Chapter 4
Molecular-Genetic Classification of Gliomas and Its Practical Application to Diagnostic Neuropathology

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Abstract Gliomas represent a broad category of tumors affecting the central nervous system of patients of all ages. Those that are diffusely infiltrative, such as the diffuse astrocytomas and oligodendrogliomas, occur most frequently in the cerebral hemispheres of adults and have a strong tendency toward clinical progression. The highest grade form, glioblastoma (GBM), WHO grade IV, has a dismal prognosis and can present either de novo or evolve from a lower grade precursor. The classification and grading of diffuse gliomas has historically been based primarily on histopathologic features, yet molecular biomarkers have now become an established component of the neuropathologic diagnosis, since molecular alterations are more reproducible classifiers and provide additional value in prognostication and prediction of therapeutic response. *Isocitrate dehydrogenase (IDH)* mutations are frequent in grade II and III diffuse gliomas of adults, as well as secondary GBMs, and are a major discriminate of biologic class. *IDH*-mutant diffusely infiltrative astrocytomas (grades II and III), as well as secondary GBMs, are characterized by *TP53* and *ATRX* mutations. Oligodendrogliomas are also *IDH*-mutant, but instead are characterized by 1p/19q co-deletion and mutations of *CIC, FUBP1*, *Notch1* and the *TERT* promoter. Primary GBMs typically lack *IDH* mutations and demonstrate *EGFR, PTEN, TP53, PDGFRA, NF1* and *CDKN2A/B* alterations and *TERT* promoter mutations. Pediatric gliomas differ in their spectrum of disease from those in adults; high grade gliomas occurring in children frequently have mutations in *H3F3A*, *ATRX* and *DAXX*, but not *IDH*. Low grade neuroepithelial tumors of childhood, such as pilocytic astrocytoma, pleomorphic xanthoastrocytoma, ganglioglioma, dysembryoplastic neuroepithelial tumor and angiocentric glioma have molecular pathogenesis and clinical behavior distinct from adult gliomas often harbor mutations or activating gene rearrangements in *BRAF, FGFR1 and MYB*.

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H. Duffau (ed.), *Diffuse Low-Grade Gliomas in Adults*,
DOI 10.1007/978-3-319-55466-2_4

Keywords Infiltrating glioma • Neuroepithelial tumor • Glioblastoma • Molecular-genetic • Biomarker • IDH mutation • 1p/19q codeletion

4.1 Introduction

The molecular genetic understanding of diffuse gliomas has improved dramatically in the past decade, and with it, the neuropathologic classification has also evolved in parallel. Not too long ago, diffuse gliomas were subdivided into oligodendrogliomas, astrocytomas and oligoastrocytomas based entirely on their histologic appearance under the microscope and graded from II to IV using morphologic criteria included within the World Health Organization (WHO) Classification of Tumors of the Central Nervous System (CNS) [1]. An abundance of evidence that emerged in the past 10 years now clearly indicates that molecular genetic subdivisions of diffuse gliomas are more reflective of their biologic properties and can be relied upon to reproducibly establish clinically meaningful diagnoses [2–4]. The revised 4th edition of the WHO Classification emphasizes the importance of molecular-genetics and establishes a new era in diagnostic neuropathology, in which genotype is incorporated into an integrated diagnosis rather than reported as an ancillary test result. While these changes remain a work in progress, a solid diagnostic platform is firmly in place [4, 5].

Bailey and Cushing's original classification of brain tumors in the early twentieth century was based on their presumed histogenesis and it introduced many of the diagnostic categories that we still recognize today [6, 7]. In their diagnostic schema and those that followed for the next 90 years, the prototypic diffuse astrocytomas exhibited irregular, hyperchromatic nuclei embedded within a fibrillary background while oligodendrogliomas were characterized by round, uniform nuclei, perinuclear halos ('fried-egg appearance') and a delicate network of branching fine capillaries ('chicken-wire' vasculature). Although the term "oligoastrocytoma" did not appear in the original classification, it did not take long for this diagnosis to gain popularity, since it was difficult to clearly place all diffuse gliomas into either the astrocytoma or oligodendroglioma category based on morphology, and some tumors appeared to contain both histologies under the microscope. The proper classification of these entities impacted tumor grading as well, since criteria for grading differed among histologic classes. For example, among astrocytomas, presence of mitoses (often just one) distinguished a diffuse astrocytoma (WHO grade II) from an anaplastic astrocytoma (WHO grade III) while necrosis and/or microvascular proliferation were required for a diagnosis of GBM (WHO grade IV) [1, 7]. For oligodendroglioma, on the other hand, the presence of florid microvascular proliferation and necrosis does not have the same grading implications, since a WHO grade IV does not exist. The distinction between a low grade oligodendroglioma (WHO grade II) and an anaplastic oligodendroglioma (WHO grade III) relied on identifying many mitoses (>6 six per ten high power fields), florid microvascular proliferation or areas of necrosis [8, 9]. The designation of oligoastrocytomas introduced additional diagnostic confusion in the past, since criteria for their classification and grading were

not agreed upon and the lack of reproducibility was considerable. Altogether, the classification of tumor lineage and grade based on morphologic criteria alone led to unacceptable levels of diagnostic discordance and caused confusion in patient care.

Molecular diagnostics, in conjunction with histopathologic and clinico-radiologic findings, are now an integral component of diagnostic surgical neuropathology and are routinely used to subdivide gliomas into diagnostic categories of clinical significance [10, 11]. In this chapter we focus on relevant emerging molecular pathways in gliomagenesis, the molecular classification of infiltrating gliomas and the diagnostic, prognostic and predictive implications of molecular biomarkers.

4.2 Isocitrate Dehydrogenase (IDH) 1/2 Mutations Divide Adult Infiltrating Gliomas Into Clinically Relevant Subsets

Isocitrate dehydrogenase genes are central to the molecular understanding and diagnosis of diffuse gliomas. The five genes encoding isocitrate dehydrogenases (*IDH1, IDH2, IDH3α, IDH3β,* and *IDH3γ*) can be further subdivided into two subclasses: (1) three are NAD(+)-dependent and localize to the mitochondrial matrix; (2) two are NADP(+)-dependent, with one localized to the mitochondria and the other to the cytoplasm. All catalyze the oxidative decarboxylation of isocitrate, producing α-ketoglutarate and carbon dioxide. The IDH3 isoform exists as a heterotetramer consisting of two alpha, one beta and one gamma subunit and is the NAD(+)-dependent isocitrate dehydrogenase that catalyzes the rate-limiting step of the tricarboxylic acid cycle (Krebs cycle) within the mitochondria. IDH1 and IDH2 are homodimers catalyzing the same reaction outside the context of the Krebs cycle and, in contrast to IDH3, use NADP+. IDH1 is the only isoform localized to the cytoplasm [12, 13].

Somatic heterozygous mutations in the *IDH1* and *IDH2* genes (chromosomes 2q33.3 and 15q26.1, respectively) are now thought to represent initiating pathogenic events in a subset of diffuse gliomas and divide them into biologically distinct subsets [14–18]. Initial studies showed that *IDH1* mutations resulted in a loss of the enzyme's role in catalyzing the conversion of isocitrate to α-ketoglutarate, yet subsequent studies demonstrated a gain-of-function that led to accumulation of the oncometabolite 2-hydroxyglutarate (2-HG) [19]. Only mutations within the enzymatic active sites of IDH1/2 confer the ability to convert α-ketoglutarate to 2-HG. Elevated levels of 2-HG inhibit enzymes that regulate cellular epigenetic status, including α-ketoglutarate-dependent histone demethylases, the TET family of 5-methylcytosine (5mC) hydroxylases and DNA demethylases, resulting in genome-wide epigenetic alterations [13, 20, 21]. Among the diffuse gliomas, the subset with the highest level of DNA methylation is referred to as CpG island methylator phenotype (G-CIMP) and these are directly related to the presence of *IDH* mutations [20–25]. Epigenetic changes set in motion by *IDH* mutations result in global changes in gene transcription that promote gliomagenesis [22].

IDH mutations are now recognized as a defining molecular event in the large majority of lower grade infiltrating gliomas and secondary GBMs. More than 80%

of WHO grades II and III astrocytomas and secondary GBMs are *IDH*-mutated, while only about 5% of primary GBMs are [14, 15, 17, 26–29]. By current definitions, all oligodendrogliomas are *IDH*-mutated and show the additional finding of chromosome 1p and 19q co-deletion. *IDH1* and *IDH2* mutations are centered at the enzyme's active site and result in a substitution for a key arginine at codons R132 and R172, respectively [15, 30, 31]. The most frequent *IDH* mutation, representing 92.7% of all mutations, occurs at codon 132 of the *IDH1* gene, and results in the substitution of arginine for histidine (R132H) [30]. *IDH1* mutations are followed in frequency by R132C (4.1%), R132S (1.5%), R132G (1.4%), and R132L (0.2%) [30]. Residue R172 in exon 4 of the *IDH2* gene is homologous to R132 in the *IDH1* gene, with R172K representing 65% of all *IDH2* mutations followed by R172M (19%), and R172W (16%) [30]. *IDH2* mutations occur at much lower frequencies (approximately 3%) than *IDH1* mutations among the diffuse gliomas, but are more frequent in oligodendrogliomas than astrocytomas [30]. Other uncommon *IDH* mutations occurring at much lower frequencies have also been reported [15, 18, 30].

Adult patients with infiltrating gliomas harboring *IDH* mutations are significantly younger than those without these mutations; however *IDH* mutations are uncommon in patients younger than 18-years-old and very rare in tumors of childhood [15, 30–35]. The median age of patients with *IDH*-mutant low grade gliomas was 36-years compared to 44-years for those harboring 'wild-type' (wt) tumors [27]. In contrast to *IDH*-wt diffuse gliomas, those that carry IDH mutations exhibit a slower rate of progression and improved clinical outcomes, grade for grade [15, 34]. The finding of an *IDH* mutation in a glioma strongly supports the diagnosis of a diffusely infiltrative glioma since they are rarely, if ever, found in other CNS neoplasms. *IDH* mutations are thought to be stable through the course of disease, but further study is needed [13]. Following an initiating *IDH* mutation, the differentiation of a diffuse glioma into the astrocytoma phenotype involves the acquisition *TP53* mutations (chromosome 17p13.1) and alterations (mutation or deletion) of *α-Thalassemia/Mental Retardation Syndrome X-linked* (*ATRX*; chromosome Xq21.1). In contrast, an oligodendroglioma phenotype is accompanied by whole arm losses of chromosomes 1p/19q, and mutations of *CIC, FUBP1* and *telomerase reverse transcriptase promoter (TERT-p)* [15–17, 26, 35–38].

4.3 The Molecular Signature of IDH-Mutant Astrocytomas

The Cancer Genome Atlas Research Network (TCGA) investigation of WHO grades II and III diffuse gliomas (morphologically diagnosed as oligodendrogliomas, astrocytomas and oligoastrocytomas) used an integrated, multiplatform whole genome approach and found that these tumors could be divided into three molecular subgroups that were best represented using two biomarkers: *IDH* mutations and co-deletion of 1p/19q. Two of the subgroups were *IDH*-mutated but separated on the basis of whole arm losses of chromosomes 1p/19q. The third subgroup harbored neither *IDH* mutations nor 1p/19q co-deletion and was referred to as *IDH*-wt.

Approximately two-thirds of the *IDH*-mutant WHO grade II and III diffuse gliomas had intact 1p/19q; of these 94% had mutations in *TP53* and 86% had inactivation of *ATRX,* a gene involved in chromatin remodeling pathways and DNA methylation [16]. Thus, the molecular signature of *IDH*-mutant astrocytoma includes *IDH* mutation, *TP53* mutation and functional loss of *ATRX* [14, 15, 28, 34, 36, 39].

The tight coupling of *IDH* and *TP53* mutations with inactivating alterations of *ATRX* has now been firmly established. Among *IDH*-mutant tumors, inactivating mutations of *ATRX* appear to be restricted to those carrying *TP53* mutations and this combination is almost mutually exclusive with co-deletion of 1p/19q [16, 38–43]. The neuropathologic diagnosis of an *IDH*-mutant diffuse astrocytoma of grade II, III or IV can be established by documenting *IDH* mutations, ATRX loss and *TP53* mutations. There are a number of ways to achieve this, including focused or whole genome sequence analysis. Immunohistochemistry for IDH-1 R132H, ATRX and p53 is also reliable and cost-effective in the routine workup of infiltrating gliomas. The finding of immunoreactivity for IDH-1 R132H, p53 (strong in over 10% of cells) together with the loss of nuclear ATRX staining is diagnostic of an *IDH*-mutant diffuse astrocytoma and grading criteria can then be applied (Fig. 4.1).

Together with *Death-domain associated protein* (*DAXX;* chromosome 6p21.3), ATRX is a core mediator of a chromatin remodeling complex necessary for the incorporation of histone variant H3.3 into the telomeres of chromosomes. Telomere maintenance is required for chromosomal integrity in the setting of numerous cell divisions associated with long-term tumor growth. ATRX/DAXX complex-mediated

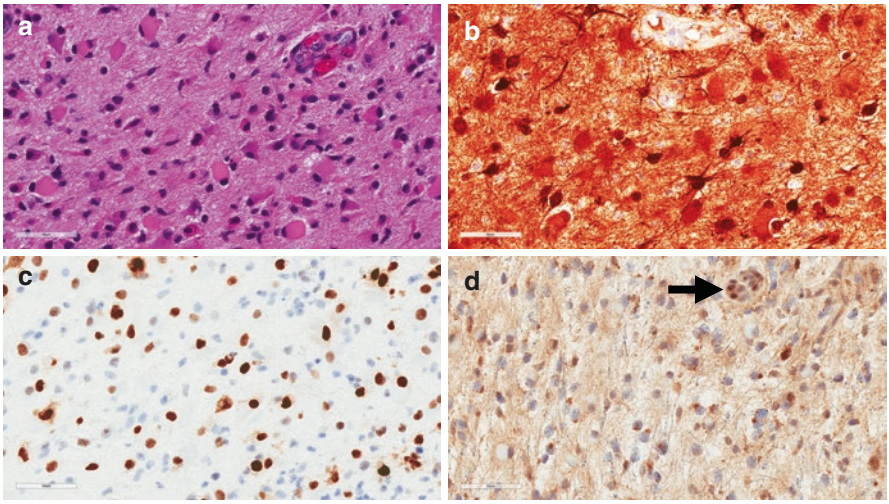

Fig. 4.1 (**a**) This infiltrating astrocytoma has hyperchromatic elongated astrocytic nuclei as often seen in the prototypic infiltrating astrocytomas but a significant proportion of tumor cells exhibit abundant globose eosinophilic cytoplasm ('gemistocytic morphology'). (**b**) The IDH-1 R132H-specific immunostain is strongly positive with diffuse cytoplasmic immunoreactivity. (**c**) A significant proportion of tumor nuclei are positive for p53 immunostain. (**d**) Loss of nuclear immunoreactivity is observed with ATRX immunostain (*arrow* shows internal positive control in endothelial cells)

chromosomal maintenance has been implicated in telomere stability and its alteration results in alternative lengthening of telomeres (ALT), a telomerase-independent pathway for telomere maintenance that has been recognized in a significant subgroup of malignancies. Mutations in *DAXX* or *ATRX* impair the heterochromatic state of the telomeres, probably because of reduced incorporation of chromatin onto H3.3 histones. *TP53* mutations play a complimentary role with genomic instability and ALT, since tumor cells presumably then have the capacity to evade apoptosis and become immortalized [38, 41, 44–49]. Nearly all *ATRX*-mutated cases of diffuse glioma also harbor *TP53* mutations and it is thought that *TP53* mutations occur first and predispose toward the acquisition of *ATRX* alterations [38]. Others have shown that the ALT phenotype in astrocytomas is correlated with a younger patient age; loss of ATRX expression by immunohistochemistry; p53 immunoreactivity; *IDH* mutations; and absence of epidermal growth factor receptor (*EGFR*) amplifications [50].

Prognostic markers for *IDH*-mutant astrocytomas will need to be better defined in order to stratify risk for this population and there is potential that additional genetic events may provide additional value. ATRX may be one such marker, since *IDH*-mutant, 1p/19q-intact WHO grade II gliomas with ATRX loss have been shown to have a longer median progression free survival (PFS; 4.4 years), and median overall survival (OS; 12.7 years) compared to *IDH*-mutant, 1p/19q-intact, *ATRX*-wt subgroup (PFS, 2.2 years and OS, 6.9 years), consistent with previous survival analyses [27, 51, 52]. A subset of *IDH*-mutant, 1p/19q-intact infiltrating gliomas have focal gains of 4q12 (*platelet-derived growth factor receptor alpha; PDGFRA*), 12q14 (*CDK4*), or 8q24 (*MYC*), providing additional markers for future investigation [16, 53].

4.4 The Molecular Signature of Oligodendrogliomas

Oligodendroglioma is the archetypal brain tumor with a molecular signature. While past studies primarily based on histomorphologic classifications emphasized the correlation of 1p/19q co-deletion with the oligodendroglioma phenotype and its chemosensitivity, more recent studies have stressed that the combination of *IDH* mutation and 1p/19q co-deletion is definitional rather than just an association [9, 16, 27, 42]. Thus, while *TP53* mutations and *ATRX* alterations characterize *IDH*-mutant astrocytomas, oligodendrogliomas are defined by *IDH* mutations and 1p/19q co-deletions, but not *ATRX* and *TP53* alterations, highlighting the relatively strict molecular dichotomy of *IDH*-mutant diffuse gliomas [16, 27, 42]. Therefore, assessment of the chromosomal arms 1p and 19q, in conjunction with *TP53, ATRX* and *IDH* mutations is essential in the diagnostic algorithm that effectively stratifies diffuse gliomas [27, 42, 51] (Figs. 4.2 and 4.3). Among the diffuse gliomas, *IDH*-mutant, 1p/19q co-deleted oligodendrogliomas have the longest median PFS (5.6 years) and OS (15.3 years), a finding supported by many [24, 27, 52, 54].

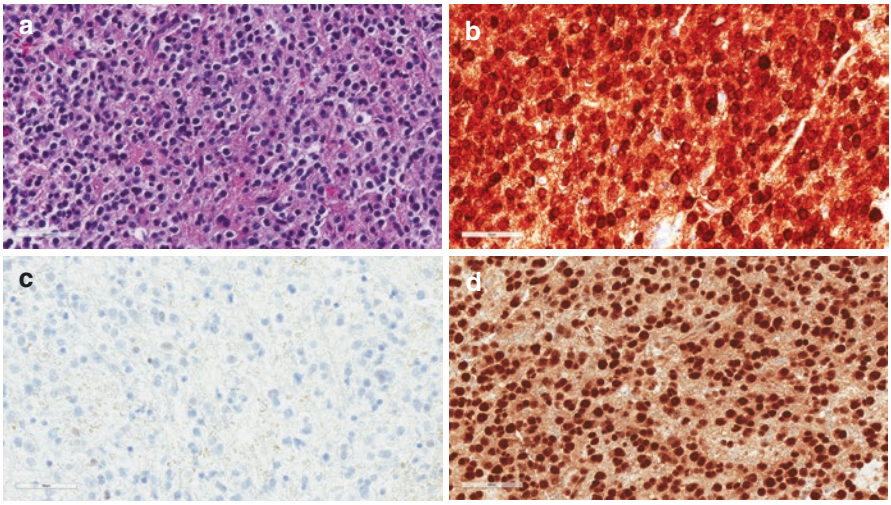

Fig. 4.2 (**a**) This low grade oligodendroglioma is comprised of tumor cells with round monomorphous nuclei and perinuclear cytoplasmic clearing ('halos'). (**b**) The IDH-1 R132H-specific immunostain is strongly positive with diffuse cytoplasmic immunoreactivity. (**c**) The p53 immunostain is negative. (**d**) ATRX immunostain highlights intact nuclear expression

Reifenberger et al. first reported that oligodendrogliomas showed a high frequency of loss of heterozygosity on the short arm of chromosome 1 (1p) and the long arm of chromosome 19 (19q) [55]. Subsequent studies demonstrated that the signature 1p/19q co-deletion is mediated through an unbalanced translocation t(1:19)(q10:p10) followed by the loss of the derivative chromosome, resulting in whole arm deletions of 1p and 19q [9, 56, 57]. Fluorescent in situ hybridization (FISH) for 1p/19q became a popular method for assessing co-deletions and is still widely used. However, it is important to realize that FISH only recognizes focal losses specific to the probes [42, 58]. Since focal losses can occur on 1p and 19q without whole arm losses, particularly in the setting of genomic instability in high grade gliomas, tests that assess only focal losses will occasionally lead to false positive results, potentially leading to the inappropriate diagnosis of oligodendroglioma [59]. Clinical tests that evaluate the entire arms, such as cytogenomic microarrays, reduce this possibility [42].

Although the R132H IDH1 mutation is the most frequent *IDH* mutation in oligodendrogliomas, there is a slightly higher frequency of *IDH2* mutations than in *IDH*-mutant astrocytomas. Other molecular-genetic alterations that occur in *IDH* -mutant, 1p/19q co-deleted oligodendrogliomas are inactivating mutations of the tumor suppressor genes *far-upstream binding protein 1* (*FUBP1*) gene and in the human homolog of the *Drosophila* capicua (*CIC*), on chromosomes 1p31.1 and 19q13.2, respectively. *FUBP1* encodes a DNA binding protein involved in c-Myc regulation and *CIC* is a downstream component of the receptor tyrosine (RTK) pathway. Mutations in *FUBP1* and *CIC* occur secondary to the unbalanced translocation with

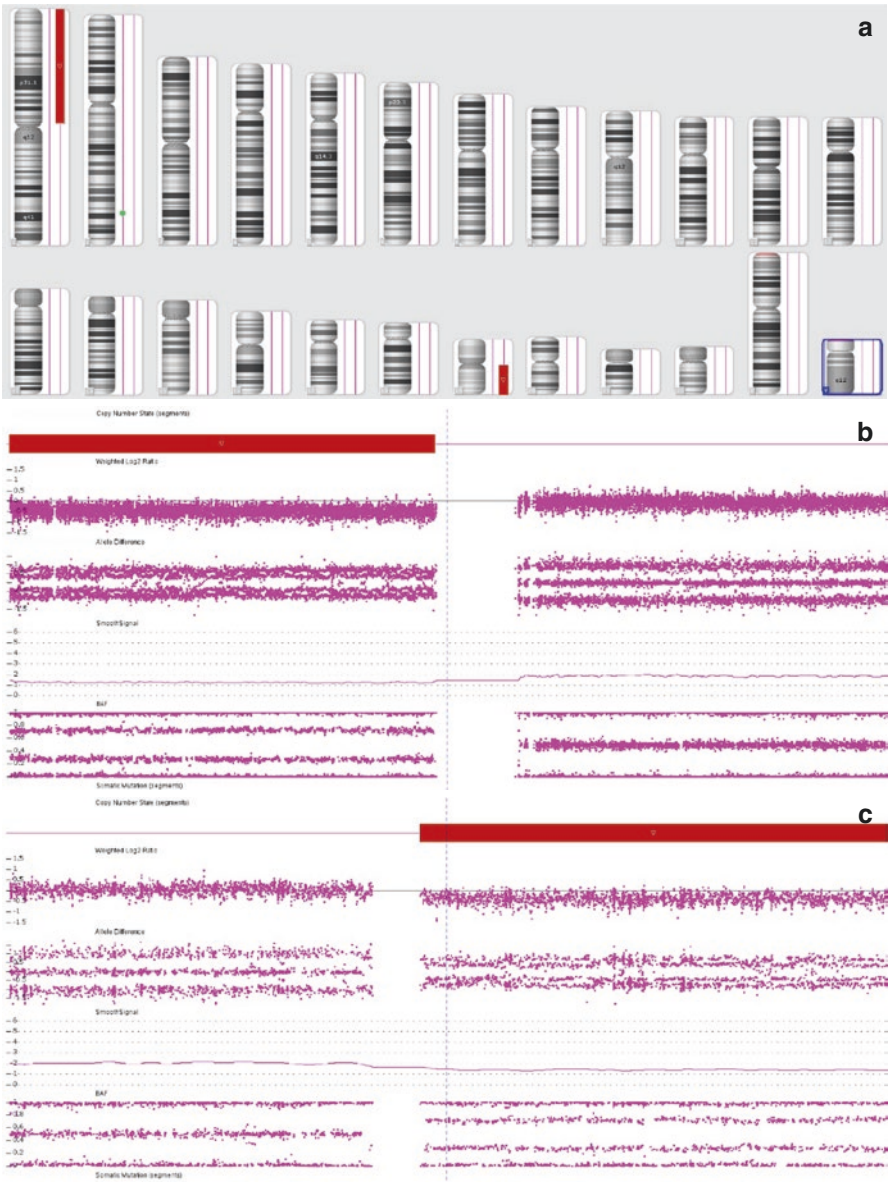

Fig. 4.3 (**a**) General view (Karyoview) of an *IDH*-mutant infiltrating glioma arising in the left frontal lobe of middle age male showing whole arm losses of 1p and 19q, and consistent with Oligodendroglioma, WHO grade II. This array detected the *IDH1* R132H mutation (*green* dot in chromosome 2). (**b** and **c**) show the detailed view of chromosomes 1 and 19, respectively, and their corresponding whole-arm losses of 1p and 19q

a frequency of 20–30% and 46–83%, respectively. These mutations are exceedingly rare in astrocytomas and are mutually exclusive with *TP53* and *ATRX* mutations [9, 16, 49, 60–63]. At present, the prognostic or predictive significance of *FUBP1* and *CIC* mutations in oligodendrogliomas remains unclear although a recent study found that outcomes of 1p/19q co-deleted gliomas were not altered by these mutations [62].

A well-known histopathologic mimic of anaplastic oligodendroglioma is the small cell variant of GBM, which needs to be distinguished since they have such differing clinical features. Small cell GBMs harbor *EGFR* (chromosome 7q12) amplifications in about 70% of the cases [9, 58, 64–66], whereas these amplifications are not seen in oligodendrogliomas and are mutually exclusive with *IDH* mutations and co-deletions of 1p/19q [59, 62]. Furthermore, imbalances of chromosome 7 and losses of chromosome 10q23 (*phosphatase and tensing homolog; PTEN*) in the context of a high grade glioma supports the diagnosis of GBM.

Nearly all *IDH*-mutated, 1p/19q co-deleted tumors also carry highly specific mutations in the *TERT* gene promoter (C228T or C250T), upstream of the *TERT* ATG start site [67–74]. *TERT-p* mutations are rare in diffuse gliomas with *ATRX* and *TP53* mutations [37, 62, 67, 74]. In distinction to ALT, activating mutations in the *TERT-p* result in enhanced telomerase activity and lengthening of telomeres. While several reports describe concordance as high as 100%, in the TCGA study of lower grade gliomas, 96% of *IDH*-mutant, 1p/19q co-deleted tumors carried *TERT-p* mutations, while only 4% of *IDH*-mutant, 1p/19q intact tumors showed this mutation [16]. However, *TERT-p* mutations are also common in up to nearly 90% *IDH*-wt GBMs [67–73]. Nearly all *IDH*-wt infiltrating gliomas with chromosome 7 gain and chromosome 10 loss harbor *TERT-p* mutations or exhibit upregulated *TERT* expression [37]. *TERT-p* mutations carry an unfavorable prognosis in the absence of *IDH* mutations (*IDH*-wt GBMs) and a favorable prognosis in the presence of *IDH* mutation and 1p/19q co-deletion (oligodendrogliomas). Although *ATRX* and *TERT-p* mutations are nearly mutually exclusive, rare cases have been reported with both or neither [67]. Among *TERT-p* mutated gliomas, there is no difference in telomere length between *IDH*-mutant and *IDH*-wt cases. However, telomeres are longer in *ATRX* altered gliomas than those with *TERT-p* mutations [37].

Thus, the molecular landscape of oligodendroglioma includes mutations in *IDH* and *TERT-p* in conjunction with whole arm losses of chromosomes 1p and 19q. Gliomas harboring these three mutations have classic oligodendroglioma phenotype and have prolonged OS [67, 75]. Other genes mutated in this subset include *NOTCH1, PIK3CA, PIK3R1, ZBTB20,* and *ARID1A*. Inactivating mutations in *NOTCH1* are only rarely identified in *IDH*-mutant, 1p/19q intact or *IDH*-wt infiltrating astrocytomas [16, 38, 61]. Other than a 1p/19q co-deletion, very few recurring whole arm copy number alterations (CNA) have been identified in oligodendrogliomas [16].

4.5 Molecular Signatures Argue Against Mixed Lineage Gliomas

The "mixed gliomas", including oligoastrocytoma and GBM with oligodendroglioma component (GBM-O), have historically suffered from considerable interobserver variability in classification and grading. The 2007 WHO Classification recognized mixed gliomas as oligoastrocytomas grades II-III, as well as GBM-O, WHO grade IV, and defined them as diffusely infiltrating gliomas composed of two distinct neoplastic cells [1]. Nevertheless, in recent years numerous investigations have concluded that mixed gliomas can be usually classified as either astrocytomas or oligodendrogliomas at the molecular-genetic level and have questioned the need for the diagnosis of oligoastrocytoma [2, 16, 26, 27, 30, 35–42, 51, 58, 60, 61, 65, 67–71, 75–78]. While *IDH*-mutant gliomas are characterized by co-deletions of 1p/19q and *TERT-p* mutations or by *TP53* and *ATRX* mutations, there is no current molecular signature for oligoastrocytoma [2, 27, 38, 42]. In the TCGA analysis, the majority of tumors diagnosed as oligoastrocytomas were *IDH*-mutant and had *TP53* mutations (*IDH*-mutant astrocytomas); the remainders were found to be *IDH*-mutant and 1p/19q co-deleted (oligodendroglioma) or *IDH*-wt [16]. Others have found that most oligoastrocytomas had molecular features of oligodendrogliomas [42]. Similarly, genomic and transcriptomic studies of GBM-O have concluded that they represent either anaplastic oligodendrogliomas, *IDH*-mutant GBMs or *IDH*-wt GBMs at the molecular level, casting doubt on the need for a GBM-O designation [3]. Only rarely are cases encountered that exhibit a genuine composite of distinct tumor types, made of discrete areas of oligodendroglioma and astrocytoma, each harboring their hallmark genetic makeup [79]. It is fully expected that the application of molecular tests will result in decreased interobserver and interinstitutional variability in the diagnosis of diffuse gliomas, as well as reduced confusion related to the clinical management that has been associated with a diagnosis of mixed gliomas. At present, oligoastrocytomas are still recognized as a histological diagnosis in the revised 4th edition of the WHO Classification but its use is discouraged and, if used, should be followed by a not otherwise specified category (NOS) classifier to highlight that molecular testing was not performed or its results were inconclusive [4].

4.6 Molecular Signatures Identify Clinically Aggressive IDH-wt Infiltrating Gliomas

The presence or absence of *IDH* mutations stratifies adult infiltrating gliomas into two distinct subsets characterized by dissimilar genetic alterations and clinical behaviors, suggesting biologically distinct diseases despite histomorphologic similarities. The majority of primary (or *de novo*) GBMs are *IDH*-wt infiltrating gliomas (95%). This is in stark contrast to the grade II and III infiltrating gliomas,

which are *IDH*-wt in only 20–25% of cases [14–16, 42]. By the currently employed histomorphologic criteria for grading infiltrating gliomas, *IDH*-wt grade II and III gliomas lack necrosis and microvascular proliferation, and therefore fall short of the histologic definition of GBM, yet their molecular-genetic profiles are strikingly similar to those of *IDH*-wt GBMs and they also display aggressive clinical behavior [16, 25]. In the TCGA analysis, grade II–III IDH-wt infiltrating gliomas had a genetic profile similar to primary (*IDH*-wt) GBM and exhibited a median OS of 1.7 years [16].

Given the clinical and genomic similarities, these lower grade *IDH*-wt astrocytomas could represent undersampled or incipient GBMs that have not yet developed the microvascular proliferation or necrosis required to be histologically diagnosed as a WHO Grade IV tumor [16, 38, 42, 80, 81]. In a recent study of 160 *IDH*-wt grade II and III astrocytomas, Reuss et al. found that 78% were molecular equivalents to conventional *IDH*-wt GBM, with similar frequencies in *TERT-p* mutations, 7p gain/10q loss, amplifications of *EGFR* or combined 10q/13q/14q co-deletion. A median survival of 19.4 months was noted, consistent with the TCGA analysis. Furthermore, if those grade II and III astrocytomas with H3 mutations were included, then 87% of these *IDH*-wt astrocytomas were molecularly and clinically indistinguishable from GBM [80]. Lower grade *IDH*-wt infiltrating gliomas have a much lower frequency of *TP53* mutations than *IDH*-mutant astrocytomas. While 94% of *IDH*-mutant, 1p/19q intact infiltrating gliomas harbored *TP53* mutations, only 25% of *IDH*-wt infiltrating grade II-III gliomas are *TP53* mutated, similar to the frequency observed in *IDH*-wt GBMs [15, 34].

Other genetic alterations frequently associated with *IDH*-wt GBMs and lower grade gliomas include those involving *PTEN, EGFR, MDM4, CDK4, NF1, PIK3CA, RB1, PTPN11, PIK3R1,* and *CDKN2A* [16]. More recently, Di Stefano et al. reported *FGFR-TACC* fusions in approximately 3% of lower grade *IDH*-wt infiltrating astrocytomas, a frequency similar to that seen in primary GBMs, providing additional evidence of the similarity of clinical behaviors between these entities. *FGFR-TACC* fusions were not present in *IDH*-mutant gliomas and were mutually exclusive with *EGFR* amplifications, but often co-occurred with *CDK4* amplifications [82]. Overall, as compared to the *IDH*-mutant counterparts, *IDH*-wt gliomas have greater activation of signaling through *EGFR, MET,* and *BRAF*; upregulated cell cycle activators; and reduced cell cycle inhibitors [81]. *IDH*-wt gliomas also have upregulation of transcription factors known as master regulators, as well as their target genes [37].

A small subset (currently estimated a less than 1%) of adult low grade infiltrating *IDH*-wt gliomas harbor *BRAF* V600E somatic mutations on chromosome 7q34 resulting from a substitution of valine by glutamic acid at codon 600 (V600E) and are thought to represent a distinct clinicopathologic entity with an improved prognosis [83–85]. *BRAF* V600E mutations, which constitutively activate the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) signaling pathway, are far more frequent in grade I, non-infiltrative gliomas, pediatric diffuse gliomas and epithelioid GBMs and may have therapeutic implications [32, 83, 84]. Lastly, Ceccarelli et al. described a novel subgroup of *IDH*-wt infiltrat-

ing gliomas that genetically and epigenetically resemble pediatric pilocytic astrocytomas and carry a favorable outcome [37]. Additional studies of IDH-wt infiltrating gliomas are necessary to address the implications of subgroups that exhibit less aggressive clinical behavior.

4.7 Primary and Secondary Glioblastomas Have Distinct Genetic Signatures

GBM is histologically defined as a high grade infiltrative astrocytoma with microvascular proliferation, necrosis or both, that has a short survival, generally less than 2 years [29, 86]. The revised 4th edition of the WHO Classification reflects the primary molecular subsets of adult GBMs by dividing them into (1) GBM, *IDH*-wt, (2) GBM, *IDH*-mutant and (3) GBM, NOS. (4). The last category is reserved for cases in which *IDH* assessment could not be performed or was not available. GBMs are often referred to as "primary" (or *de novo*) when they present to medical attention as grade IV disease as the first manifestation, and as "secondary" when they have evolved over time from a grade II or III infiltrating astrocytoma. Primary and secondary GBMs are histologically indistinguishable except for larger extents of necrosis more frequently found in the former [28]. Despite their morphologic overlap, primary and secondary GBMs differ in their genetic and epigenetic landscape, with *IDH* mutations being much more frequent in secondary GBMs. Secondary GBMs arise in younger patients (usually less than 45 years) and are associated with longer survivals [14, 28, 29]. Primary GBMs represent the vast majority of cases (over 90%), are nearly all *IDH*-wt, and are characterized by a rapid clinical onset of symptoms, most often in an elderly patient.

The genetic hallmark alterations of primary, *IDH*-wt GBMs include mutations of *PTEN* and *TERT-p*, gain of chromosome 7/loss of chromosome 10, deletions of *CDKN2A,* and amplifications of proto-oncogenes, most notably, *EGFR, PDGFRA* or *c-MET*. Although the sequence of oncogenic events in *IDH*-wt primary GBMs has not been determined, it has been suggested that *TERT-p* mutations, present in up to 90% of adult GBMs, may precede the characteristic combined gain of chromosome 7/loss of chromosome 10, seen in 60% of primary *IDH*-wt GBM, followed by additional oncogenic events [37, 87]. Three core signaling pathways are nearly always altered in primary, *IDH*-wt GBM and include (1) the receptor tyrosine kinase pathway [RTK/RAS/phosphoinositide 3-kinase (PI3K)], (2) the P53 pathway and (3) the Retinoblastoma (Rb) pathway, which are altered in 88%, 87% and 78% of GBMs, respectively [86, 88]. The most frequently altered genes in the RTK/RAS/PI3K pathway include *PTEN, neurofibromin-1 (NF1), EGFR, PIK3R1, PIK3CA,* and *PDGFRA*. Alterations in the Rb pathway include *CDK4, CDK6, CCND2, CDKN2A/B* and *RB1*. The genes frequently altered in the p53 pathway include *MDM2, MDM4* and *TP53* [14, 28, 29, 54, 78, 86, 88, 89]. More recently, Morris et al. reported recurrent somatic mutations in the *FAT* tumor suppressor (*Drosophila*)

homolog 1 (*FAT1;* chromosome 4q35.2) in 20.5% of GBMs, resulting in its inactivation and leading to aberrant Wnt activation and tumorigenesis [90].

Brennan et al. has shown that 57% of GBM had evidence of mutation, rearrangement, altered splicing and/or focal amplification of *EGFR,* reflecting its status as a key oncogenic event in this disease [89]. Furthermore, approximately 50% of *EGFR* amplified tumors also harbor the variant III (EGFRvIII) deletion that leads to constitutive tyrosine kinase activation [4, 78, 91]. Approximately15–18% of primary, *IDH*-wt GBMs carry *PDGFRA* amplifications while *MDM2* and *CDK4* amplifications are present in 5–15% and 14–18% of the cases [4, 14, 29, 78, 89].

Deletions of the *CDKN2A* gene (chromosome 9p21), encoding the tumor suppressor proteins p16 (INK4a) and p14 (ARF) (activators of Rb and p53, respectively) are seen in up to 50% of GBMs [4, 14, 76–78]. *TP53* and *RB1* mutations and deletions are seen in 28–35% and 8–12% of primary GBMs, correspondingly [4, 14, 78, 89]. Activating mutations of *PI3K* are present in 12–25% of primary GBMs, with mutations in either *PIK3CA* or *PIK3R1* driving increased enzymatic activity. Deletions or mutations in *PTEN*, the primary negative regulator of the PI3K/AKT signaling pathway, occur in approximately 25–35% of GBMs. Mutations or deletions of *NF1*, a Ras antagonist, have been identified in up to 10–18% of primary GBMs [4, 14, 29, 78, 86, 88, 89].

BRAF V600E mutations are present in less than 5% of all GBMs, but are overrepresented in epithelioid GBM with approximately 50% harboring the mutation [29]. Approximately, 5% of adult primary GBMs carry *H3F3A* mutations. While the frequency of *BRAF* and *H3F3A* mutations is much lower in adult gliomas than those of children, it is important to remember that both *H3F3A* mutations and BRAF mutations will be present in tumors that do not have *IDH* mutations (i.e., *IDH* testing will reveal an "*IDH*-wt" status) and testing for these mutations will need to be performed in the relevant clinical setting in order to document these distinct diseases [29, 42]. Mutation specific immunostains against the *BRAF* V600E (VE1) and the H3 (H3K27M) mutations are readily available and clinically useful for practical diagnostic neuropathology (Fig. 4.4).

IDH mutations occur at a very low frequency in clinically diagnosed primary GBM (less than 5%) [4, 28, 92]. It is likely that *IDH*-mutant primary GBMs progressed from a non-symptomatic lower grade glioma that evaded diagnosis [28, 92]. As a corollary, secondary GBMs can occasionally be *IDH*-wt when they arise following the diagnosis of lower grade glioma; not surprisingly, secondary GBMs that lack *IDH* mutations usually have a poor prognosis [92]. Regardless, the *IDH* mutation status is more relevant to the clinical behavior of the GBM than the primary or secondary designation. Similar to the molecular-genetic makeup of their precursor lesions, *IDH*-mutant, secondary GBM frequently harbor *TP53* and *ATRX* alteration: 85% of secondary GBMs are *IDH*-mutated and *TP53* and *ATRX* mutations are seen in 81% and 71%, respectively [4, 40, 92]. Since these mutations occur early in gliomagenesis, additional alterations identified within *IDH*-mutant, secondary GBMs are likely acquired during biological progression and can serve as prognostic markers [93]. Secondary *IDH*-mutant GBMs contain the highest number of alternating,

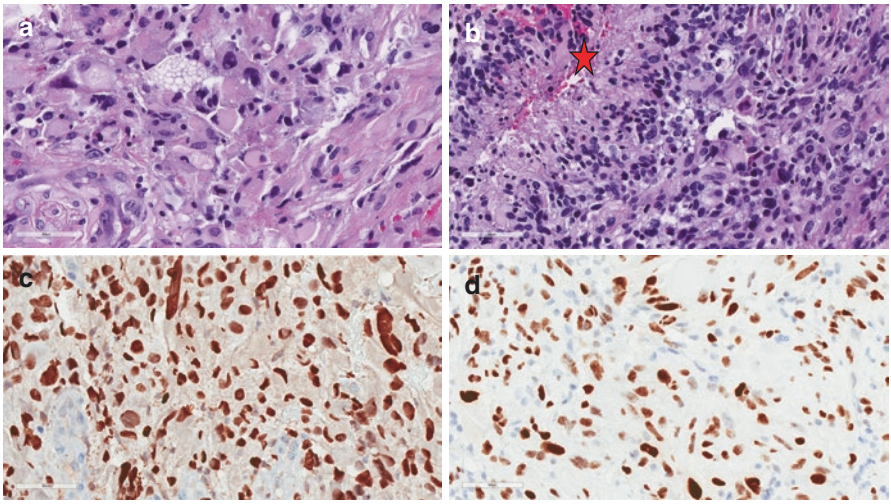

Fig. 4.4 (**a** and **b**) This GBM arose in the thalamus of a middle-aged man and was morphologically heterogeneous. The tumor was highly cellular with abundant pleomorphic cells. Pseudopalisading necrosis is evident (star in **b**). (**c**) The K27M immunostain shows strong diffuse nuclear positivity. Therefore this is best classified as a K27M-Midline GBM, WHO grade IV. (**d**) p53 immunostain is strongly positive as well. This GBM was *IDH*-wt and ATRX immunostain showed nuclear retention (not shown). *TP53* and *ATRX* mutations often co-occur with H3K27M mutations but have the highest correlation in G34R/V-mutated GBMs

intrachromosomal breakpoints, consistent with chromothripsis [81]. Thus, the GBM genotypes account for biologic differences in histologically indistinguishable tumors and improves the ability to predict patient outcomes [86].

4.8 Pediatric Gliomas Are Genetically and Biologically Distinct from Their Adult Counterparts

Pediatric gliomas are most frequently either low grade and circumscribed, or high grade and diffusely infiltrative. The low grade, well circumscribed astrocytomas (most often pilocytic astrocytomas) frequently arise in the cerebellum, followed by the cerebral hemispheres, deep midline structures, optic pathway, brainstem and spinal cord [94]. Non-infiltrative or poorly infiltrative gliomas with an affinity for the temporal lobe, are also more frequent in children and include pilocytic astrocytomas (PA, WHO grade I), gangliogliomas (WHO grade I), dysembryoplastic neuroepithelial tumor (DNET, WHO grade I) and pleomorphic xanthoastrocytomas (PXA, WHO grade II or III) [4]. The histologic findings of Rosenthal fibers, eosinophilic granular bodies (EGB's) and a low grade glioma with a biphasic appearance usually points to a diagnosis of PA and the finding of a *KIAA1549:BRAF* fusion is typical. This fusion event results from tandem duplications in the chromosome 7q34

region and is observed in more than 70% of PA's, predominantly in those arising within the cerebellum, but also in other locations [95]. A temporal lobe-predominant glioma with a relatively solid growth pattern exhibiting a combination of spindle-shaped and xanthomatous cells and pleomorphic, multinucleated astrocytes in association with EGBs points to a diagnosis of PXA and the presence of a *BRAF* V600E mutation is supportive [96]. *BRAF* V600E mutations are frequent events in pediatric CNS neoplasia including gangliogliomas (20–40%), PXA's (60–70%), DNET (30%), diffuse astrocytomas (23%) and PA's (5–10%) [32, 97].

Most low grade neuroepithelial tumors of children have only one dominant somatic genetic event that affects protein coding. In the majority, such solitary alterations are mutually exclusive and include *NF1, RAF* or *RAS*, the receptor tyrosine kinases *fibroblast growth factor receptor 1* (*FGFR1*; chromosome 8p11.23), and *V-Myb avian myeloblastosis viral oncogene homologue* (*MYB;* chromosome 6q23.3) or in its homologue, *MYBL1* (chromosome 8q13.1) [32]. In a study of 249 pediatric low grade gliomas, which included multiple histologic entities, 90% showed recurrent somatic alterations and 83% showed rearrangements or structural alterations [98]. The most frequent genetic alterations were found in genes encoding *FGFR1,* the *neurotrophic tyrosine receptor kinase 2* (*NTRK2;* chromosome 9q21.33), *KRAS* (chromosome 12p12.1), the *receptor tyrosine kinase adaptor tyrosine-protein phosphatase non-receptor type 11* (*PTPN11;* chromosome 12q24), *NF1* (chromosome 17q11.2), and *BRAF* (chromosome 7q34) [97]. Alterations of *BRAF, FGFR1, PTPN11,* and *NTRK2* all lead to the activation of the MAPK/ERK signaling pathway, making it a primary driver of pediatric low grade gliomas [32, 99]. The most specific genotype-phenotype association was the tight linkage between angiocentric glioma and the *MYB-QKI* translocation [98].

Others studies have also emphasized the significance of alterations in *MYB/MYBL1, FGFR1* and *BRAF* V600E in pediatric low grade gliomas and suggest a relationship between tumor histology and genetic alterations [32, 43, 84, 100, 101]. Qaddoumi et al. reported a high frequency of *FGFR1* alterations those tumors dominated by round, regular bland "oligodendroglial-like" tumor cells, including 82% of DNETs and 40% of diffuse oligodendroglial tumors. Tumors with astrocytic differentiation and "diffuse" patterns were more frequently characterized by *MYB* alterations, with 41% of pediatric diffuse astrocytomas showing structural rearrangements and 87% of angiocentric gliomas showing the specific *MYB-QKI* fusion [100]. These findings clearly demonstrate that low grade infiltrating gliomas arising in the pediatric population are distinct from those in adults, since the *IDH* mutations of adult diffuse gliomas are rare in the pediatric diseases and the mutations in the pediatric diseases are not present in those of adults [43]. However, the *IDH*-wt status of pediatric infiltrating low grade gliomas does not imply a more biologically aggressive behavior; the rate of progression of lower grade gliomas in the pediatric population is significantly lower than their histologically comparable adult counterparts [43, 100, 102].

Among the pediatric low grade gliomas, oligodendrogliomas represent a diagnostic challenge, since they are histologically similar to adult tumors, yet do not often harbor their defining genetic alterations of *IDH* mutations and 1p/19q

co-deletion. Only 18% of pediatric oligodendrogliomas harbor an *IDH* R132H mutation and only 25% exhibit 1p/19q co-deletion. Those that were *IDH*-mutant and 1p/19q co-deleted ('adult-type') occurred in older children and adolescents [102]. As described above, *FGFR* alterations are more frequent in pediatric oligodendrogliomas, but occur in less than half [32, 102]. *BRAF* alterations are absent in pediatric oligodendrogliomas, but the diffuse leptomeningeal glioneuronal tumor (known also as disseminated oligodendroglial-like leptomeningeal tumor), which was recently codified in the revised WHO Classification, are reported to harbor concurrent *KIAA1549:BRAF* gene fusions and 1p deletions [4, 9, 103, 104]. The precise relationship of this entity to other pediatric brain tumors, such as pilocytic astrocytoma or oligodendroglioma will require further investigation.

The high grade gliomas of childhood are also clinically and genetically distinct from those of adults. Pediatric high grade gliomas nearly always arise *de novo* and very rarely are the result of progression from a lower grade glioma. Although they differ from their adult counterparts in terms of location, clinical behavior, mutational landscape and gene expression profiles, they can similarly be separated into molecular subclasses [78]. Mutations targeting RTK/RAS/PI3K pathway, histone modification or chromatin remodeling and cell cycle regulation have been respectively found in 68%, 73% and 59% of these tumors, including diffuse intrinsic pontine gliomas (DIPG) and non-brainstem gliomas [105]. One of these classifications that included both adult and pediatric GBMs and used DNA methylation profiles identified six molecular classes: IDH, K27, G34, RTK I (PDGFRA), Mesenchymal and RTK II (Classic) [78, 106]. Two of these classes —the K27 and G34— were dominated by pediatric cases that harbored the respective *H3F3A* mutations. Korshunov et al. recently performed a large scale genomic and epigenetic integrated analysis of 202 pediatric GBMs which unexpectedly showed that 20% displayed methylation profiles similar to either low grade gliomas or PXA's, had a better OS and were also enriched for PXA-associated molecular alterations including *BRAF* V600E mutations and homozygous deletions of 9p21 (*CDKN2A*). The remaining 162 pediatric GBMs stratified into the following four subgroups: *IDH1*-mutant (6%), H3.3 G34-mutant (15%), H3.3/H3.1 K27-mutant (43%), and those GBMs that were wild type for H3 and *IDH* (36%) [107].

A genetic signature of pediatric high grade gliomas (and a smaller subset that occur in adults) includes mutations that arise in the histone variant H3.3 encoded by the genes *H3F3A* (chromosome 1q42.12) and *H3F3B* (chromosome 17q25.1), or H3.1 genes (*HIST1H3B* and *HIST1H3C,* both located on chromosome 6p22.2) [107, 108]. Two specific histone mutations in H3.3 in pediatric GBMs are mutually exclusive with *IDH* mutations; one is present at amino acid 27 resulting a substitution of lysine for methionine (K27M) and the second at position 34 resulting in a substitution of glycine for either arginine or valine (G34R/V) [109, 110]. *H3F3A* K27M is strongly aligned with high grade gliomas of the midline of younger children, with the classic presentation in the pons or thalamus. The G34R/V variant is more typical of supratentorial high grade astrocytomas and is observed in older children and young adults. The presence of an H3K27M mutation correlates with malignant behavior and shorter survival regardless of its histologic features [43,

106, 109–111]. *TP53* and *ATRX* mutations co-occur with H3.3 mutations, with the highest correlation in G34R/V GBMs and with lower, yet significant, overlap with K27M mutations [29, 110]. *H3F3A* K27M mutations have been described in high grade astrocytomas of the spinal cord in the pediatric and young adult population, further supporting the associations with younger age, aggressiveness and midline location [112].

DIPG represents a specific form of pediatric high grade glioma that typically presents between 6 and 7 years of age and has a dismal median survival of 10 months [111]. *H3F3A* mutations are present in over 70% of these tumors and *PDGFRA* amplifications are present in 28–36% [111]. Other alterations that may prove to be clinically significant include missense mutations in *ACVR1* (also known as *ALK2*; chromosome 2q23-q24) in up to 32% [105]. To date, IDH mutations have not been identified in DIPG's [111, 113]. In comparison to the pediatric counterparts, adult brainstem infiltrating gliomas occur less frequently and have a better outcome, most likely because they represent a distinct disease process or include a combination of dissimilar diseases [113].

4.9 Ancillary Testing for Biomarker-Driven Diagnosis of Infiltrating Gliomas

Distinguishing glioma lineage based on histomorphologic criteria alone can be challenging, since tumors frequently exhibit overlapping features and numerous studies have documented substantial intra- and interobserver discordance. As noted above, molecular biomarkers are objective and reproducible classifiers that can be used to complement and improve morphology-based diagnoses. Many of the biomarkers discussed above have been developed for routine use in diagnostic neuropathology and are included in immunohistochemical, molecular-genetic and cytogenomic testing platforms [114].

One of the most important prognostic and predictive biomarker used in the clinical management of patients with high grade gliomas is the methylation status of the promoter for *O6-methylguanine-DNA methyltransferase (MGMT)*. MGMT is a DNA repair enzyme with the ability to restore guanine from O6-methylguaninie induced by alkylating agents such as temozolomide (TMZ) [29, 87]. Hence, low levels of MGMT would be expected to correlate with an improved response to alkylating agents. *MGMT* promoter methylation, which occurs in about 40% of GBMs and correlates with low protein expression levels of MGMT, is consistent with enhanced response to therapy and improved overall survival. Promoter methylation is typically assessed by methylation-specific PCR [78, 115, 116]. MGMT immunohistochemistry is currently not recommended for clinical practice [108].

Gene sequencing is becoming more widely available and can be accomplished in a focused, single gene approach, a targeted gene panel, or whole exome or whole genome approach. As noted above, many genetic alterations are specific to the development and progression of glial neoplasms. From a diagnostic perspective,

genes of interest include, but are not limited to, *IDH1, IDH2, TP53, ATRX, CIC, FUBP1, TERT, NOTCH1, DAXX, EGFR, PTEN, NF1, RB1, BRAF, MYB, MYBL1, MYC, FAT, FGFR1, NTRK, ACVR1, H3F3A, HIST1H3B, PDGFRA,* and *SETD2*. Depending on the gene and its specific type of alteration, it can be assessed by immunohistochemistry, FISH or cytogenomic microarray, focused or high-throughput sequencing technologies, or multiplexed platforms.

Many gliomas are characterized by highly recurrent genomic alterations that are best assessed by a focused analysis. For example, cerebellar pilocytic astrocytomas are enriched by *KIAA1549:BRAF* gene fusions and angiocentric gliomas exhibit *MYB* alterations, most notably *MYB-QKI* rearrangements [95, 98]. While FISH probes can be used to test for some gene rearrangements, sequencing may be required in others. However, given the growing number of driver genes involved in gliomagenesis and the genomic variability of CNS tumors, next-generation sequencing (NGS) platforms are gaining application in diagnostic neuropathology [97]. NGS panels have been developed that include genes relevant to CNS neoplasms for the detection of single nucleotide variations, fusions and CNAs and have shown high sensitivity and specificity with concordance as high as 98% when compared to well-established single biomarker methods [117, 118].

Assessment of CNAs has great diagnostic utility in surgical neuropathology and both single-nucleotide polymorphism array and array comparative genomic hybridization technologies have been employed. The quality and quantity of CNAs among gliomas tend to correlate with classification, grade, progression, and prognosis [119]. FISH is a commonly employed technique to assess for CNAs at single locus including, for example, amplifications of *EGFR* and *PDGFRA*, as well as deletions of *PTEN* and *CDKN2A* in high grade astrocytomas [78]. Similarly, FISH for 1p/19q co-deletion has been used as a diagnostic marker of oligodendroglioma.

Whole genome methods (cytogenomic microarray) for assessing CNAs are increasingly being employed due to the abundance of diagnostically relevant information that is obtained. The assessment of whole arm losses of 1p and 19q is becoming critical for *IDH*-mutant glioma, since the event is definitional for oligodendroglioma (Fig. 4.3). Since the detection of 1p and 19q losses by FISH documents only focal deletions on these chromosome arms rather than the whole chromosomal arm losses associated with the unbalanced translocation that is the signature of oligodendroglioma, it is expected that false positives may result, especially in genomically unstable high grade gliomas [42, 87]. For example, Clark et al. showed that 5.7% of GBMs showed 1p/19q co-deletion by either FISH and/or PCR-based LOH but that the vast majority of these (over 90%) also had 10q LOH and/or *EGFR* amplifications, which virtually never occur in the setting of *IDH* mutations and whole arm losses of 1p/19q [59]. Thus, a false positive detection rate of approximately 6% would be expected using FISH as a marker for whole arm losses of 1p and 19q in the setting of high grade gliomas. Whole genome assessment of CNA also reliably detects gain of chromosome 7 and loss of chromosome 10, which are typical of *IDH*-wt GBMs and have been shown to correlate with a tendency of shorter survival when occurring in conjunction with 9p losses [119].

Among *IDH*-mutated gliomas, CNAs have diagnostic and potentially prognostic value. Gains of 7q are an early event in a subset of *IDH*-mutant astrocytomas and are mutually exclusive with loss of 1p/19q [119]. *IDH*-mutant gliomas with *TP53* mutations typically have at least one of the following CNAs: +7q, +8q, −9p, −11p and +12p. These CNAs are associated with a poor prognosis and/or progression and may be related to the gains or losses of *MET, MYC, CDKN2A, CDKN1C,* and *KRAS*, respectively [120]. Other losses potentially related to astrocytoma progression include 17p, the site of *TP53* and 10q, the site of *PTEN* [93]. *IDH*-mutant GBMs have been shown to harbor higher levels of CNAs and increased incidence of chromothripsis in comparison to their precursor lesions and to *IDH*-wt tumors of all grades [81].

Amplification events are often prognostically significant and are viewed as potential targets of therapy in both pediatric and adult glioma [107]. Common amplification events in primary, *IDH*-wt GBMs include several regions of interest (ROI) that contain oncogenes on the following chromosomes: 7p11.2, 7q21.2, 7q31.2 for *EGFR/CDK6/MET*, respectively; 12q14 and 12q15 for *CDK4*/*MDM2*, correspondingly; and 4q12 (*PDGFRA*). Among IDH-mutant high grade gliomas, *PDGFRA* amplifications are noted with increased frequency with higher grade and also are an independent prognostic factor in de novo *IDH*-mutant GBMs [121]. Homozygous and hemizygous deletion events that commonly occur in *IDH*-wt GBMs include the following ROI's: 17q11.2, 10q23, 9p21.3 and 13q14, corresponding to *NF1, PTEN, CDKN2A/CDKN2B*, and *RB1* genes, respectively [4, 29, 54, 87, 89, 91]. *BRAF* alterations at 7q34 can also be detected using cytogenomic microarrays.

Immunohistochemistry (IHC) is a cost-efficient method that is widely available and can be used for determining the protein expression patterns that correlates with genetic alterations. Commonly used IHC stains used to classify gliomas include IDH1 R132H, p53, ATRX, H3K27M, BRAF, CIC, and FUBP1 [42, 80, 108]. *IDH* mutations are critical for distinguishing between subtypes of gliomas and can also be used to distinguish between glioma and reactive gliosis. *IDH1* R132H mutation accounts for more than 90% of all *IDH* mutations and a monoclonal mutant-specific antibody recognizes the mutant protein with cytoplasmic immunoreactivity with high sensitivity and specificity [87, 122]. Since other rare non-R132H *IDH1/2* mutations will not be recognized with this immunostain and as the designation of a diffuse glioma in adults as *IDH*-wt has gained important clinical and therapeutic significance, gene sequencing analysis of *IDH1* codon 132 and *IDH2* codon 172 is recommended in the event of a negative or indeterminate result with *IDH1* R132H immunostain [78, 108]. It has recently been suggested that sequencing may not be warranted in the setting of a negative R132H immunostain in GBMs arising in patients older than 55 years due to the rarity of non-R132H IDH1 mutations [4, 5].

TP53 mutations are frequent in lower grade, *IDH*-mutant infiltrating astrocytomas and are almost mutually exclusive with 1p/19q co-deletions. The detection of p53 by IHC can be used as a surrogate for *TP53* mutations and in support of an astrocytic lineage, but only with some significant caveats. The p53 immunostain recognizes the normal protein and is not specific for mutations. *TP53* mutations

leads to reduced degradation of the protein and nuclear accumulation of both mutant and wild-type gene products [78]. Strong nuclear p53 positivity in >10% tumor nuclei is a predictor of *TP53* mutations, but should be evaluated in the context of morphology and other test results [108]. Inactivating mutations in *ATRX* commonly co-occur with *TP53* mutations in the setting of *IDH*-mutant lower grade infiltrating astrocytomas [16]. In combination with 1p/19q and *IDH1/2* mutational status, *ATRX* alterations have become part of the molecular diagnostic algorithm for the refinement of diffuse glioma lineage. *ATRX* mutation results in a truncated protein and in abrogated protein expression, which correlates very well with loss of nuclear immunoreactivity of ATRX [42, 78]. Of note, it is important to evaluate the immunoreactivity of non-neoplastic nuclei, such as those of endothelial cells, as an internal positive control in order to correctly assess ATRX status [108]. Several studies have highlighted its prognostic value since better clinical outcomes have been noted in *IDH*-mutant astrocytomas with ATRX loss as compared to *ATRX*-wt subsets [27, 123].

The revised 4th edition of the WHO Classification recognizes the entity of diffuse midline glioma, H3 K27M-mutant, highlighting the tight coupling of this mutation to a specific form of high grade glioma [4]. K27M mutations involving either the H3.3 or H3.1 histones can be detected by nuclear staining using H3K27M immunohistochemistry with a sensitivity and specificity of 100% [124] (Fig. 4.4). Another clinically useful immunostain is the mutation specific *BRAF* V600E (VE1) which has a sensitivity of 100% and a specificity of 98% for the mutation, but only if strong cytoplasmic positivity is considered as positive [108].

Both *CIC* and *FUBP1* mutations are specific to oligodendrogliomas and are found only in the setting of *IDH* mutations and 1p/19q co-deletion. Loss of nuclear CIC expression by IHC suggests a loss-of-function mutation but the sensitivity and specificity is relatively low (69% and 87%, respectively) [108]. Loss of nuclear FUBP1 by IHC correlates with inactivating mutations with a sensitivity of 100% and specificity of 90% in oligodendrogliomas. However, there is currently no consensus for evaluating these immunostains and their prognostic values have not been completely elucidated [87, 108].

4.10 Conclusion

Gliomas are common brain tumors that are highly variable with respect to location, histomorphology, molecular-genetic signatures, clinical behavior and treatment responses. The diagnosis of gliomas in the past has suffered from low reproducibility due to intraobserver, interobserver and interinstitutional variability [125]. Specific molecular alterations, or their combinations, are now known to carry diagnostic, prognostic and/or predictive value. Molecularly defined subsets of gliomas are more cohesive and reproducible, capturing the biologic features better than histopathology alone. We are in transition from a histology-based practice to an integrative, biomarker-driven diagnosis that will optimize patient stratification and

treatment and enhance research efforts. With the revised 4th edition of the WHO Classification, molecular parameters have been added to histologic class to define many entities [4]. Other entities have been dropped or discouraged, such as gliomatosis cerebri and oligoastrocytomas, which can both be unequivocally assigned to other molecularly defined subgroups [2, 126].

Important progress has been made by integrating molecular-genetic alterations with tumor classification, but new questions that have arisen require further attention. In particular, risk stratification within genetic subsets of disease will need to be re-evaluated and optimized, a subject of ongoing investigations. Recent studies suggest that histologic grade (II vs III) and mitotic activity are not highly informative among *IDH*-mutant infiltrating gliomas for predicting outcome [52]. It has also been demonstrated that there are little differences in age at presentation and survival between grade II and III *IDH*-mutated astrocytomas [127]. Furthermore, *IDH*-wt anaplastic astrocytomas, which are a WHO grade III neoplasm, have a poorer outcome than *IDH*-mutant GBMs, a WHO grade IV neoplasm [128]. As molecular platforms evolve and become more sophisticated, the field of diagnostic neuropathology will undergo further maturation and the need for comprehensive molecular analysis of CNS tumors will increase with the identification of clinically significant genetic biomarkers.

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