Chapter 2
Molecular Pathogenesis

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Introduction

Several decades of concentrated efforts have improved the understanding of the molecular biology and pathogenesis of primary brain tumors. Historically, primary brain tumors have been diagnosed and graded based on histopathologic criteria. However, as more data have accumulated regarding the molecular alterations underlying specific tumors, it has become clear that a number of key molecular alterations are associated with the initiation, progression, and clinical outcome of specific tumor histologies and grades. In addition, recent large-scale efforts at high-throughput molecular profiling of large cohorts of tumors, such as The Cancer Genome Atlas (TCGA) effort focused on glioblastoma (GBM) tumors, have identified novel molecular features that define specific clinically important tumor subgroups. Thus, in addition to contributing to a more detailed understanding of the origins and biology of primary brain tumors, these findings have contributed more refined definitions and knowledge of their molecular heterogeneity, and this knowledge plays an increasingly important role in the current approaches for diagnosis, grading, and treatment decisions. Together, these efforts have further refined our understanding of the molecular pathogenesis of these tumors and provided a basis for potential “personalization” of therapy based on the molecular features of individual tumors.
Gliomas

Gliomas are the most common primary tumors in adults. The most common histopathologic subtypes of gliomas are astrocytomas and oligodendrogliomas. These tumors are named for morphologic and immunohistochemical similarities between the normal glial cell types (astrocytes and oligodendrocytes) and the tumor cells in these histologies. Under the current WHO criteria, these tumors are also graded based on histopathologic features and criteria [1]. As described below, while these tumors share histologic features of glial lineages, the molecular alterations, biology, and natural history observed in the different histologies and different grades of tumors are quite distinct. These findings indicate that there are biologic and clinically important differences in either the cell of origin, initiating events, and/or molecular pathogenesis of progression between tumor types.

Low-Grade and Intermediate-Grade Gliomas

Diffuse Astrocytoma, WHO Grade II

p53

The tumor suppressor gene TP53 which encodes for the p53 protein has been clearly implicated in the pathogenesis of diffuse low-grade astrocytomas. This multifunctional molecule has been called a “guardian of the genome” (PMID: 7475551) and plays an important role in multiple cellular pathways related to oncogenesis including cellular response to DNA damage, cell cycle regulation, and cell death (apoptosis). Cells without functional p53 are thus prone to DNA instability and lack several of the key regulatory pathways that prevent survival and proliferation of cells with significant DNA damage.

The potentially important role of p53 in the molecular oncogenesis of low-grade astrocytoma is suggested by several observations. First, diffuse astrocytomas (along with higher-grade astrocytomas) occur at increased frequency in patients with inherited mutations in TP53 or Li–Fraumeni syndrome. Second, data from mouse models indicate that p53 mutations alone can contribute to immortalization and increased proliferation of astrocytes [2] and in combination with other genetic alterations can result in glioma formation [3, 4]. Perhaps most importantly, inactivating mutations in p53 are observed in a majority (>50%) of WHO Grade II astrocytomas [5, 6] and a slightly lower percentage of anaplastic astrocytomas (WHO Grade III). Together, these data suggest that p53 mutation or inactivation is an early and causative event in astrocytoma pathogenesis.

In addition to the previously described role of p53 in low-grade astrocytoma, new data suggest that this gene also plays a larger role in high-grade astrocytomas than previously recognized [7]. While prior data had suggested that p53 alterations were relatively rare in glioblastoma, recent results from the TCGA study reported a higher than expected rate of p53 mutation in these tumors. These findings suggest that either a larger percentage of GBM tumors arise from lower-grade tumors
(see discussion of primary vs secondary GBM below) or that p53 alteration plays a more significant role in de novo high-grade astrocytoma than previously thought. Alternatively, a bias for secondary tumors in the sample set profiled by TCGA could also explain a higher p53 mutation rate in this data set compared to others.

Platelet-Derived Growth Factor (PDGF)

PDGF is a secreted protein that binds to the PDGFR tyrosine kinase. PDGF and PDGFR are often expressed by the same cells, resulting in the potential for both autocrine and paracrine stimulatory activities. PDGF plays an important part in neural development, particularly in the regulation of glial precursors [8]. PDGF has also been implicated in the pathogenesis of low-grade astrocytoma in several ways. Exogenous addition or overexpression of PDGF can result in increased proliferation or transformation of glial cells [9, 10]. Overexpression of both PDGF and PDGFR is also observed in low-grade astrocytomas [11].

However, it appears that PDGF overexpression alone is not sufficient for astrocytoma initiation and may depend on interactions with other molecular alterations. For example, alterations in p53 are highly correlated with PDGF overexpression in low-grade astrocytoma, suggesting a cooperative interaction [11]. In addition, the effects of PDGF overexpression in mouse models of tumor initiation depend on other alterations that are present. Overexpression of PDGF alone in glial progenitors led to tumors with an oligodendroglial phenotype, while overexpression of PDGF along with activation of Akt in these same glial progenitors resulted in a shift in tumor histology toward an astrocytic phenotype [12]. Thus, these data suggest that PDGF may play an important role in astrocytoma pathogenesis and that the effects of PDGF on tumor initiation and histology may be dependent on both the cell of origin and the status of the p53 and other signaling pathways.

IDH1 and IDH2 Mutations in Astrocytoma

Recent efforts at comprehensive sequencing to detect gene mutations in glioblastoma led to the novel observation of mutations in the isocitrate dehydrogenase genes IDH1 and IDH2 in a subset of these high-grade tumors [13, 14]. Additional studies demonstrated that mutations in these genes were also found in a large percentage (>50–80%) of low-grade astrocytomas and oligodendrogliomas [15–18]. High rates of mutations (>70%) were also observed in anaplastic astrocytomas (WHO Grade III) and glioblastomas (WHO Grade IV) that arose from lower-grade tumors, but generally not in primary glioblastomas that had no known lower-grade precursor. In addition, IDH1 mutations are highly correlated with both p53 mutation and 1p/19q loss (see below) in individual tumors [16]. While the biologic effect of IDH1/IDH2 mutation on oncogenesis remains unclear, these data indicate that mutation in IDH1 and/or IDH2 may be an important early step in the molecular pathogenesis of low-grade astrocytoma and oligodendroglioma, as well as secondary higher-grade gliomas and is a focus of ongoing investigations [19].
Other DNA Changes

Although much less prominent than in higher-grade astrocytoma, there are several less common alterations at the DNA and epigenetic level that have been noted in low-grade astrocytoma. These include the gain of chromosome 7q, loss of regions of chromosome 22q, and gains of regions of chromosomes 5p, 9, and 19p [20]. Combined loss of chromosomes 1p and 19q has also been observed in low-grade astrocytoma, although at much lower rates than in oligodendroglioma [21].

Recent genome-wide studies of DNA alterations associated with risk of glioma development have identified single nucleotide polymorphisms (SNPs) in several genes. These individual genes include \textit{CDKN2A} and \textit{CDKN2B}, which are involved in the regulation of cell cycle, and genes involved in regulation of telomeres including \textit{RTEL1} and \textit{TERT} [22, 23]. The finding of increased risk of glioma in patients with germ line alterations in these genes strongly suggests a mechanistic role of these genes in glioma development.

Prognostic and Predictive Factors in Low-Grade Astrocytoma

While the list of potentially mechanistically important molecular alterations in low-grade gliomas continues to grow, the prognostic or predictive power of these markers to date has been less important in low-grade versus high-grade gliomas. Clinicopathologic factors that have been demonstrated to impact survival in low-grade astrocytoma include patient age, extent of resection, radiographic enhancement, and performance status [24–26]. Overall, neither mutation of p53 nor overexpression of PDGF has significant prognostic impact within low-grade astrocytomas [27, 28]. However, molecular markers of proliferation including MIB-1/Ki-67 labeling and phosphorylated histone H3 (pHH3) staining are prognostic in low-grade and intermediate-grade astrocytomas, with higher indices of proliferation associated with worse prognosis [29, 30]. Although data are still accumulating, the high rate of mutation of \textit{IDH1/IDH2} in astrocytoma and oligodendroglioma suggest that these alterations may be associated with tumor initiation. Whereas these mutations do not have prognostic significance for low-grade tumors, they are associated with a favorable prognosis in high-grade tumors (WHO Grades III and IV) [18, 31, 32].

Anaplastic Astrocytoma

The majority of anaplastic astrocytomas (WHO Grade III) appear to arise from lower-grade tumors and thus share many of the signature molecular alterations observed in Grade II astrocytomas [1]. However, the more aggressive histopathology and clinical course of anaplastic astrocytoma are associated with several important molecular alterations that are also seen at higher frequencies in a subset of GBM (Grade IV) tumors (see Refs. [1, 33, 34] for review). These alterations associated with progression from low to intermediate grade include defects in the Rb pathway (discussed in more detail below) including mutations in \textit{RBI} and loss of the
Ink4a/Arf locus on chromosome 9. In addition, loss of chromosomes 19q and 11p is seen at higher frequency in anaplastic astrocytoma than in low-grade tumors. A subset of anaplastic astrocytomas also demonstrate loss of chromosome 10 and/or PTEN mutations similar to many GBM tumors.

**Oligodendroglioma and Anaplastic Oligodendroglioma**

Overall, oligodendroglial lineage tumors demonstrate a significantly different spectrum of molecular alterations compared to astrocytic tumors. By far the most common alteration seen in both low-grade and intermediate-grade oligodendrogliomas is concurrent deletion of chromosomes 1p and 19q that is observed in up to 50–80% of cases [35, 36]. In the majority of tumors this is due to an unbalanced translocation [37]. These findings are potentially consistent with a central role of this alteration in the molecular pathogenesis of oligodendroglial tumors, however, the specific role of a number of candidate genes in these regions remains to be elucidated. Loss of chromosomes 1p and 19q is also an important prognostic factor in these tumors. Combined loss is associated with better prognosis and improved response to both radiation and chemotherapy and is often used clinically for treatment decision-making [38, 39]. More rarely, isolated loss of chromosome 1p or 19q is observed in oligodendroglioma, although these isolated losses are less prognostic than combined loss. Indeed, isolated loss of 19q is much more common in astrocytic tumors. Oligodendroglial tumors also demonstrate a high frequency of increased expression of both EGFR and PDGF/PDGFR and high rates of methylation of several genes including p14, RB1, CDKN2A/CDKN2B, and MGMT.

**Glioblastoma**

Glioblastoma (WHO Grade IV) represents the highest grade and most clinically aggressive form of glioma. Although these tumors represent a single histopathologic entity defined by WHO criteria and are distinguished from Grade III astrocytomas primarily by the presence of pseudopalisading necrosis and/or vascular proliferation [1], it is becoming increasingly clear that this single histopathologic entity actually comprises a surprisingly complex variety of molecular phenotypes.

**Secondary GBM**

Classically, glioblastomas have been described as primary or secondary based on their natural history and a series of findings over many years defining differences in molecular phenotype [1, 34]. Analyses of molecular alterations in glioblastoma have found that a minority of tumors demonstrated molecular alterations consistent with an evolution from a low-grade tumor and these tumors have been called “secondary” GBMs to describe their origins from lower-grade precursors. As described above, there are several molecular features found at high rates in low-grade tumors such as p53 [40] mutation or more recently IDH1 mutation [31, 41]. The origin of secondary
GBMs from lower-grade tumors is highlighted by the presence of these signature alterations in a minority of GBMs, in addition to evidence of additional important molecular alterations associated with tumor progression [40–42]. Secondary GBM is observed at higher frequencies in younger patients and is associated with better overall prognosis. Important molecular alterations associated with progression to anaplastic astrocytoma and finally secondary GBM include alterations in the Rb pathway and loss of parts of chromosome 10 and/or mutation of the tumor suppressor gene PTEN.

**Primary GBM**

In contrast, the majority of GBMs appear to arise de novo without any prior lower-grade lesion identified and have been called “primary” GBMs [1, 40]. These tumors typically lack the signature low-grade molecular alterations including p53 and IDH1/IDH2 mutation. Instead primary GBMs display a distinct spectrum of molecular alterations that at least to some extent are mutually exclusive with those of secondary GBM.

Secondary GBMs demonstrate a frequency of signature molecular alterations that several lines of data suggest are involved in the molecular pathogenesis of these tumors. First, these tumors have a high frequency of amplification of portions of chromosome 7 that include the epidermal growth factor receptor (EGFR) [43]. A subset of GBMs with EGFR amplification also demonstrate mutation of the receptor that results in loss of multiple exons and constitutive activation called the vIII mutant (EGFRvIII) [44]. In addition, primary GBMs have a high frequency of loss of the long arm of chromosome 10. This chromosome contains the important tumor suppressor gene PTEN that was initially identified based on its high rate of mutation in gliomas and other high-grade epithelial tumors [45–47]. In addition to loss of chromosome 10 in a high percentage of tumors, many GBMs also demonstrate mutation or loss of PTEN on the other chromosome. These observations along with the importance of this gene in key signaling pathways and mouse models of GBM demonstrate its importance in the molecular pathogenesis of these tumors. In addition to playing a role in primary GBM, alterations on chromosome 10 and in the PTEN gene are also seen in the progression of anaplastic astrocytoma to GBM, thus also implicating PTEN in the progression to secondary GBM. Primary GBMs are observed at higher frequencies in older patients and are associated with a worse prognosis.

**Key Functional Pathways in Glioma Pathogenesis**

The central molecular alterations described above impact several key signaling pathways in gliomas. Alterations at the DNA level (including chromosomal loss, gene amplification, or gene mutation) modulate pathway activity by virtue of gene dosage effects. Pathway activity may also be impacted by changes in gene expression levels or activation or inhibition of receptor-linked or intracellular signaling cascades.
These signaling pathways have been implicated in the molecular pathogenesis of gliomas through data from human tumor samples as well as preclinical and mouse models.

The p53 Pathway

As described above, the TP53 gene is an important tumor suppressor gene located on chromosome 17p13.1 that is centrally involved in multiple pathways regulating DNA integrity, cell cycle, and cell death [48]. In addition to mutation of the TP53 gene itself, the p53 pathway can be affected by alterations in several other regulatory genes/proteins. The MDM2 and MDM4 genes are antagonists of p53 that bind to the p53 promoter and inhibit its transcription. Amplification of both MDM2 and MDM4 has been identified in a subset of gliomas with intact p53 indicating an alternate mechanism for downregulation of this pathway [49]. In addition, a high proportion of gliomas contain deletions or mutations in the Ink4a/Arf locus which encodes regulators of both the p53 and Rb pathways [50–52]. Taken together, these data suggest that essentially all astrocytomas harbor important molecular alterations that affect the p53 pathway, although the frequency of specific alterations can vary by grade.

The Rb Pathway

Like TP53, the retinoblastoma (RB1) gene is a key tumor suppressor that has been implicated in the pathogenesis of many cancer types. The Rb protein plays a key role in regulating cell cycle progression and proliferation. Thus, cells with alterations in the Rb pathway have impaired regulation of these functions, resulting in increased cellular proliferation and a lack of response to anti-growth signals. The important role of several components of the Rb pathway in gliomagenesis has been demonstrated by alterations in these genes in human tumors and/or their mechanistic association with gliomagenesis in mouse models. The Rb pathway contains several positive and negative regulatory elements.

In normal, non-dividing cells, the Rb protein is inactive (hypophosphorylated) and binds to its regulatory partner E2F. Mutation or activation of Rb results in the dissociation from E2F and subsequent transcription of multiple genes involved in cell cycle progression and cellular proliferation. While mutations in the RB1 gene itself are relatively rare in low-grade astrocytoma, they can be seen in up to 30% of high-grade astrocytomas [53]. Alterations in other key genes/proteins in this pathway observed at significant rates in gliomas include the following: (1) amplification of the positive regulators CDK4, CDK6, and Cyclin D and (2) mutation, deletion, or decreased expression of the negative regulators p16, p15, and p27 [50–52, 54]. As with p53, the data suggest that while the specific component of the pathway altered in specific tumors can vary (and be mutually exclusive) essentially all gliomas demonstrate functional lesions in at least one key element of the Rb pathway.
Growth Factor Receptor Signaling and Angiogenesis

Receptor Tyrosine Kinases

A number of signaling pathways important in brain tumor pathogenesis involve specific growth factors and their associated receptor tyrosine kinases. Many of these growth factor receptors are cellular homologues of viral oncogenes and have been implicated in tumor initiation and progression of gliomas based on data from human tumors and mouse models. One example discussed above is PDGF, which is the cellular homologue of the oncogene v-sis. Another example is EGFR, the cellular homologue of the viral oncogene vErbB. EGFR binds the growth factors EGF and tumor growth factor (TGF-α). As discussed above, EGFR is implicated in the pathogenesis and progression of high-grade astrocytomas. As a result of their important role in tumor initiation and biology, these signaling pathways have also become central in the search for novel therapeutic agents for brain tumors and other cancers. A number of novel drugs and biologic agents have thus been developed to target individual or multiple signaling pathways that are in clinical trials or have been FDA approved for treatment of brain tumors. These therapeutic developments have been summarized in a number of reviews [55–62].

Downstream Signaling Pathways

Activation of EGFR, PDGFR, and other transmembrane receptors as a result of ligand binding or mutation affects a number of key intracellular signaling cascades. Three of the most important pathways in gliomas include the Ras/Raf/MAPK pathway, the PI3K/PTEN/AKT/mTOR pathway, and the angiogenesis pathways.

Ras/Raf/MAPK  Increased activation of the Ras/Raf/MAPK pathway has been implicated in promoting cell cycle progression and proliferation. One mechanism of activation of Ras in gliomas is the loss or negative regulatory activity of the NF1 protein as a result of mutations. While mutations in NF1 associated with development of low-grade pilocytic astrocytomas have been well described as part of the neurofibromatosis type 1 syndrome, they had not been described as prominent in high-grade astrocytomas. However, recent data from the TCGA effort have described mutation or somatic deletion of NF1 in 23% of sporadic GBM tumors [7, 63, 64]. The potential role of NF1 in the pathogenesis of high-grade astrocytomas is also demonstrated by the results of a number of mouse glioma model studies.

PI3K/PTEN/AKT/mTOR  The AKT pathway is also activated by a combination of transmembrane receptor signaling acting through PI3K and alterations at the DNA level involving PI3K and AKT in addition to PTEN. PTEN is a negative regulator of AKT and as described above, loss of chromosome 10 (containing the PTEN locus) and/or mutation or deletion of PTEN is seen in a high percentage of high-grade astrocytomas. In addition, recent data from the TCGA effort demonstrated lower percentages of mutations in PI3K subunits and AKT as potential novel mechanisms of activation of this pathway in GBM [7].
Angiogenesis  Increased vascularity and microvascular proliferation are important histopathologic features of GBM that separate it from lower-grade astrocytomas. One of the most important signaling factors associated with vascular proliferation in GBM and other tumors is VEGF which acts through multiple VEGF receptors (VEGFRs). VEGF can be secreted by tumor cells and is expressed at particularly high levels in areas of hypoxia and necrosis. Secreted VEGF binds VEGF receptors on vascular endothelial cells, resulting in growth and maturation of new blood vessels. Other signaling pathways including PDGF, EGF, and AKT can also promote angiogenesis both through activation of the VEGF/s VEGFR pathway and other mechanisms. Angiogenesis and angiogenesis-inducing signaling pathways have become an important therapeutic target in GBM and represent some of the most significant successes for targeted therapies in this disease [58, 59].

Molecular Classification and Clinically Relevant Subtypes of Glioblastoma
The molecular alterations associated with different histologies and grades of gliomas described above are derived from the work of many investigators over several years. However, recent technological developments have enabled more rapid and comprehensive investigation of the molecular characteristics and alterations of these and other tumors. While this global molecular understanding of glioma biology is still in evolution, these studies have already led to a number of novel observations that have potentially important clinical implications.

One of the key findings to come out of these global molecular profiling studies is that the single histopathologic entity defined by the WHO criteria as GBM actually consists of multiple molecular phenotypes that are characterized by significant differences in the molecular alterations observed at the DNA, RNA, and protein levels [7, 63–70]. One of the most powerful of these approaches has been global gene expression profiling in which the expression levels of 20,000 or more genes can be measured simultaneously from a single tumor sample. Application of various bioinformatic approaches to large data sets consisting of gene expression data from multiple tumors can be used to identify robust subgroups of tumors that share similar gene expression profiles. This approach has been taken by a number of groups [65–70]. While the names of the subtypes and the specific genes that define them vary between these studies, there are a number of aspects that are common across many analyses. In particular, the majority of these studies have identified at least one molecular subtype of GBM that is characterized by increased levels of expression of genes associated with mesenchymal differentiation, extracellular matrix, invasion, and angiogenesis. These “mesenchymal” tumors are associated with worse prognosis in most studies and may be associated with “primary” GBMs. Another molecular subtype of GBM that has been identified and validated in multiple data sets is characterized by increased expression of genes associated with normal neural tissues or neural development. These “proneural” tumors have been associated with better prognosis and “secondary” GBMs (and see below). Figure 2.1a and b highlights examples of gene expression subtypes identified in independent data sets.
Fig. 2.1 Gene expression and integrated molecular subclassification of GBM. (a) and (b) show figures from Phillips et al. [66] and Verhaak et al. [63], respectively, demonstrating gene expression subtypes of GBM identified from gene expression studies. Genes with high expression are shown in red, while genes with low expression are shown in green. The Proneural (Pn) and Mesenchymal (Mes) subtypes appear to be particularly robust and have been observed and validated in multiple data sets. (c) shows the association of other molecular alterations with the gene expression subsets from Verhaak et al. [63]. Higher frequency of TP53 and IDH1 mutations is evident in the Proneural type, while EGFR amplification is more common in classical and NF1 mutation is observed at higher frequency in the mesenchymal subtype.

The identification of robust molecular subtypes of GBM has been accelerated even further by the comprehensive effort to molecularly characterize a large cohort of GBM tumors as part of The Cancer Genome Atlas (TCGA) Network effort. Integrated analyses of GBM DNA copy number, mutation, and gene expression from this effort further highlight the potentially important links between molecular phenotype and clinical outcome [7, 63, 64]. These studies suggest that GBM tumors with proneural gene expression patterns were associated with high rates of point mutations in IDH1, amplification of PDGFRα, and mutation or loss of heterozygosity of TP53 [63]. Classical tumors in this gene expression classification were associated with high rates of amplification of chromosome 7 and EGFR amplification, while mesenchymal tumors were associated with higher rates of loss of chromosome 17q11.2 (containing NF1) and mutation of the NF1 gene [63]. These
data are summarized in Fig. 2.1c adapted from Verhaak et al. [63]. In addition, analysis of clinical outcomes in this retrospective study suggested that there was a differential response to therapy as a function of molecular subgroups.

Taken together, these data suggest that the prior classification of GBM tumors as primary or secondary can be further refined based on the prevalence of key molecular alterations observed within each subtype. The finding that a high proportion of low-grade astrocytomas and proneural GBM tumors demonstrate similar rates of p53 mutation, IDH1 mutation, and PDGF/PDGFR overexpression strongly suggests that the vast majority of secondary GBMs are actually proneural (and vice versa). On the other hand, primary GBMs appear to consist of several gene expression subtypes that are all associated with higher frequency of amplification of chromosome 7 and loss of chromosome 10 than proneural tumors. In addition, the distinct subtypes of primary GBM are also associated with additional specific alterations. For example, the mesenchymal subtype is associated with higher frequencies of chromosome 17q11 deletion (containing the NF1 gene) as well as higher frequencies of NF1 mutation. The newly described classical subtype is associated with higher level amplification of chromosome 7p11.2 and EGFR and loss of chromosome 9p21. A summary of this updated view of the pathogenesis of primary and secondary GBMs is shown in Fig. 2.2. Thus, while these data continue to evolve, it is highly likely that better understanding of these and other molecular alterations will eventually lead to both a more detailed understanding of the tumor biology and the identification of novel therapeutic targets for individual tumor subtypes.

**Fig. 2.2** An updated view of the development of primary and secondary glioblastoma highlighting key molecular alterations associated with specific tumor grades and/or molecular subtypes
Prognostic and Predictive Markers

In addition to a better understanding of the molecular pathogenesis of gliomas, the growing molecular profiling data have led to the identification of molecular biomarkers that are beginning to be used to help guide patient therapy. Clinically relevant biomarkers can be roughly divided into those that are prognostic and predictive. Prognostic markers are associated with patient outcome or natural history of the disease in patients without treatment or in patients receiving non-targeted therapies. Predictive biomarkers are associated with differential outcome to treatment with a specific targeted therapy. Recent studies have identified several molecular markers prognostic of patient outcome to standard therapy (radiation and temozolomide [71]) in glioblastoma. These include MGMT promoter methylation [72] and a multigene predictor based on gene expression levels of multiple genes, many of which define the proneural and mesenchymal tumor types [73, 74].

Recent evidence from TCGA also identified an epigenetically defined tumor subset that appears independent of MGMT. The majority of low–intermediate-grade gliomas have been found to exhibit the CpG methylator phenotype (CIMP), analogous to prior descriptions of CIMP in colon cancer and other tumors [75]. CIMP-positive tumors are positively associated with \textit{IDH1} mutation and show improved prognosis, compared to CIMP-negative tumors. Future work will be required to define the causative role of CpG island methylation and gliomagenesis in CIMP-positive tumors.

The identification of molecular markers predictive of outcome to targeted therapies for GBM has been more problematic, due in part to the relative paucity of effective agents for this disease and the statistical difficulty of identifying responsive subgroups from the relatively small sample sizes in most GBM studies. For example, some studies found that high expression of EGFR [76] or presence of the \textit{vIII} mutant of EGFR [77] was associated with improved outcome to EGFR inhibitors, while high levels of phosphorylated Akt [76] or low expression of PTEN [77] was associated with worse outcome to these agents. However, several subsequent studies in different types of GBM patients have failed to demonstrate a clear predictive power of these markers for treatment outcome with EGFR inhibitors [38]. Thus, the attempts to integrate and apply the growing body of high-throughput molecular profiling data in GBM to identify robust predictive biomarkers of outcome and sensitive or resistant molecular subtypes to specific targeted agents remain an active area of investigation in neuro-oncology.

Cell of Origin and the Tumor Stem Cell Hypothesis

\textit{Mouse Models}

While our knowledge regarding important molecular events in the pathogenesis of gliomas is expanding exponentially, it is perhaps surprising that the cell of origin and key initiating events for initiation of specific tumor types remain obscure.
However, data from normal development, animal models, and recent identification of tumor stem cells from multiple primary brain tumors have shed some light on these important questions.

Gliomas are classified pathologically based on their resemblance to astrocytes and oligodendrocytes. While these cells have historically been hypothesized to be the cell of origin for astrocytomas and oligodendrogliomas, respectively, more recent data have brought this hypothesis into question. In particular, several lines of evidence from mouse models suggest that gliomas can originate from less differentiated normal cells (stem cells or progenitors) in addition to more differentiated cells (astrocytes or oligodendrocytes). Furthermore, both the cell of origin and initiating molecular events have a significant impact on the likelihood of tumorigenesis and ultimate tumor histopathology. For example, activation of Ras and AKT in neural progenitors resulted in high-grade astrocytoma but not when the same molecular alterations were targeted to more differentiated cells [78]. However, the addition of Ink4a/Arf deletion allowed tumor initiation in more differentiated cells with the addition of Ras, Ras + AKT [79], or EGFR [80]. Thus, tumor initiation depends not only on the oncogenic “hit” but also on the cell of origin. Additional oncogenic alterations in mouse models that result in different outcomes based on cell of origin and cooperating alterations include mutation of NF1, p53, and PTEN [81, 82], PDGF [83], and v-ErbB [84].

**Tumor Stem Cells**

Recent developments have highlighted the potentially important role of a subpopulation of cells within a tumor called tumor stem cells in many tumors including gliomas [85, 86]. The tumor stem cell hypothesis proposes that all the cells within a tumor are not equal, but rather that tumors contain a functional hierarchy of cells similar to that observed in the normal development of multiple human organs [87]. In this model, a small percentage of cells in the tumor called tumor stem cells possess the ability to self-renew and give rise to daughter cells that maintain the stem cell pool within the tumor. In addition, these tumor stem cells have the capacity to initiate tumors and divide and “differentiate” into a heterogeneous mix of cells that not only make up the bulk of the tumor but also actually lack the key stem cell properties of self-renewal and tumor initiation. Recent studies have identified populations of cells from GBM and other brain tumors with stem cell properties [88–98]. In some studies, these stem cells were defined by expression of the surface marker CD133 [89, 90]. However, other data suggest that while CD133 may be an enrichment marker for tumor stem cells, CD133-negative cells maintain tumor initiation properties in some cases [99, 100] and thus additional markers of brain tumor stem remain to be discovered. Other lines of evidence suggest that tumor cells with stem cell properties are associated with increased resistance to radiation and chemotherapy [101, 102], are associated with a perivascular location in human tumors [103], and may even promote angiogenesis [104]. Thus, while significant
controversy remains regarding the optimal functional and surface marker definitions of tumor stem cells in gliomas, there is increasing evidence that cells with stem cell properties play an important role in tumor initiation and treatment resistance in these and other tumors.

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