 (MMP-2) transcription [37, 103]. The NF-κB level is elevated in actively migrating glioma cells in vitro and in vivo where it plays an important role in cell survival [148].

Actin cytoskeleton rearrangements

Cell migration requires dynamic remodeling of the actin cytoskeleton through assembly, disassembly, and organization of actin filaments into functional networks, which direct protrusion at the front of the cell and retraction at the rear. One of the first steps in cell migration is the formation of actin-rich structures, termed lamellipodia, at the leading edge of the motile cell [142]. These lamellipodia are broad, sheet-like protrusions containing short-branched actin filaments [133]. In addition to lamellipodia, more slender cytoplasmic protrusions containing bundels of cross-linked actin filaments (filopodia) can be formed [142]. Members of the Rho family of small GTP binding proteins, esp. Rac and cell division cycle protein Cdc42, are pivotal regulators of these processes. When bound to GTP, these proteins can interact with downstream target proteins, including protein kinases, phosphatases, and WASP/WAVE proteins (Wiskott-Aldrich Syndrome protein/Wiskott-Aldrich Syndrome protein family members). These latter proteins are activators of the Actin-related protein Arp2/3 complex, a nucleator of new actin filaments at the leading edge of the cell and thereby instrumental for protrusion of lamellipodia and filopodia [47, 124, 142]. Several studies showed that inhibition of Rac1, one of the three Rac isoforms, inhibits glioma cell migration and invasion in vitro [32, 34]. Depletion of the phosphoinositide phosphatase synaptojanin-2 (another effector of Rac1) using small interfering RNA was reported to inhibit glioma cell invasion through Matrigel and rat brain slices in vitro [34]. Interestingly, Rac is one of the downstream targets of phosphatidylinositol 3-kinase (PI3K), and the effect of PI3K [i.e., phosphorylation of PI-4,5-bisphosphate (PIP2)] is counteracted by the tumor suppressor protein phosphatase and tensin homolog (PTEN) [29]. By dephosphorylating PIP3, PTEN may inhibit glioma cell invasion in two ways: by modulation of glioma cell motility by inactivating Rac and Cdc42 as well as by suppression of extracellular matrix (ECM) degradation via MMPs [53]. As loss of chromosome 10q, which contains the PTEN gene (locus: 10q23.3), is a frequent event in esp. GBMs [42, 67], such loss may thus result in increased migration.

Scaffold for migration

In normal brain, common ECM components such as collagens, laminin, and fibronectin are essentially restricted to

the vessel walls and the perivascular and subpial glial limiting membrane [60]. The exact composition of the ECM in the neuropil is not yet fully elucidated, but hyaluronan, glycosaminoglycans, and proteoglycans are considered to be major ECM components in this compartment [13, 60, 63]. Due to the dense network of cell processes the volume of the extracellular space in the normal neuropil is limited. In diffuse gliomas, this space increases in volume, becomes more irregular, and abnormal ECM components accumulate in this space [204]. The fact that, in contrast to almost all other tumors, glioma cells have the capacity to diffusely infiltrate in the neuropil suggests that unique cell–ECM or cell–cell interactions are involved [179]. Glioma cells may create their own microenvironment by synthesizing and depositing ECM molecules such as vitronectin, tenascin-C, and laminin [21, 74, 84, 112, 128, 153, 180, 202, 204]. The glycoprotein vitronectin is preferentially expressed at the advancing margins of gliomas, and its expression level was described to correlate with glioma grade [180]. Additionally, vitronectin was reported to confer a survival advantage for tumor cells at the advancing tumor margin [180]. Increased expression of tenascin-C was described to correlate with higher malignancy grade [74] as well as to promote endothelial cell adhesion, spreading, and migration, which are critical steps in the process of angiogenesis [202]. Laminin deposits were found in the border zone between the normal brain and the migrating glioma cells in an orthotopic glioma animal model [112, 128]. Also in vitro, glioma cells express and secrete laminin. It was suggested that laminin production by glioma cells is stimulated by growth factors and gangliosides [84]. Other ECM components that show an increased expression in gliomas include osteopontin, secreted protein acidic and rich in cystein (SPARC), thrombospondin, and brain enriched hyaluronic acid binding protein [13]. Apart from ECM components, glioma cells may also use the surface of neighboring neuronal and glial cells (including myelin sheaths) as a scaffold for diffuse infiltration in the neuropil. Interestingly, myelin was reported to be one of the most permissive substrates for attachment and migration of glioma cells [58]. This phenomenon may at least partly explain the histopathological finding that glioma cells preferentially migrate in white matter tracts.

Cell–ECM and cell–cell interactions

Glioma cell migration requires dynamic expression of adhesion molecules, adequate positioning of these molecules, attachment to a relevant substrate, and detachment when the cell moves on. CD44 and integrins are considered to play a major role in glioma cell–ECM adhesion. CD44 is a hyaluronan receptor with a high expression in gliomas that was described to correlate with glioma grade [4, 13]. Engagement of CD44 with its ligand activates the small GTP binding protein Rac1, leading to actin cytoskeleton rearrangements and redistribution of CD44 to membrane ruffles. Proteolytic cleavage of CD44 by a disintegrin and metalloproteinase 10 produces an intramembranous cleavage product which acts as signal transduction molecule that in turn enhances invasion of glioma cells [120]. Integrins are a family of calcium-dependent, transmembrane molecules that mediate cell–ECM and cell–cell adhesion and consist of a non-covalently linked α and β subunit. ECM binding integrins bind esp. to the RGD sequence in the ECM components. Through the cytoplasmic domain of the β subunit, integrin activation can lead to activation of FAK, and of its intracellular signal transduction pathway [77, 142]. Subsequently, cytoskeletal rearrangements may occur and lead to cell movement [52]. Integrins that were described to be upregulated on glioma cells are $\alpha 3\beta 1$, $\alpha v\beta 1$, $\alpha v\beta 3$, $\alpha v\beta 5$, the two latter integrins being receptors for vitronectin. In addition, $\alpha v\beta 3$ can also bind to laminin, fibronectin, and tenascin-C [97]. The poliovirus receptor CD155/PVR, which is recruited to the leading edge of migrating cells where it co-localizes with actin and αv integrins and binds to vitronectin, was shown to be highly expressed in GBMs [94]. Expression of this adhesion molecule leads to increased FAK signaling and adhesion-induced activation of paxillin. Forced expression of CD155 in glioma cells resulted in increased dispersal of these cells in mice brains, while knock down of this receptor caused a decrease in migration of U87 cells in vitro [164, 165]. Other examples of adhesion molecules with a changed expression pattern in gliomas include adhesion molecule on glia/$\beta 2$ subunit of Na,K-ATPase (AMOG/$\beta 2$), ephrin receptor tyrosine kinases (EphB2-B3), fibroblast growth factor inducible 14 receptor (Fn14), and protein tyrosine phosphatases zeta/beta [50, 121, 122, 158, 178]. For cell–cell interactions in glioma migration cadherins and neural cell adhesion molecules (NCAM) may be important. Cadherins are calcium-dependent transmembrane cell–cell adhesion glycoproteins that form adherens junctions by homophilic interactions. Intracellularly, they link to the actin cytoskeleton via catenins (p120 catenin) [79]. Instability and disorganization of cadherin-mediated junctions lead to increased migration and invasiveness of glioma cells in vitro [5]. NCAM is a member of the glycoprotein immunoglobulin receptor superfamily and mediates strong interactions between cells via homophilic binding. The finding that expression of NCAM is inversely correlated with glioma grade suggests that loss of this adhesion molecule allows tumor cells to detach from neighboring (tumor and/or non-neoplastic) cells and to migrate into the brain parenchyma [125, 155]. Increased invasion of polysialylated C6 rat glioma cells into the murine corpus callosum may be explained by attenuation of homophilic NCAM interactions [174].

Proteases

In analogy with invasion of other cancer cells it is often hypothesized that glioma cells remodel their microenvironment by degrading the surrounding ECM to render it permissive for migration. Based on in vitro studies, several glioma derived proteolytic enzymes involved in cell migration were discovered, such as Cathepsin B; MMP-2 (synonym: Gelatinase A); MMP-9 (Gelatinase B); MMP-12; urokinase-type plasminogen activator (uPA) [20, 64, 137, 153, 193, 198]. These proteases are synthesized and secreted as inactive pro-enzymes and activated by proteolytic cleavage outside the cell. For some of these proteases a role in glioma invasion has been confirmed in in vivo studies [64, 90, 156], their expression being correlated with glioma grade and infiltrative capacity. The expression of these proteases is tightly regulated and can, for example, be activated by interaction of the glioma cell with the surrounding ECM. Several studies showed that the activation of ERK and Akt pathways stimulates secretion of MMP-2 and -9 [81, 187]. In an in vitro study, tenascin-C was reported to increase invasiveness of glioma cells through up-regulation of MMP-12 [153]. Overexpression of SPARC by glioma cells was described to cause increased expression of uPA, uPAR, MMP-2 and -9, which then leads to upregulation of PI3K and RhoA [85].

Growth factors and related signaling molecules

While in vitro studies revealed that Epidermal Growth Factor (EGF), basic Fibroblast Growth Factor (bFGF), and Transforming Growth Factor β significantly affect invasion of glioma cells (for review see [33]), many questions remain about the origin (tumor cells? inflammatory cells? pre-existent brain cells?) and exact role of such growth factors in vivo. Esp. GBM cells often show mutation or amplification of the EGF receptor gene and overexpression of this receptor on the cell surface [105, 123]. Other studies indicate that Scatter Factor/Hepatocyte Growth Factor (HGF) is important for glioma cell migration. HGF binds to the tyrosine kinase c-Met receptor, and both HGF and its receptor are frequently overexpressed in gliomas. HGF-binding to c-Met results in autophosphorylation of the receptor, subsequent activation of several signaling pathways (e.g., MAPK-, Jak/Stat-, PI3K-pathways), and various cellular reactions including migration [66]. Recently, it was shown that hypoxia-induced HIF-1α causes up-regulation of c-Met and thereby enhances the effect of HGF on glioma migration [44]. Insulin-like Growth Factor (IGF) binds with high affinity with IGF-Binding Protein 2 (IGFBP2), a soluble protein that is frequently overexpressed in high-grade gliomas. Overexpression of IGFBP2 results in upregulation of invasion related genes such as MMP-2 [186]. The Invasion Inhibitory Protein (IIp45) inhibits glioma invasion in vitro as well as in vivo in an orthotopic xenograft model by binding to IGFBP2 [168]. Iip45 was reported to be underexpressed in GBMs due to inactivation by tumor-specific alternative splicing [169]. The expression of the cell surface chemokine receptor CXCR4 is much higher in invasive than in non-invasive glioma cells [45]. Binding of its ligand, SDF-1/CXCL12, leads to activation of Akt and ERK1/2 signaling pathways and, subsequently, to increased survival, proliferation, and (via activation of proMMP-2) to increased invasion [145, 195]. Expression of the angiogenic factor angiopoetin-2 (Ang2) was found to be high in esp. the invasive areas of gliomas and to induce upregulation of MMP-2 in vivo and in vitro [68, 76, 83, 90].

Inflammatory cells and other factors

While high-grade malignant gliomas were described to contain large numbers of microglial cells and macrophages, lower numbers of microglial cells were found in low-grade diffuse gliomas [143]. These cells are able to produce cytokines and growth factors and may contribute to evasion of immune attack as well as stimulate tumor growth, but the exact effect of such inflammatory cells in gliomas is not known [188]. The findings that glioma patients show an increased number of immune-suppressive regulatory T-cells (not only in the tumor tissue, but also in peripheral blood) and that expression of MHC class I and II molecules is downregulated on invading glioma cells may explain that diffuse infiltrative glioma cells can evade an immunere-sponse (a phenomenon that has been called “stealth invasion of the brain”) [46, 202].

Therapy

Like for guerilla warriors, conventional methods to fight diffuse infiltrative glioma cells have limited effect or cause too much collateral damage, and a “search & destroy” tactic may be needed.

Conventional therapies

The fact that diffuse infiltrative glioma cells tend to blend in extensively in the brain microenvironment makes it hard to plan an effective counterattack. Whereas surgery of most other tumors aims at complete resection (with or without a margin of normal tissue), the diffuse growth of gliomas in the brain parenchyma precludes complete tumor removal. Already in the early days of neurosurgery, Dandy and Gardner noticed that even after performing a hemispherectomy glioma patients were not necessarily cured [38, 56].

Still, for patients with a high-grade malignant glioma maximal removal of the contrast-enhancing tissue without worsening neurological impairment is an independent prognostic factor for overall survival [73, 140]. Intraoperative assessment of the extent of resection by the neurosurgeon is, however, inaccurate [2, 170]. Also, although radiotherapy was proven to be beneficial for malignant glioma patients, eradicating diffuse infiltrative glioma cells by radiotherapy without significantly damaging the infiltrated brain parenchyma has been difficult to achieve [80, 93, 95]. Up till now limited field irradiation (generally with an arbitrary 2 cm beyond the contrast enhancing mass) rather than whole brain irradiation is the standard treatment [95]. The success of chemotherapy is hampered by the marked intratumoral heterogeneity of gliomas [140]. Esp. in areas where the original tissue architecture is relatively preserved, the blood-brain barrier may form an obstacle for optimal delivery of chemotherapeutics to diffuse infiltrative tumor cells. Patients with malignant oligodendroglial tumors [esp. those with loss of the short arm of chromosome 1 and of the long arm of chromosome 19 (-1p/-19q)] often show response to chemotherapy using alkylating agents [28, 182]. Recently, temozolomide treatment (concomitant and adjuvant with radiotherapy) was shown to result in modest improvement of median overall survival and increased 2 years survival in GBM patients up to 70 years of age [171]. However, up till now diffuse glioma patients are far from being cured by conventional therapies, and there is an urgent need for other therapeutic approaches [140].

"Anti-invasive" therapies

Interference with glioma cell motility may be exploited as a novel therapeutic approach [59]. We will now discuss some examples of experimental studies interfering with different aspects of glioma cell migration. Inhibition of FAK activation by TAE226 not only led to reduction of glioma cell adhesion, migration, and invasion through an artificial ECM, but also to reduced proliferation and enhanced apoptosis of these cells [161]. In an in vitro study, the Ras inhibitor S-trans, trans-farnesyl thiosalicylic acid was reported to reduce migration and anchorage-dependent proliferation of GBM cells by inhibiting PI3K signaling and Rac1 activity [62]. Application of the $\alpha v\beta 5$ integrin antagonist SJ749 not only reduced adhesion of glioma cells to fibronectin but also proliferation of these cells in vitro [107], while the $\alpha v\beta 3$ inhibitor IS20I exhibited strong anti-mitotic and anti-migratory effects in vitro and reduced glioma growth in vivo in subcutaneous and intracerebral glioma models [15]. In a recent phase I trial including 51 malignant glioma patients that were treated with the αv integrin inhibitor EMD 121974 (cyclo Arg-Gly-Asp-D-Phe-(N-methyl)-Val, a cyclic RGD pentapeptide) complete response was seen in two patients and partial response in three patients [196]. In preclinical trials using orthotopic U87 glioma lesions in nude mice, this inhibitor was described to induce anoikis (apoptosis supposedly induced by detachment from the ECM) in angiogenic blood vessels and brain tumor cells [175].

Application of the anti-tenascin antibody 81C6 has been studied in a phase II clinical trial. Injection of ^{131}I-m81C6 (44 Gy) in the surgically created resection cavity of patients with recurrent malignant glioma followed by standardized chemotherapy resulted in prolonged median survival [139]. Downregulation of SPARC in glioma cells using short interfering RNA decreased tumor cell survival and invasion in vitro by reducing phosphorylation of AKT, FAK, and integrin-linked kinase [160]. Downregulation of uPA, uPAR, and MMP-9 by RNA interference was reported to result in decreased invasion in both Matrigel and spheroid-assays in vitro, and in regression of orthotopic gliomas in nude mice [64]. The synthetic MMP inhibitors batimastat and marimastat reduced glioma invasion *in vitro* [177]. Local treatment of intracerebral glioma models in mice with an anti-c-Met antibody (OA-5D5) resulted in major growth inhibition of U87 lesions, but not of G55 lesions. As G55 tumors express c-Met but lack HGF expression, only gliomas where HGF drives tumor growth may thus respond to anti-c-Met-therapy [110]. EGFR, which is frequently overexpressed in GBMs, can be targeted with EGFR kinase inhibitors like gefitinib and erlotinib. Only a small number of the GBM patients that were treated with such inhibitors showed response (esp. those in which the glioma cells co-expressed EGFRvIII and PTEN) [115].

The tyrosine kinase inhibitors emodin and aloe emodin have been shown to induce anti-cancer effects in various tumor types. Emodin was reported to inhibit secretion of MMP-2 and -9 by glioma cells, invasion through a Matrigel coated chamber, phosphorylation of FAK, ERK1/2 and Akt/PKB, and inhibition of glioma invasion in vitro and in vivo [81, 116]. PEX, a fragment of MMP-2, is an endogenous inhibitor of angiogenesis, cell proliferation, and migration. The expression level in gliomas was described to be correlated with glioma grade and with expression of $\alpha v\beta 3$ integrin to which it is bound. One study showed that, while endogenous PEX expression was not sufficient to inhibit glioma growth, administration of PEX inhibited cell migration in vitro as well as angiogenesis and glioma cell proliferation in subcutaneous and intracranial human glioma xenografts [14].

Other therapeutic approaches

Driven by the failure of conventional therapeutic approaches for diffuse glioma patients, several other therapeutic strategies for these tumors are being developed or

already introduced in the clinic. Because of the striking microvascular changes in high-grade gliomas, these tumors have since long been considered as good candidates for anti-angiogenic therapy [51]. However, as in diffuse gliomas many intratumoral vessels may be incorporated rather than newly formed, the actual effect of anti-angiogenic therapy remains to be seen. Anti-VEGF therapy in experimental orthotopic GBM models resulted not only in reduction of vascular changes but also in increased vessel co-option by the tumor [86, 92].

Unravelling the stem cell aspects of gliomas may provide new targets for therapy [185]. Furthermore, as non-neoplastic NSCs were shown to be able to migrate toward and induce apoptosis of glioma cells, such stem cells may be used as vehicles to target infiltrating glioma cells ("search & destroy") [108, 197]. Further research now concentrates on how to optimally arm stem cells for this purpose. In orthotopic glioma models injection of NSCs transduced with the gene for cytosine deaminase led to a strong reduction of tumor burden [1]. Similarly, a promising result was obtained in a study in which orthotopic C6 rat glioma models were injected with NSCs transduced with herpes simplex virus-thymidine kinase gene (NSCtk) followed by systemic ganciclovir administration. The NSCs were shown to migrate actively from injection sites toward the C6 tumor cells in both the ipsi- and contralateral hemisphere and caused marked inhibition of tumor growth and increased survival [100].

With convection-enhanced delivery (CED), one or more small-caliber catheters are placed through a burr hole into the target tissue under image guidance, and an infusate is actively pumped into the brain parenchyma. This infusate will then disperse through the interstitial space [136, 150, 183]. Using CED, different therapeutics (e.g., chemotherapeutics, endotoxins, radioisotopes, chimeric products) may reach diffuse infiltrative glioma cells in the brain parenchyma [118, 119]. Although the pre-clinical results are promising [87, 126, 190], it is clear that the positioning of the catheter is crucial for the success of this approach, and that the distribution of the infusate should be closely monitored during treatment [151].

Glioma patients can be vaccinated with dendritic cells that are loaded with tumor-associated peptides. Ideally such dendritic cells then stimulate a cytotoxic T-cell response against the tumor. In gliomas, this approach is hampered by the large inter- and intratumoral antigenic heterogeneity and the lack of a universally expressed tumor antigen. Alternatively, dendritic cells can be loaded with a cell lysate derived from the patient's own glioma. Using this latter method, an overall prolonged median survival was found [39]. As an increased number of immune-suppressive, regulatory T cells was found in GBMs [46], interference with such cells also represents a potential target for immunotherapy of malignant gliomas [65]. Several studies investigated the potential of oncolytic viruses as vectors for gene therapy in the treatment of gliomas. Up till now, the success of virotherapy is limited, partly because of the host immune response which attenuates the distribution of the viruses and is difficult to control. Furthermore, as diffuse infiltrative glioma cells may show limited proliferative activity, and virus replication occurs preferentially in proliferating cells, the effect of virotherapy on the diffuse infiltrative part of gliomas remains to be elucidated [48, 159, 167].

Concluding remarks and future perspectives

The unique diffuse infiltrative growth of gliomas in the brain parenchyma has important diagnostic, prognostic, and therapeutic implications. While the understanding of different aspects of this growth pattern may be facilitated by using guerilla war as a metaphor, the analogy is of course not perfect. For instance, up till now it is unclear if and how the brain tissue "fights back". Furthermore, it is important to realize that the diffuse infiltrative growth pattern is not just the result of malignant progression as both low- and high-grade diffuse gliomas display this phenomenon. Esp. the high-grade lesions frequently show marked phenotypical heterogeneity with spatial differences in cellular phenotype and malignancy grade. Therefore, in a clinical setting combination of clinical, radiological, and pathological information is warranted to avoid diagnostic inaccuracy, particularly in cases where only small biopsy specimens are available for histopathological diagnosis.

Unravelling the mechanisms that allow glioma cells to diffusely infiltrate in the neuropil may provide novel therapeutic targets for recognizing, attacking, and killing these cells. Investigations using glioma models have already provided a wealth of information on the biological mechanisms responsible for glioma cell migration. However, many of these experiments were performed in in vitro and in vivo models that poorly recapitulate the glio ma cell-microenvironment interactions of human glioma cells in brain tissue [153]. Moreover, the exact culture conditions may have a major influence on the results obtained. For example, it was shown that cultured glioma cells may show a "mesenchymal drift" due to transdifferentiation [127], and that GBM cells cultured in vitro with FGF and EGF more closely mirror the phenotype and genotype of the original tumors than GBM cells cultured in serum [98].

Even the results obtained in orthotopic animal models for gliomas should be interpreted with caution, as such models not always mimic the genotype and/or phenotype of the original human gliomas. In fact such models often show a compact, expansive rather than diffuse infiltrative growth

pattern in the brain [86, 196]. Furthermore, it is important to realize that different mechanisms may underlie perivascular growth versus diffuse infiltrative growth of glioma cells in the neuropil, e.g., because conventional ECM components are absent in the latter compartment [146].

As has been suggested before [25], diffuse gliomas are unlikely to be cured by techniques that cannot selectively destroy the neoplastic cells. While interference with the mechanisms underlying diffuse glioma growth may be exploited as a novel therapeutic approach, up till now the crucial prerequisites for this growth pattern are far from clarified. Ideally, studies aiming at further elucidation of these mechanisms should be performed in original human glioma tissue and in (orthotopic) models that genotypically and phenotypically closely mimic the situation in human glioma patients [147, 154]. It is to be expected that esp. such studies will ultimately disclose the Achilles heel of the diffuse infiltrative glioma cells.

Acknowledgments We would like to thank Dr Mathé Prick, Department of Neurology, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands for providing the images of patients illustrating radiological aspects of glioma growth, and Sandra Boots-Sprenger, Department of Neurology and Radiology, RUNMC, for her excellent support in creating Figs. 1 and 3. AC and PW are supported by Dutch Cancer Society grant KUN 2003-2975. AJI is supported by EU the 6th framework grant LSH-2002-2.2.0-5 (eTUMOUR).

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