Astrocytic Tumors

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Abstract Astrocytic gliomas are the most common primary brain tumors and account for up to two thirds of all tumors of glial origin. In this review we outline the basic histological and epidemiological aspects of the different astrocytoma subtypes in adults. In addition, we summarize the key genetic alterations that have been attributed to astrocytoma pathogenesis and progression. Recent progress has been made by interpreting genetic alterations in a pathway-related context so that they can be directly targeted by the application of specific inhibitors. Also, the first steps have been taken in refining classical histopathological diagnosis by use of molecular predictive markers, for example, *MGMT* promoter hypermethylation in glioblastomas. Progress in this direction will be additionally accelerated by the employment of high-throughput profiling techniques, such as array-CGH and gene expression profiling. Finally, the tumor stem cell hypothesis has challenged our way of understanding astrocytoma biology by emphasizing intratumoral heterogeneity. Novel animal models will provide us with the opportunity to comprehensively study this multilayered disease and explore novel therapeutic approaches in vivo.

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1.1 Introduction

Astrocytic gliomas are the most common group of primary central nervous system (CNS) tumors and account for up to two thirds of all tumors of glial origin. The World Health Organization (WHO) classification of CNS tumors (Louis et al. 2007) recognizes a total of seven distinct histological entities of astrocytic neoplasms (Table 1.1). These may be roughly separated into two major groups: (1) the more common group of diffusely infiltrating astrocytic tumors, comprising diffuse astrocytoma, anaplastic astrocytoma, and glioblastoma multiforme, as well as (2) the less common group of astrocytic tumors exhibiting a more circumscribed growth, namely, pilocytic astrocytoma, pleomorphic xanthoastrocytoma (PXA), and subependymal giant cell astrocytoma. In the new WHO classification of 2007 (Louis et al. 2007), gliomatosis cerebri was added to the astrocytic tumor group because it is no longer considered as a distinct entity but as a variant of diffusely growing astrocytic glioma with unusually extensive infiltration of large parts of the brain and occasionally even the spinal cord. In addition, the pilomyxoid astrocytoma has been newly recognized as a histologically and clinically distinct variant of pilocytic astrocytoma with less favorable prognosis (Tihan et al. 1999). However, because pilocytic astrocytomas constitute the most common primary brain tumors in children,

Andreas von Deimling (Ed.), *Gliomas*, Recent Results in Cancer Research 171,
DOI: 10.1007/978-3-540-31206-2_1, © Springer-Verlag Berlin Heidelberg 2009

Table 1.1 Classification and grading of astrocytic tumors according to the WHO classification of tumors of the central nervous system

Tumor type	WHO grade
Diffusely infiltrating astrocytic gliomas	
Diffuse astrocytoma	II
Fibrillary astrocytoma	II
Protoplasmic astrocytoma	II
Gemistocytic astrocytoma	II
Anaplastic astrocytoma	III
Glioblastoma	IV
Giant cell glioblastoma	IV
Gliosarcoma	IV
Glioblastoma with oligodendroglial component	IV
Gliomatosis cerebri	(III)[a]
Astrocytic gliomas with more circumscribed growth	
Pilocytic astrocytoma	I
Pilomyxoid astrocytoma	II
Pleomorphic xanthoastrocytoma	II
Pleomorphic xanthoastrocytoma with anaplastic features	Not determined
Subependymal giant cell astrocytoma	I

[a]Gliomatosis cerebri usually corresponds to WHO grade III. However, in most instances, grading has to be performed on small biopsy samples, which in the classic (type 1) lesions without any solid focus may underestimate grade due to the sometimes rather low density of diffusely infiltrating tumor cells. In contrast, biopsies from the solid area of a type 2 lesion often corresponds to anaplastic astrocytoma (WHO grade III) or glioblastoma (WHO grade IV)

they will not be covered here but are described in detail in Chap. 4 on "Pediatric Gliomas."

Historically, the German pathologist Rudolf Virchow (1821–1902) was the first to separate the gliomas from other primary brain tumors, such as the meningiomas, the melanomas and so-called sarcomatous tumors of the CNS. The first systematic classification of gliomas according to defined histological criteria and their putative histogenetic origin dates back to the seminal publication of Bailey and Cushing in 1926 (Bailey and Cushing 1926). These authors also systematically related histological features to patients' outcome, thereby providing the first basis for brain tumor grading. Today, the astrocytic tumors, like all other CNS tumors, are worldwide uniformly diagnosed and graded according to the criteria of the WHO classification of CNS tumors, which were published in an updated form in 2007 (Louis et al. 2007).

1.2 Epidemiological, Neuroradiological, and Clinical Features

The incidence of astrocytic tumors tends to be highest in western countries. Caucasians are more frequently affected than people from African or Asian descent (Ohgaki and Kleihues 2005). The Central Brain Tumor Registry of the United States (CBTRUS 2005) indicates incidence rates of astrocytic gliomas being twice as high in whites as in blacks. In Japan, however, gliomas are only about half as frequent

as in the United States (Kuratsu et al. 2001). Males are slightly more commonly affected, with a male to female ratio of about 1.3:1. A potential increase in brain tumor incidence of about 1–2% per year has been reported, which primarily seems to affect the elderly population (Greig et al. 1990). However, it cannot be excluded that this increase may be mainly due to the refinement and widespread availability of modern neuroradiological imaging techniques, which have greatly facilitated tumor detection (Legler et al. 1999).

According to CBTRUS data, diffuse astrocytoma of WHO grade II has an annual incidence rate of 1.3/1 million population. The mean age of diagnosis is 46 years and the 5-year survival rate is about 45%. Anaplastic astrocytoma has a slightly later median age of onset of about 50 years. The annual incidence is about 4.9/1 million population and prognosis is significantly worse with a 5-year survival rate of only 28%. Glioblastoma manifests at a median age of 64 years with an annual incidence of about 3/100,000 population. Thus, glioblastoma is the most frequent primary brain tumor. It accounts for approximately 12–15% of all intracranial neoplasms and 50–60% of all astrocytic tumors. Despite aggressive multimodal treatment, the prognosis of glioblastoma is very poor, with nearly all patients succumbing to their disease within the first 2 years (2-year survival rate is 8.2%, 5-year survival rate 2.9%).

So-called long-term glioblastoma survivors are patients who survive for longer than 36 months after diagnosis. Although a number of studies have reported on such patients, it cannot be excluded that at least in some of the reports, long-term survival may be related to the inclusion of patients with malignant oligodendroglial tumors or pleomorphic xanthoastrocytoma (Kraus et al. 2000). A recent study of the German Glioma Network reported on the thus far largest series of 55 glioblastoma long-term survivors (Krex et al. 2007). While no specific clinical, socioeconomic, or molecular parameters could be directly linked to long-term survival, this study clearly indicated that young age at diagnosis, good initial clinical performance score, and the presence of *MGMT* promoter methylation in the tumor cells are favorable factors overrepresented in glioblastoma long-term survivors.

Diffusely infiltrating astrocytomas may arise in all parts of the brain. However, they are mostly observed in the cerebral hemispheres and most frequently affect the frontal and temporal lobes. Tumor infiltration can extend into the adjacent cortex, the basal ganglia, or even the contralateral hemisphere. A diffuse astrocytic glioma or, less commonly, a diffuse oligodendroglial or mixed glioma, that infiltrates the brain extensively, involving three or more lobes (frequently bilaterally), often extending to infratentorial structures and to the spinal cord is referred to as "gliomatosis cerebri."

On computed tomography (CT) scans, diffuse astrocytomas of WHO grade II present as ill-defined, homogeneous masses of low density. On magnetic resonance imaging (MRI), they are typically hypointense on T1-weighted and hyperintense on T2-weighted images. While gadolinium enhancement is not common in low-grade diffuse astrocytomas and generally suggestive of malignant transformation, at least partial contrast enhancement is commonly seen in WHO grade III lesions. However, a subset of anaplastic astrocytomas lack contrast enhancement on MRI, thus underlining the crucial importance of histology for the correct grading of these neoplasms. Glioblastoma typically presents with ring enhancement on neuroimaging, with the contrast enhancement corresponding to the vital and highly vascularized peripheral area of the neoplasm surrounding a central area of tumor necrosis that lacks enhancement.

Glioblastoma (WHO grade IV) may arise from diffuse astrocytoma (WHO grade II) and anaplastic astrocytoma (WHO grade III) through malignant progression. These tumors usually develop in younger patients (< 45 years of age) and are referred to as secondary glioblastoma.

Time of progression may range from less than 1 year to more than 10 years with a median time interval of about 4–5 years. Primary glioblastoma, which develops de novo without a history of a lower-grade lesion, accounts for the vast majority of cases. Patient age in these cases is significantly higher, with a median age of diagnosis around 55–60 years and a clinical history usually shorter than 3 months. In the past few years there has been increasing evidence that the terms "primary" and "secondary" glioblastoma do not only describe subgroups of patients with different clinical history but may even account for distinct disease entities that are associated with distinct patterns of genetic aberrations (Kleihues and Ohgaki 1999). However, prognosis seems to be equally poor in both cases and histological features do not differ between primary and secondary glioblastomas.

Clinically, patients with astrocytic gliomas often initially present with epileptic seizures. Focal clinical symptoms are variable and depend on tumor location. While for low-grade lesions neurological deficits may be subtle, higher-grade tumors may present with symptoms that appear more severe. Clinical history in these cases is often short and rapid tumor growth may coincide with perifocal edema causing mass shift and symptoms of increased intracranial pressure.

Pleomorphic xanthoastrocytoma (PXA) belongs to the group of astrocytic gliomas exhibiting a more circumscribed growth pattern and accounts for less than 1% of all astrocytic neoplasms. Histologically, the vast majority of PXA cases correspond to WHO grade II and patients demonstrate a relatively favorable prognosis (Giannini et al. 1999). However, rare cases show histological features of anaplasia, in particular increased mitotic activity and/or necrosis. Such tumors are referred to as "PXA with anaplastic features" and are more likely to demonstrate a less favorable outcome. On recurrence, some PXA may progress to high-grade gliomas, including glioblastoma multiforme, as demonstrated in several case reports (Giannini et al. 2007). While the tumor usually initially manifests within the first 2 decades of life, occasionally older patients are affected. PXAs are typically located superficially in the cerebral hemispheres, most often the temporal lobe, with frequent involvement of the leptomeninges. This is also the reason why patients often present with a long-standing history of seizures. In contrast to diffuse astrocytoma of WHO grade II, PXA radiologically presents as a contrast-enhancing, often cystic mass lesion.

Subependymal giant cell astrocytoma (SEGA) is closely associated with tuberous sclerosis, with an estimated 6–16% of tuberosis sclerosis patients developing one or more of these tumors. Neuroimaging typically demonstrates a contrast-enhancing intraventricular tumor, most often located in the region of the foramen of Monro. Obstructive hydrocephalus is a common feature. The most common clinical symptoms are either worsening of a preexisting epilepsy or symptoms of increased intracranial pressure. Patients diagnosed with a SEGA should be clinically checked for the presence of other manifestations of tuberous sclerosis, if not already known to have the syndrome.

1.3 Histopathology

Diffuse Astrocytoma. Histologically, diffuse astrocytomas are well-differentiated tumors lacking signs of anaplasia. They consist of neoplastic astrocytic cells embedded in a loosely structured, often microcystic fibrillary tumor matrix (Fig. 1.1d). According to the prevailing cell type, three major variants of diffuse astrocytoma are distinguished. The most common subtype is *fibrillary astrocytoma*, which is composed of multipolar tumor cells with scant cytoplasm and fine cell processes. Nuclear atypia may be present, distinguishing tumor cells from reactive astrocytes, but mitoses are generally rare or completely absent. Occasional or regional gemistocytic

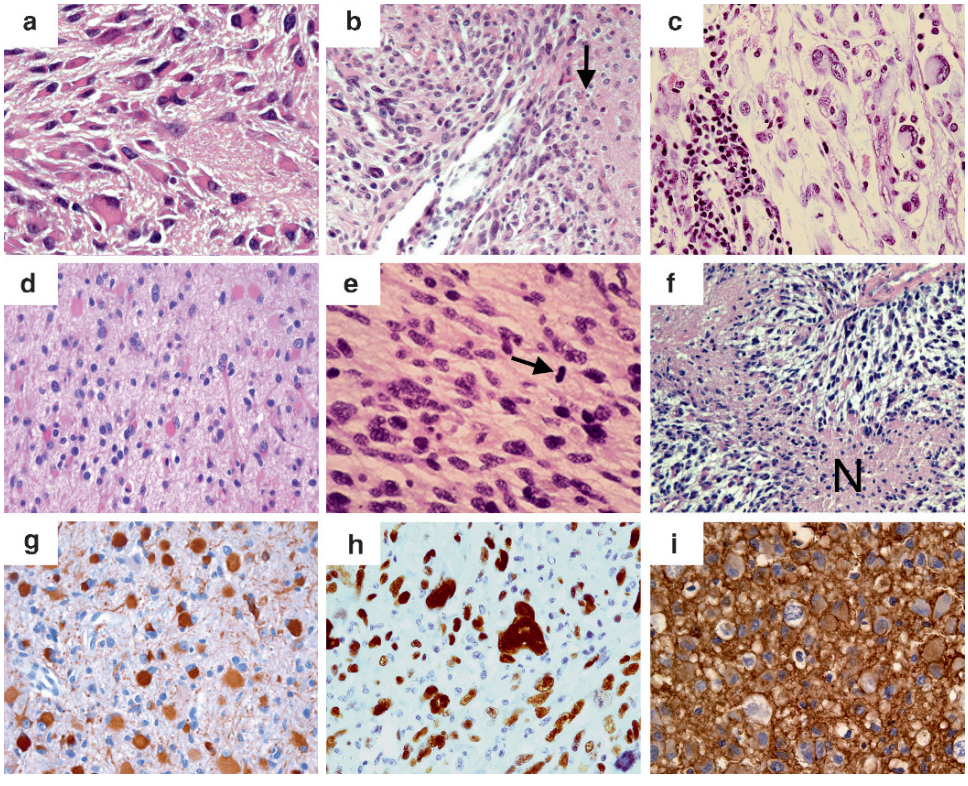

Fig. 1.1 Histological and immunohistochemical features of astrocytic tumors. (**a-c)** Astrocytomas with circumscribed growth. (**a)** Subependymal giant cell astrocytoma is composed of mainly large and plump, polygonal cells with abundant, glassy cytoplasm within a variably fibrillated matrix. (**b, c)** Cells in pleomorphic xanthoastrocytoma show nuclear and cytoplasmatic pleomorphism and xanthomatous change. Note the relatively sharp border between the tumor and the surrounding brain tissue (*arrow* in **b**) and reactive lymphocytic infiltration (**c**). (**d–f)** Diffusely infiltrating astrocytomas. (**d)** Diffuse astrocytoma of WHO grade II is a moderately cellular tumor composed of uniform fibrillary or gemistocytic astrocytic tumor cells with no signs of anaplasia. (**e)** Anaplastic astrocytoma shows increased cellularity, nuclear atypia, and mitotic activity (*arrow*). (**f)** Glioblastoma with the histological hallmarks of prominent microvascular proliferation and pseudopalisading necrosis (*N*). (**g–i)** Typical immunohistochemical features in diffusely infiltrating astrocytomas: Staining of gemistocytic astrocytoma cells for the glial fibrillary acid protein (GFAP, **g**), strong nuclear positivity for the p53 tumor suppressor protein in a giant cell glioblastoma (**h**) and overexpression of the epidermal growth factor receptor (EGFR) in a case of glioblastoma (**i**)

astrocytes can be observed in fibrillary astrocytoma. These cells exhibit a characteristically large eosinophilic cytoplasm with eccentric nuclei and strong immunohistochemical expression of glial fibrillary acid protein (GFAP; Fig. 1.1g). Tumors consisting of more than 20% gemistocytic astrocytes are classified as *gemistocytic astrocytoma*. Several reports indicate that gemistocytic tumor cell differentiation is a prognostically unfavorable feature as these tumors tend to undergo malignant progression more rapidly (Schiffer et al. 1988; Peraud et al.

1998). A rare astrocytoma variant is *protoplasmatic astrocytoma*. In these cases, neoplastic astrocytes exhibit a small cell body with few, flaccid cell processes and only weak GFAP expression. Mucoid degeneration or formation of microcysts is commonly observed.

Due to their high degree of cellular differentiation, diffuse astrocytomas are referred to as low-grade lesions and correspond to WHO grade II. However, they have an inevitable tendency for recurrence and malignant progression to anaplastic astrocytoma and, finally, secondary glioblastoma.

Anaplastic Astrocytoma. Basic histological features and tumor cell types are similar to those described for diffuse astrocytomas. However, anaplastic astrocytoma is characterized by a higher degree of nuclear pleomorphism, increased cellularity, and an elevated mitotic activity (Fig. 1.1e). Hypercellularity may be regional. Occasional multinucleated tumor cells and atypical mitoses may be observed. Necrosis is generally absent and, if present, demands for the diagnosis of glioblastoma. Small tumor vessels are still lined with a single, flat layer of endothelial cells. However, the beginning of microvascular proliferation may be observed, but is still limited to occasional tumor vessels and not glomerulum- or garland-like, as observed in glioblastoma. Anaplastic astrocytomas correspond to WHO grade III and tend to recur and progress to secondary glioblastoma.

Glioblastoma. Key histological features that distinguish glioblastomas from lower-grade astrocytic lesions are the presence of prominent microvascular proliferation and necroses (Fig. 1.1f). Pathological vessels are most commonly found around necrotic areas and exhibit a typical glomerulum- or garland-like appearance. In addition, vascular thrombosis is frequently observed and may contribute to the formation of ischemic tumor necrosis. Necroses can either appear as large areas of destroyed tumor tissue or can manifest in small, band-like foci surrounded by radially orientated, densely packed tumor cells in a "pseudopalisading" pattern.

The tumor cells in glioblastoma are highly pleomorphic, including relatively well-differentiated fibrillary or gemistocytic astrocytes, spindle cells, small cells with pathologic nuclear/cytoplasmic ratio, as well as multinucleated giant cells. Mitotic activity is high and atypical mitoses may be numerous. Metaplastic changes are occasionally present leading to epithelial differentiation (so-called glioblastoma with epithelial differentiation or adenoid glioblastoma) or formation of bone or cartilage. Further uncommon differentiation patterns include the presence of numerous PAS-positive granular tumor cells ("granular cell glioblastoma") or prominent tumor cells with lipidization ("heavily lipidized glioblastoma"). Occasional glioblastomas are composed of a monomorphic population of small anaplastic cells with sparse cytoplasm and round or carrot-shaped hyperchromatic nuclei (Miller and Perry 2007). These *small cell glioblastomas* should be distinguished from highly anaplastic oligodendrogliomas and cerebral primitive neuroectodermal tumors.

Gliosarcoma comprises up to 2% of all glioblastomas and displays a biphasic pattern with both glial and mesenchymal (sarcomatous) differentiation. Interestingly, molecular genetic studies clearly demonstrated that both tumor components are of monoclonal origin (Actor et al. 2002).

Another variant is *giant cell glioblastoma*, which accounts for less than 5% of all glioblastomas. While multinucleated giant cells may appear in a high fraction of classic glioblastomas, they dominate the histological picture in the giant cell variant. The giant cells are extremely bizarre and the number of nuclei may reach up to more than 20. In some cases, the cells are embedded in a reticulin fiber-rich matrix. Giant cell glioblastomas frequently show a more circumscribed growth, which may contribute to their somewhat better prognosis. Interestingly, giant cell glioblastoma, though clinically manifesting as a primary glioblastoma, shares a high incidence of *TP53* muta-

tions with secondary glioblastoma and frequent *PTEN* mutation with primary glioblastoma (Meyer-Puttlitz et al. 1997; Peraud et al. 1999; Fig. 1.1h).

A fraction of glioblastomas present histological features associated with oligodendroglial differentiation. These cases are referred to as *glioblastoma with oligodendroglial component*. There is evidence that these tumors clinically behave better than classic glioblastoma but worse than anaplastic oligodendroglioma and anaplastic oligoastrocytoma without necrosis (Miller et al. 2006).

Gliomatosis Cerebri. Gliomatosis cerebri is rare and preferentially develops in adults with an age peak between 40 and 50 years. The diagnosis of gliomatosis cerebri is established by a tissue biopsy combined with neuroimaging findings, which demonstrate extensive tumor growth involving three or more cerebral lobes, frequently bilateral tumor spread, and infiltration of the basal ganglia, brain stem structures, cerebellum, and sometimes even spinal cord. The biopsy specimens show an infiltrating glioma typically composed of monomorphic, often elongated tumor cells that grow diffusely in the brain parenchyma. The vast majority of cases exhibit astrocytic features, while cases of oligodendroglial or mixed oligoastrocytic gliomatosis have also been reported. The formation of so-called secondary structures, such as perineuronal satellitoses as well as perivascular and subpial aggregates, is often seen in cortical infiltration areas. The classic form of gliomatosis cerebri (type I) presents without any solid mass lesion and does not show marked microvascular proliferation and necrosis. In contrast, type II lesions are characterized by the presence of a focal mass lesion, most frequently corresponding to anaplastic astrocytoma or glioblastoma, in addition to the diffusely infiltrating areas of gliomatosis.

Pleomorphic Xanthoastrocytoma. Histologically, pleomorphic xanthoastrocytomas are relatively compact and well-circumscribed tumors growing in the cerebral cortex and invading the meninges. The adjacent cortex often shows dysplastic features. The tumors are composed of pleomorphic astrocytic tumor cells, including bipolar spindle cells growing in fascicles, epithelioid cells, as well as multinucleated giant cells, with variable subsets of the neoplastic cells displaying cytoplasmatic lipidization (Fig. 1.1b, c). A pericellular or perilobular reticulin network, eosinophilic protein droplets, and prominent lymphocytic infiltrates are further characteristic features. Rare histologic variants include tumors with angiomatous, epithelioid, or gangliocytic components. While the vast majority of pleomorphic xanthoastrocytomas correspond to WHO grade II, a few cases exhibit five or more mitoses per ten HPF (microscopic high-power fields) and/or necrosis. These tumors are designated *pleomorphic xanthoastrocytoma with anaplastic features* (Giannini et al. 1999). No definite WHO grade has been assigned to these rare cases, but compared to classic cases their prognosis appears less favorable.

Subependymal Giant Cell Astrocytoma. Histology reveals a circumscribed, moderately cellular tumor composed of pleomorphic large astrocytic cells with abundant glassy eosinophilic cytoplasm, round ganglioid nuclei, and distinct nucleoli (Fig. 1.1a). Smaller spindle cells growing in streams as well as calcifications are also commonly encountered. Mitoses are usually absent or rare. Subependymal giant cell astrocytoma corresponds to WHO grade I. Occasionally, increased mitotic activity and/or necrosis can be noted, but are not necessarily linked to malignant behavior.

1.4 Immunohistochemistry

Astrocytic tumors stain generally positive for a couple of more or less lineage-specific markers, with glial fibrillary acid protein

(GFAP) being the diagnostically most relevant marker. Expression of vimentin, S-100 protein, microtubule-associated protein 2 (MAP2), and alpha B-crystallin is also commonly observed in astrocytic tumors; however, these antigens are also expressed in most other glial tumors and many non-glial neoplasms. The fraction of GFAP-positive tumor cells varies considerably from case to case. As mentioned before, protoplasmatic astrocytomas are only weakly GFAP-positive, while the fibrillary and gemistocytic astrocytoma variants show more consistent and stronger GFAP staining (Fig. 1.1g). In gemistocytic astrocytes, GFAP immunoreactivity is often accentuated in the subplasmalemmal region due to the intracytoplasmatic distribution of intermediate filaments. With increasing malignancy, GFAP immunoreactivity may become weaker or even get completely lost, as in small anaplastic glioma cells. In gliomatosis cerebri, the infiltrating tumor cells may also be GFAP-negative. Thus, differential diagnosis may become challenging in such cases or in gliomas displaying metaplastic changes. For example, adenoid glioblastomas occasionally exhibit expression of epithelial markers, such as cytokeratins, and thus have to be distinguished from intracerebral metastases. Also, rare cases of gliosarcomas primarily may appear as spindle-cell sarcomas, with glial differentiation difficult to detect even by means of immunohistochemistry.

Apart from the expression of glial lineage markers, several transformation-associated proteins are expressed in astrocytomas. For example, nuclear positivity for the p53 tumor suppressor protein is present in about 60% of WHO grade II and III astrocytomas, but absent in pilocytic astrocytomas, subependymal giant cell astrocytomas, and the vast majority of pleomorphic xanthoastrocytomas. In glioblastomas, p53 immunoreactivity is also commonly observed in secondary glioblastomas and giant cell glioblastomas (up to 80%; Fig. 1.1h), while primary glioblastomas only stain positive in about 30% of cases. In contrast, immunoreactivity for the epidermal growth factor receptor (EGFR) is a common feature in primary glioblastomas (about 60% of the cases; Fig. 1.1i) and rare in secondary glioblastomas as well as other astrocytic neoplasms (Kordek et al. 1995). In pleomorphic xanthoastrocytomas, expression of the CD34 antigen is often found not only in vascular endothelial cells but also in tumor cells (Reifenberger et al. 2003). Subependymal giant cell astrocytomas show variable expression of GFAP and S-100. In addition, immunoreactivity for neuronal markers such as synaptophysin or neurofilaments may be detectable.

Labeling indices for the proliferation-associated antigen Ki-67 (MIB-1) differ considerably from tumor to tumor, and mean values determined for the individual WHO grades have large overlap. However, in diffuse astrocytic gliomas a threshold value of more than 5% is often used as an additional criterion to distinguish between WHO grade II and III lesions. The Ki-67 index in pleomorphic xanthoastrocytomas and subependymal giant cell astrocytomas usually does not exceed the 5% level.

1.5 Molecular Genetics

Diffuse Astrocytoma. The *TP53* tumor suppressor gene at 17q13.1 is mutated in about 60% of diffuse astrocytomas (Ichimura et al. 2000). Even higher frequencies of up to 80% of *TP53* mutations are detectable in the gemistocytic variant (Watanabe et al. 1998). Interestingly, *TP53* mutations in most cases are already present in the first biopsy and their frequency does not increase in recurrences, suggesting that *TP53* mutations are among the earliest events in astrocytoma development (Fig. 1.2). This hypothesis is supported by the fact that brain tumors in patients harboring a *TP53* germline mutation predominantly correspond to astro-

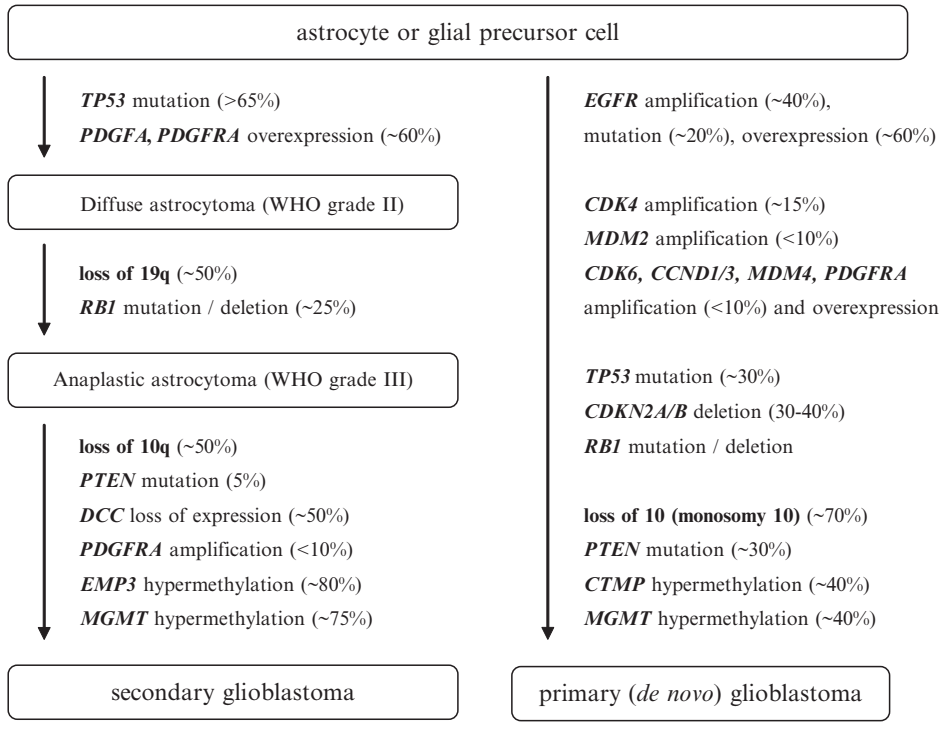

Fig. 1.2 Schematic representation of the molecular pathogenesis of primary and secondary glioblastomas (According to Ohgaki and Kleihues 2007 with modifications)

cytic tumors, usually anaplastic astrocytoma or glioblastoma. In line with Knudson's double-hit hypothesis, *TP53* mutations in diffuse astrocytomas are commonly associated with loss of heterozygosity (LOH) at polymorphic loci on 17p resulting in complete loss of wild-type p53 in the tumor cells. Diffuse astrocytomas without *TP53* alterations frequently exhibit promoter methylation and loss of expression of the $p14^{ARF}$ gene at 9p21, the gene product of which regulates MDM2-mediated degradation of p53 (Watanabe et al. 2007). Other genes that have been reported to be epigenetically silenced in more than 50% of diffuse astrocytomas include the *MGMT* gene at 10q26 (Watanabe et al. 2007), the protocadherin-gamma subfamily A11 (*PCDH-gamma-A11*) gene at 5q31 (Waha et al. 2005), and the *EMP3* gene at 19q13 (Kunitz et al. 2007). Interestingly, *MGMT* hypermethylation was found to be associated with *TP53* mutation but is mutually exclusive to $p14^{ARF}$ hypermethylation (Watanabe et al. 2007).

Another common alteration in diffuse astrocytomas is overexpression of the platelet-derived growth factor receptor alpha (PDGFRA) and its ligand PDGFalpha, thereby enabling an autocrine growth stimulation of the tumor cells (Hermanson et al. 1992). *PDGFRA* amplification, however, is restricted to a small subset of high-grade gliomas, in particular glioblastomas (Fleming et al. 1992).

Karyotyping and comparative genomic hybridization analyses revealed trisomy 7 or gains of chromosome 7q as a common genomic imbalance, which is detectable in up to 50% of diffuse astrocytomas. Further chromosomal aberrations

comprise losses on 22q, 19q, 13q, 10p, 6, and the sex chromosomes as well as gains on 5p, 9, and 19p (for review, see Reifenberger and Collins 2004). In contrast to oligodendrogliomas, combined losses on 1p and 19q are rare in diffuse astrocytomas.

Anaplastic Astrocytoma. Anaplastic astrocytomas share a similarly high frequency of gains on chromosome 7, allelic losses on 17p, and *TP53* mutations with diffuse astrocytomas. In addition, anaplastic astrocytomas often carry deletions on chromosomes 6, 9p, 11p, 19q, and 22q. The deletions on 9p preferentially target the cell-cycle regulatory genes *CDKN2A, p14^{ARF}*, and *CDKN2B* at 9p21. Inactivation of *p14^{ARF}* serves as an alternative means to impair the p53 pathway in cases without *TP53* mutations (Ichimura et al. 2000). Deletions or mutations of *CDKN2A* (coding for p16^{INK4a}) and *CDKN2B* (coding for p15^{INK4b}), the gene products of which function as inhibitors of complexes between D-type cyclins and the cyclin-dependent kinases CDK4 and CDK6, alter cell cycle regulation at the G_1/S-phase transition by aberrantly activating the retinoblastoma (pRB) pathway. Up to 20% of anaplastic astrocytomas carry homozygous deletions involving *CDKN2A* and *CDKN2B*, while amplification and overexpression of the *CDK4* gene at 12q13-q14 is present in up to 10% of cases (Reifenberger et al. 1994). Furthermore, about 25% of anaplastic astrocytomas have mutations in the retinoblastoma gene (Ichimura et al. 1996). In contrast to glioblastomas, *EGFR* amplification is only rarely observed in anaplastic astrocytomas (< 10% of cases). Similarly, mutations in the *PTEN* tumor suppressor gene at 10q23 are rare. If present, however, *PTEN* mutations are associated with poor prognosis (Smith et al. 2001).

Glioblastoma. Glioblastomas are characterized by complex chromosomal, genetic, and epigenetic changes affecting a variety of tumor suppressor genes and proto-oncogenes. The most common chromosomal aberrations detected by conventional karyotyping are monosomy 10, trisomy 7 and, in about 50% of the cases, "double minute chromosomes" or "homogenously staining regions," which are cytogenetic correlates of gene amplification (Bigner and Vogelstein 1990).

The concept of *primary glioblastoma* (glioblastoma arising de novo) and *secondary glioblastoma* (glioblastoma arising from a lower-grade precursor lesion) has shed light on different genetic pathways in glioblastoma formation (Ohgaki and Kleihues 2007; Fig. 1.2). The more common primary glioblastomas bear frequent *EGFR* amplification as well as *PTEN* tumor suppressor gene mutations. *TP53* mutations are found in only 30% of the cases; however, *p14^{ARF}* alterations as well as *MDM2* or *MDM4* amplification can serve as alternative means to bypass p53-regulated growth control in primary glioblastoma. Secondary glioblastomas carry *TP53* mutations in more than 60% of cases, while *EGFR* and *MDM2* or *MDM4* amplification as well as *PTEN* mutations are rare. Allelic losses on 19q and 13q, promoter hypermethylation of the *RB1* gene, and overexpression of *PDGFRA* are more common in secondary than in primary glioblastoma (Ohgaki and Kleihues 2007). In addition, epigenetic silencing of various genes, including *MGMT* and *EMP3*, is more common in secondary than in primary glioblastomas. Collectively, these data suggest that primary and secondary glioblastomas constitute genetically different disease entities (Fig. 1.2). However, as indicated before, both entities share comparable histological features and an equally poor prognosis. The fact that the different alterations eventually target the same cellular pathways, namely, the p53, pRb1, PTEN/PI3K/AKT, and mitogen-activated protein kinase pathways, and thereby lead to the similar functional aberrations, may explain this phenomenon (Reifenberger and Collins 2004).

Giant cell glioblastomas clinically manifest as primary glioblastomas but share features of both primary and secondary glioblastoma. While they carry *PTEN* mutations in the same frequency as primary glioblastomas (about 30–40%), *TP53* mutations are detectable in up

to 90% cases and *EGFR* amplification and/or overexpression is usually absent (Meyer-Puttlitz et al. 1997; Peraud et al. 1999; Fig. 1.1h).

The molecular genetics of *gliosarcoma* is fairly similar to that of primary glioblastomas, except for *EGFR* amplification, which seems to be less frequent (Reis et al. 2000). Microdissection of the gliomatous and sarcomatous tumor components followed by CGH analysis revealed common genetic aberrations in both components, thus arguing for a monoclonal origin of both components (Actor et al. 2002).

Combined deletions of 1p and 19q, i.e., the characteristic genomic aberration in oligodendroglial tumors, are rare in glioblastomas (less than 10% of the cases), and not overrepresented in glioblastomas from long-term survivors (Krex et al. 2007). However, some studies reported that 1p/19q deletion may be more common in glioblastomas with an oligodendroglial component, which in part may account for the better survival associated with this particular glioblastoma subgroup (Miller et al. 2006).

Gliomatosis Cerebri. Molecular studies on gliomatosis cerebri have identified *TP53* mutations in 11–44% of the cases (Herrlinger et al. 2002; Mawrin 2005). Individual tumors demonstrated a *PTEN* mutation and/or *EGFR* amplification, while *CDK4* or *MDM2* amplifications as well as homozygous *CDKN2A* deletions were not detected. In line with a monoclonal tumor origin, molecular analysis of tissue samples from multiple spatially distinct regions in gliomatosis cerebri revealed identical *TP53* mutations (Kros et al. 2002). However, specific molecular changes driving the widespread tumor infiltration in gliomatosis cerebri remain to be uncovered.

Pleomorphic Xanthoastrocytoma. Loss on chromosome 9 is the most common genomic imbalance in pleomorphic xanthoastrocytoma, which is detectable by CGH analysis in 50% of cases. Other losses affect chromosomes 17 (10%), 8, 18, and 22 (4% each). Chromosomal gains could be identified on chromosomes X (16%), 7, 9q, 20 (8% each), 4, 5, and 19 (4% each) (Weber et al. 2006). *TP53* mutations are seen in a small fraction of tumors (< 10% of cases; Giannini et al. 2001; Kaulich et al. 2002). A recent study reports on frequent homozygous deletions of the tumor suppressor genes *CDKN2A, p14*^{ARF}, and *CDKN2B* on 9p21.3. Interestingly, transcript levels of the *TSC1* gene on 9q were also found to be consistently low in PXAs; however, the causative mechanism still remains unclear, as there was no evidence for *TSC1* mutations or promoter methylation (Weber et al. 2006).

Subependymal Giant Cell Astrocytoma. Biallelic inactivation of either the *TSC1* or the *TSC2* tumor suppressor gene is typical for these tumors (Chan et al. 2004). Since the corresponding gene products have an inhibitory function on the mTOR pathway, their mutational inactivation leads to aberrant activation of mTOR signaling, which in turn represents an interesting novel target for specific pharmacologic inhibition. A comparative genomic hybridization study on subependymal giant cell astrocytomas indicated that chromosomal imbalances are rare or absent (Rickert and Paulus 2002).

1.6 Molecular Diagnostics

Correct histopathological diagnosis is of utmost importance for evaluating prognosis and providing patients with an adequate therapeutic regimen. However, when merely employing conventional histology and immunohistochemistry many astrocytoma cases are diagnostically challenging. In addition, it is well known that survival of individual glioma patients may vary considerably within each histological group, even after adjustment for relevant prognostic factors such as age, performance score, and extent of resection. This points to a role of tumor-inherent molecular factors in the response to therapy and eventually survival. However, despite the identification of numerous chromosomal and genetic aberrations in the different types of astrocytic gliomas, the molecular diagnostics of these tumors is just beginning to

become of clinical relevance. Particularly in regard to the prediction of chemosensitivity to alkylating drugs commonly used to treat malignant gliomas, such as the nitrosoureas and temozolomide, considerable progress has been made in the last few years. The *MGMT* (O^6-methylguanine–DNA methyltransferase) gene on chromosome 10q26 encodes a DNA repair protein that removes alkyl groups from the O^6 position of guanine, an important site of DNA alkylation (Gerson 2004). Chemotherapy-induced alkylation in this location triggers cytotoxicity and apoptosis. High levels of the MGMT repair protein thus may counteract the therapeutic effect of alkylating agents and thereby lead to treatment failure. Epigenetic silencing of *MGMT* by means of promoter hypermethylation has been identified as the main mechanism reducing MGMT expression and thereby diminishing its DNA repair activity. Importantly, *MGMT* promoter methylation has been associated with the response of glioblastomas to alkylating chemotherapy using nitrosourea compounds (Esteller et al. 2000), temozolomide (Hegi et al. 2005), or a combination of both (Herrlinger et al. 2006). Based on *MGMT* promoter methylation analysis in glioblastomas from patients treated in a large prospective clinical trial, patients whose tumors had a methylated *MGMT* promoter survived significantly longer than patients whose tumors lacked *MGMT* promoter methylation when treated with combined radio-/chemotherapy (Hegi et al. 2005). In patients treated with radiotherapy alone, *MGMT* promoter methylation did not influence survival, thus indicating that the *MGMT* promoter status is a predictive factor for response to chemotherapy but not radiotherapy. As *MGMT* promoter methylation can be easily assessed by methylation-specific polymerase chain reaction (MSP) analysis, it is now a frequently requested molecular assay at neuropathological centers.

High-Throughput Approaches. Advances in high-throughput profiling techniques nowadays allow for the simultaneous screening of thousands of genes within a single tumor. Thereby, characteristic molecular signatures can be assessed at the genomic level by means of array-based comparative genomic hybridization (array-CGH), as well as at the transcript level by mRNA expression profiling. After employing bioinformatic approaches, these signatures may be used to assign tumors to defined molecular subgroups providing refined diagnostic and prognostic information. In this regard, it was shown that gene expression-based classification of morphologically ambiguous high-grade gliomas correlates better with prognosis than the histological classification (Nutt et al. 2003). Furthermore, molecular classification of gliomas on the basis of genomic profiles obtained by array-CGH closely parallels histological classification and was able to distinguish, with few exceptions, between different astrocytoma grades as well as between primary and secondary glioblastomas (Roerig et al. 2005). Another array-CGH study suggested that glioblastomas can be subdivided into clinically relevant subsets on the basis of genomic profiles (Korshunov et al. 2006). Along these lines, comprehensive molecular profiling at the gene and transcript levels identified distinct prognostic subclasses of high-grade astrocytomas, which could be assigned to different stages in neurogenesis (Phillips et al. 2006). Interestingly, tumors displaying neuronal lineage markers presented with longer survival, while patients whose tumors expressed neural stem cell markers had shorter survival times. Markers of proliferation, angiogenesis, and mesenchyme contributed to the definition of the prognostically poor astrocytoma subgroup. Moreover, the authors could derive a two-gene (*PTEN* and *DLL3*) expression signature from their profiles suggesting that markers within the Akt and Notch signaling pathways can be employed as meaningful prognostic markers (Phillips et al. 2006). Expression profiling of pediatric glioblastomas similarly identified at least two tumor subsets, one being associated with poor prognosis and Ras and Akt pathway activation as well as increased expression of

genes related to proliferation and to a neural stem-cell phenotype. The other subset showed a better prognosis, lacked Akt and Ras pathway activation, and is speculated to originate from astroglial progenitors (Faury et al. 2007).

The main drawback of large-scale profiling techniques, however, is that their use in routine neuropathological diagnostics is limited as these methods are quite expensive and not generally available. Thus, it seems desirable to identify single diagnostically or prognostically relevant genes or defined genetic signatures, merely comprising a small number of genes or proteins, respectively.

1.7 Pathways to Astrocytoma and Targeted Therapies

The Cell-Cycle Regulatory Pathways pRB and p53. The pRB pathway plays a central role in regulating G_1/S transition and is commonly affected in anaplastic astrocytomas and glioblastomas. Under mitogenic stimuli, CyclinD expression is upregulated and the CyclinDs bind to Cdk4 or Cdk6, thereby phosphorylating the Rb1 protein and releasing E2F transcription factors, resulting in the activation of S-phase genes like CyclinE. The formation of the CDK4/CyclinD complex can be negatively regulated by *CDKN2A* (encoding $p16^{INK4a}$) and *CDKN2B* (encoding $p15^{INK4B}$), which are two of the INK4 family of CDK inhibitors that specifically bind to Cdk4 and Cdk6, competing with and thereby blocking their binding to the CyclinDs. Specific pRB pathway alterations in astrocytomas comprise *RB1* mutations and loss of expression as well as amplifications of *CDK4* found in the same frequency in both primary and secondary glioblastomas (Schmidt et al. 1994; He et al. 1995; Ichimura et al. 1996; Table 1.2). Deletions of *CDKN2A* and *CDKN2B* are more frequently detected in primary glioblastomas (Jen et al.

Table 1.2 Synopsis of the most relevant tumor suppressor genes and proto-oncogenes involved in astrocytoma pathogenesis

Gene	Location	Typical alteration	Function of the protein
Tumor suppressor genes			
TP53	17p13	Mutation	Regulation of apoptosis, cell cycle progression and DNA repair
RB1	13q14	Mutation, hypermethylation	Cell cycle regulation
CDKN2A	9p21	Homozygous deletion, hypermethylation	Cell cycle regulation by inhibition of CDK4 and 6
$p14^{ARF}$	9p21	Homozygous deletion, hypermethylation	Cell cycle regulation by inhibition of Mdm2
PTEN	10q23	Mutation	Negative regulation of PI3K
TSC1/TSC2	9q34/16p13	Mutation, phosphorylation	Negative regulation of mTOR
Proto-oncogenes			
EGFR	7p11	Amplification and overexpression	Growth factor receptor
PDGFRA	4q12	Amplification and overexpression	Growth factor receptor
MET	7q31	Amplification and overexpression	Growth factor receptor
CDK4	12q13	Amplification and overexpression	Promotion of G_1/S-phase progression
CDK6	7q21–22	Amplification and overexpression	Promotion of G_1/S-phase progression
CCND1	11q13	Amplification and overexpression	Cyclin D_1, G_1/S-phase progression
CCND2	6p21	Amplification and overexpression	Cyclin D_3, G_1/S-phase progression
MDM2	12q15	Amplification and overexpression	Inhibition of p53
MDM4	1q32	Amplification and overexpression	Inhibition of p53
MYCC	8q24	Amplification and overexpression	Transcription factor

1994), and promoter hypermethylation may also account for the inactivation of these two genes (Schmidt et al. 1997).

The p53 pathway regulates a plethora of cell functions, including responses to DNA damage, hypoxia, apoptosis, inappropriate oncogene activation, and defects in DNA methylation (Prives and Hall 1999). Alterations in the p53 signaling pathway are a common finding in diffuse astrocytomas of all WHO grades. While mutation or loss of the p53 tumor suppressor gene is frequent already in diffuse astrocytomas of WHO grade II and recurrences arising from these tumors, including secondary glioblastomas (Watanabe et al. 1997), alterations of other pathway components more often substitute for *TP53* mutations in primary glioblastomas. A subset of glioblastomas and anaplastic astrocytomas exhibits amplification of the *MDM2* and *MDM4* genes (Reifenberger et al. 1996; Riemenschneider et al. 1999, 2003), which can inhibit p53 function through inhibitory binding to p53. Thus, *MDM2* and *MDM4* amplification/overexpression constitutes an alternative mechanism to escape from p53-regulated cell cycle control. Another way to impair p53 function is deletion or methylation of the tumor suppressor gene $p14^{ARF}$ on chromosome 9q21 (Nakamura et al. 2001). $p14^{ARF}$ (the human homologue of $p19^{ARF}$) is encoded through an alternative reading frame from the same chromosomal locus as exon 1 of the *CDKN2A* ($p16^{INK4A}$) tumor suppressor gene and has the ability to inhibit MDM2-mediated degradation of p53 (Quelle et al. 1995; Pomerantz et al. 1998; Table 1.2).

Growth Factor Receptor Signaling and the PI3K/AKT Pathway. Activated EGFR and PDGFRA signaling pathways are a common finding in diffuse astrocytomas and affect multiple cell functions such as cell proliferation, growth, differentiation, migration, and survival. Many diffuse astrocytomas of WHO grade II exhibit overexpression of *PDGFRA* (Hermanson et al. 1992), while amplification and overexpression of *EGFR* and *PDGFRA* are a finding characteristic of high-grade lesions, in particular glioblastoma (Table 1.2; Figs. 1.1i and 1.2). Ligands of both EGFR and PDGFRA are secreted by the tumor cells themselves and can stimulate their receptors in an autocrine fashion (Ekstrand et al. 1991). In about half of the cases, *EGFR* amplification is associated with a structural rearrangement of the *EGFR* gene resulting in the formation of a deletion-mutant receptor, which is referred to as EGFRvIII. The *EGFRvIII* gene has an in-frame deletion of 801 base pairs, corresponding to exons 2–7 in the mRNA, resulting in the deletion of amino acids 6–273 in the extracellular ligand-binding domain and the generation of a glycine at the fusion point (Wikstrand et al. 1998). Functionally, the mutated vIII receptor is constitutively active, thus mimicking the effects of ligand-stimulated EGFR in increasing cell proliferation. Clinically, recent data indicate that EGFRvIII mutant glioblastomas constitute a distinct subset of tumors with more aggressive behavior, in which established prognostic factors in glioblastoma were not predictive of outcome (Pelloski et al. 2007).

One of the major pathways involved in signal transduction downstream of growth factor receptors is the PI3-kinase/AKT pathway, which has attracted considerable attention within recent years. Activated growth factor receptors can bind and activate the PI3-kinase, which then phosphorylates phosphatidylinositol-4,5-diphosphate (PIP2) to phosphatidylinositol-3,4,5-triphosphate (PIP3) leading to activation of protein kinaseB/AKT. This process is controlled by the tumor suppressor PTEN (phosphatase and tensin homolog deleted on chromosome 10), which has the ability to dephosphorylate PIP3 to PIP2 and thereby inhibits AKT (Knobbe et al. 2002). Another recently identified negative regulator of AKT is the carboxyl-terminal modulator protein (CTMP), which was reported to demonstrate hypermethylation and transcriptional downregulation in up to 40% of glioblastomas (Knobbe et al. 2004). In conveying the downstream signaling effects of AKT, the

serine/threonine protein kinase mammalian target of rapamycin (mTOR) has been proposed to play a central role. AKT can either directly or indirectly (via the TSC-complex) phosphorylate mTOR, leading to subsequent activation of S6K and STAT3, as well as suppression (i.e., phosphorylation) of 4E-BP1 with the effects of cell cycle progression and inhibition of apoptosis (Bjornsti and Houghton 2004; Riemenschneider et al. 2006).

Growth factor receptor signaling pathways have gained attention recently in regard to targeted molecular therapies. In non-small cell lung cancers it could be demonstrated that the presence of certain activating mutations of *EGFR* conveyed responsiveness to the tyrosine kinase inhibitor Gefitinib (Bell et al. 2005). In contrast to the frequent vIII mutations in the extracellular ligand-binding domain of *EGFR* in glioblastomas, those activating mutations affect the ATP-binding pocket of the tyrosine kinase domain, leaving the receptor ligand-dependent (Lynch et al. 2004). Also among patients with glioblastoma, about 10–20% appear to benefit from the EGFR inhibitors erlotinib and gefitinib (Table 1.3). However, the infrequency of mutations in the *EGFR* kinase domain of glioblastomas suggests that such mutations do not account for responsiveness to EGFR kinase inhibitors. A recent study reported that coexpression of EGFRvIII and PTEN, as detected by immunohistochemistry, may serve as a predictor of responsiveness to EGFR kinase inhibitors in glioblastomas (Mellinghoff et al. 2005).

Another example on why careful pathway dissection is necessary for advancing molecular targeted therapies is the use of mTOR inhibitors in patients whose tumors have loss of *PTEN*. Rapamycin, a complex macrolide and potent fungicide, immunosuppressant, and anticancer

Table 1.3 Selected molecular targets and specific inhibitors under evaluation in glioma therapy (Modified from Rich and Bigner 2004)

Target	Function	Specific inhibitor	Drug type
EGFR	Tyrosine kinase growth factor receptor	Gefitinib (ZD1839)	Tyrosine kinase inh.
		Erlotinib (OSI774)	Tyrosine kinase inh.
		AEE788	Tyrosine kinase inh.
PDGFRA	Tyrosine kinase growth factor receptor	Imatinib mesylate (STI571)	Tyrosine kinase inh.
		SU6668	Tyrosine kinase inh.
		MLN518/608	Tyrosine kinase inh.
VEGFR	Tyrosine kinase growth factor receptor	SU5416	Tyrosine kinase inh.
		PTK787/ZK222584	Tyrosine kinase inh.
PKC	Protein kinase	Tamoxifen	Anti-estrogen
		LY317615	Small molecule
RAS	Proto-oncogene	Tipifarnib (R115777)	Farnesyl transferase inh.
		Lonafarnib (SCH66366)	Farnesyl transferase inh.
PI3K	Lipid kinase	Wortmannin	Antibiotic
		LY294002	Antibiotic
MTOR	Serine/threonine protein kinase downstream of AKT	Rapamycin	Antibiotic
		CCI-779	Antibiotic
		RAD001	Antibiotic
		AP23573	Antibiotic
Integrins avß3 and avß5	Cell adhesion molecule	Cilengitide (EMD121974)	Cyclic RGD peptide

agent, is a highly specific inhibitor of mTOR (Singh et al. 1979). A phase II study of CCI-779, an ester of rapamycin, in patients with recurrent glioblastoma led to radiographic improvement in 36% of patients, and was associated with a significantly longer median time to progression (Table 1.3). Interestingly, high levels of phosphorylated p70S6 kinase in baseline tumor samples appeared to predict a patient population more likely to benefit from treatment (Galanis et al. 2005). While these results may represent a further promising step in the treatment of glioblastoma patients, they also show quite plainly that no single agent is likely to produce striking results in these aggressive tumors and that better therapeutic results may only be achieved by identification of novel therapeutic targets and the combination of different agents.

Invasion- and Adhesion-Associated Pathways. Another reason why the efficacy of treating malignant gliomas remains largely unsatisfying is that astrocytoma cells have the ability to invade deeply into the surrounding brain tissue, thus making local therapeutic approaches ineffective. Many extracellular factors regulating glioma cell invasion have been well established. Astrocytoma cells have been shown to modulate their microenvironment by secreting proteolytic enzymes, like matrix-metalloproteinases (Rao 2003), as well as extracellular matrix components, like fibronectin, laminin, vitronectin, and collagen type IV (Friedlander et al. 1996; Mahesparan et al. 1999). Complex signaling pathways are also involved in the regulation of astrocytoma cell migration and invasion. Activation of EGFR and/or PTEN/PI3K/AKT signaling can enhance tumor cell invasion (Guha and Mukherjee 2004; Rao and James 2004). Similar effects can be achieved by binding of various different integrin subtypes to autocrinely secreted extracellular matrix components (Friedlander et al. 1996; Goldbrunner et al. 1996). Of note, integrin and growth factor receptor signaling pathways have been shown to overlap in their activating effects on the focal adhesion kinase (FAK), which serves as central relays in integrating different upstream pathways in their effects on migratory and invasive cell properties making FAK an ideal candidate molecule for novel targeted molecular therapies (Riemenschneider et al. 2005). Small molecule inhibitors against FAK have recently demonstrated potent antimigratory effects in various cancer cell lines (Huang et al. 2005; Choi et al. 2006a, b). In addition, the blockade of integrins may lead to indirect targeting of FAK. EMD121974, for example, is a potent antagonist to αvß3 and αvß5 integrins and is currently being evaluated in phase I and II studies in adults with recurrent anaplastic gliomas or newly diagnosed GBMs (Eskens et al. 2003).

Except for FAK, the proline-rich tyrosine kinase (Pyk2) interacts with many of the same proteins, although the consequences of these interactions remain to be elucidated (Lipinski et al. 2003). Other important molecules involved in astrocytoma cell migration and invasion are members of the Rho family of small GTPases (RhoA and Rac1), including signaling by lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P), as well as the nuclear factor (NF)-κB family of transcriptions factors (for review, see Salhia et al. 2006).

1.8 Novel In Vitro and In Vivo Astrocytoma Models

The Tumor Stem Cell Hypothesis. Modeling of astrocytic gliomas has made tremendous advances in the past few years and has accelerated our insight into the molecular and cellular mechanisms of astrocytoma growth. A recent milestone in the field is the identification of a potential tumor stem cell (TSC) fraction (Vescovi et al. 2006), which is characterized by expression of the CD133 antigen and other stem cell-associated markers. Dirks and colleagues isolated the CD133-positive cell subpopulation from human

brain tumors and could demonstrate that these cells exhibited stem cell properties, i.e., self-renewal, in vitro. Moreover, when transplanted into NOD-SCID (non-obese diabetic, severe combined immunodeficient) mouse brains, only CD133-positive cells produced tumors, which were exact phenocopies of the patients' original lesions, while CD133-negative cells – even when injected in higher concentrations – were not able to produce tumors (Singh et al. 2004).

However, it has to be mentioned that there is some controversy in the field regarding the exceptional tumor initiating capabilities of CD133-positive glioma cells. In a recent study, tumor cells from 22 primary and secondary glioblastomas were cultured under medium conditions favoring the growth of neural and cancer stem cells (Beier et al. 2007). Remarkably, only a subset of primary glioblastomas contained a significant CD133-positive subpopulation and both CD133-positive and -negative tumor cells were similarly tumorigenic in nude mice, suggesting that CD133-negative tumor cells may also exhibit stem cell properties. Nevertheless, CD133-positive cells were characterized by higher proliferation indices, thus suggesting a possible prognostic significance of this cell fraction.

Recent evidence suggests that TSCs may also be accountable for the radioresistance of glioblastomas through preferential activation of the DNA damage checkpoint response and an increase in DNA repair capacity (Bao et al. 2006). In comparison to their negative counterparts, CD133-positive TSCs were enriched after radiation in both cell culture and the brains of immunocompromised mice. Radioresistance of CD133-positive glioma stem cells could be reversed with a specific inhibitor of the Chk1 and Chk2 checkpoint kinases. Thus, the CD133-positive tumor cell fraction may represent the cellular population that confers glioma radioresistance and could be the source of tumor recurrence after radiation. Consequently, targeting DNA damage checkpoint response in cancer stem cells may provide a novel therapeutic glioblastoma model.

Further studies indicate that primary human tumor-derived TSCs and their matched glioma cell lines showed marked phenotypic and genotypic differences (Lee et al. 2006). In contrast to the traditionally serum-cultured cell lines, tumor stem cells derived from glioblastomas and cultured in bFGF and EGF more closely recapitulated the genotype, gene expression patterns, and in vivo biology of human glioblastomas. Thus, TSC cultures may serve as a more reliable model than many commonly utilized glioma cell lines for understanding the biology of primary human tumors.

Novel Animal Models. Recent progress has also been made in regard to genetically engineered mouse models. Their detailed description is beyond the scope of this review and the reader is referred to a number of excellent review articles specifically addressing this issue (Holland 2001; Reilly and Jacks 2001; Begemann et al. 2002; Gutmann et al. 2003; Hesselager and Holland 2003). Mutant mice can be generated either by genetic germ-line modifications or somatic gene transfer and provide a powerful tool for investigating the importance of single molecular alterations or pathways in astrocytoma pathogenesis. By such experiments it could be shown that Ras- and AKT-dependent pathways, but also inactivation of pRB- and p53-signaling, are of essential importance for astrocytoma formation in vivo (Holland et al. 2000; Uhrbom et al. 2002; Xiao et al. 2002). The histology of CNS tumors generated in such models has been reviewed by an international consortium and guidelines for the classification of these tumors have been defined (Weiss et al. 2002). Most interestingly, those engineered tumors appear to more and more realistically mimic their human counterparts. In this regard, a novel animal model was generated by the double knockout of the *TP53* tumor suppressor gene and the neurofibromatosis type 1 (*NF1*) gene, which leads to the activation of Ras signaling

(Zhu et al. 2005). The resultant mice developed astrocytomas with 100% penetrance. The murine tumors exhibited key features of human astrocytomas with diffuse infiltration of the surrounding brain tissue and malignant progression over time. Of note, early presymptomatic lesions resided within the subventricular zone (SVZ) of the lateral ventricle, one region of the CNS that is supposed to contain neurogenic stem cells.

Taken together, these findings clearly indicate that these novel animal astrocytoma models may be exploited as powerful tools in the preclinical evaluation of novel therapies. Moreover, they may also help to provide further insight into the pathways and molecular alterations underlying astrocytoma pathogenesis and may even help to address the yet unresolved issue of identifying the astrocytoma cell of origin.

1.9 Conclusion

This review outlines the tremendous advances that have been made over the last 2 decades regarding our understanding of the molecular alterations underlying astrocytoma oncogenesis and progression. Classification of astrocytic tumors according to WHO criteria is still primarily based on the recognition of key histological features. In terms of molecular diagnostics, however, *MGMT* promoter hypermethylation has been established as a first predictive molecular marker in glioblastomas, which is more and more commonly implemented in the diagnostic procedure in neuropathological centers and provides important predictive information on chemosensitivity. No doubt, the facilitated accessibility of high-throughput profiling techniques will further accelerate the progress of molecular diagnostics and may also refine our knowledge about the key pathogenic pathways involved in astrocytomas. These pathways can then be targeted by the use of novel specific inhibitors, enabling us to provide patients with more individualized therapeutic approaches in addition to surgery, radiotherapy, and conventional chemotherapy. Several of these drugs are already in clinical phase I and II trials. The development of novel animal models will allow us to test new agents in a preclinical setting and most realistically access their therapeutic potential for subsequent clinical trials. Finally, another future challenge will be to address the issue of tumor heterogeneity by, for example, specifically targeting the infiltrating cells in the invasive rims of the tumors or a potential tumor stem cell fraction with high pathogenic ability.

Note added in proof

During the production of this review two novel large-scale multi-dimensional studies were released reporting on the integrative genomic analysis of human glioblastoma:
The Cancer Genome Atlas Research Network investigated 91 human glioblastomas for mutations in 601 selected genes (Cancer Genome Atlas Research Network 2008). Major novel findings were the detection of NF1 mutations in 14% and ERBB2 mutations in 8% of tumors. Parsons and colleagues sequenced 20,661 genes in 22 human glioblastomas and thereby identified recurrent mutations in the active site of isocitrate dehydrogenase 1 (IDH1) in a large fraction of young patients and in most patients with secondary glioblastomas (Parsons DW et al. 2008). Direct sequencing in a series of 685 brain tumors revealed highest frequencies of somatic IDH1 mutations in diffuse astrocytomas (68%), oligodendrogliomas (69%), oligoastrocytomas (78%) and secondary glioblastomas (88%). Primary glioblastomas and other entities were characterized by a low frequency or absence of mutations in amino acid position 132 of IDH1 (Balss et al. 2008). The very high

frequency of IDH1 mutations in WHO grade II astrocytic and oligodendroglial gliomas suggests a role in early tumor development and may be exploited for differential diagnostic purposes.

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