The Molecular Biology of Diffuse Low-Grade Gliomas

8

Nicholas F. Marko and Robert J. Weil

Abstract

The World Health Organization (WHO) grading scheme for glial neoplasms assigns grade II to three infiltrating (non-circumscribed) gliomas: diffuse astrocytomas, oligodendrogliomas, and oligoastrocytomas. Although commonly referred to collectively as among the "low-grade gliomas", these three tumors represent molecularly and clinically unique entities. Each is the subject of active basic research aimed at developing a more complete understanding of its molecular biology, and the pace of such research continues to accelerate. Additionally, because prognostication and management of these tumors has historically proven challenging, translational research regarding grade II infiltrating gliomas continues in the hopes of identifying novel molecular features that can better inform diagnostic, prognostic, and therapeutic strategies. Unfortunately, the basic and translational literature regarding the molecular biology of WHO grade II infiltrating gliomas remains nebulous. Our goal for this chapter is to present a comprehensive discussion of current knowledge regarding the molecular characteristics of these three WHO grade II tumors on the chromosomal, genomic, and epigenomic levels. Additionally, we discuss the emerging evidence suggesting molecular differences between adult and pediatric low-grade, infiltrating gliomas. Finally, we present an overview of current strategies for using molecular data to classify low-grade, infiltrating gliomas into clinically relevant categories based on tumor biology.

N.F. Marko, MD (✉)
Department of Neurosurgery,
University of Texas MD Anderson Cancer Center,
1515 Holcombe Blvd, FC7,
Houston, TX 77030, USA
e-mail: nfmarko@mdanderson.org

R.J. Weil, MD
Department of Neurosurgery,
Brain Tumor and Neuro-Oncology Center,
Cleveland Clinic,
Cleveland, OH, USA

H. Duffau (ed.), *Diffuse Low-Grade Gliomas in Adults*,
DOI 10.1007/978-1-4471-2213-5_8,

Keywords
Neuro-oncology • Infiltrating • Astrocytoma • Oligodendroglioma • Oligoastrocytoma • Molecular biology • Genetics • Karyotype • Epigenetic • Epigenomic • Classification

Introduction

The term "diffuse low-grade glioma" is commonly used to refer to one of three glial neoplasms assigned to World Health Organization (WHO) grade II: diffuse astrocytoma, oligodendroglioma, or oligoastrocytoma [1]. The WHO system is a purely histologic system that is the most common strategy for classifying gliomas, and "low grade" is often used to describe those gliomas with a microscopic appearance that is "histologically benign". Use of the terms "low-grade glioma" and "histologically benign", however, are falling out of favor, the former because it aggregates a number of dissimilar disease processes with unique molecular, phenotypic, and clinical characteristics and the latter because the absence of aggressive histologic features does not necessarily correlate with a "benign" clinical course in glioma patients. Nonetheless, these entities differ both clinically and molecularly from WHO grade III and IV gliomas, and so they are often discussed together.

Molecular investigation of WHO grade II astrocytomas, oligodendrogliomas, and oligoastrocytomas is an area of active research and occupies a unique position in the world of translational oncology. Because prognostication and management of these tumors has historically proven challenging, the translational research paradigm has been embraced by investigators working on these tumors in the hopes of identifying novel molecular features that can better inform diagnostic, prognostic, and therapeutic strategies. Arguably the most notable translational achievement in neuro-oncology has come from research on WHO grade II gliomas, where chromosomal characteristics are now being routinely used to inform discussions of prognosis and strategies for adjuvant therapy.

Despite these translational successes, the literature regarding the molecular biology of diffuse, low-grade gliomas remains nebulous. The goal of this chapter is to present a comprehensive discussion of the current knowledge regarding the molecular characteristics of these tumors on the chromosomal, genomic, and epigenomic levels. We have endeavored to clarify the many points of potential confusion and apparent contradiction that exist among this body of work, and we have attempted to organize this data into a logical and organized framework through which it can be more readily understood. We first discuss the specific chromosomal, genomic, and epigenomic features of WHO grade II astrocytomas and oligodendrogliomas. Next, we briefly address the oligoastrocytomas, or "mixed gliomas," whose molecular biology generally represents a combination of that of the astrocytoma and oligodendroglioma. Finally, we make additional comments regarding the pediatric diffuse gliomas and provide an overview of the current literature discussing potential molecular strategies for classifying the diffuse, low-grade gliomas.

Background

Diffuse Astrocytoma

The synonymous terms "diffuse astrocytoma" and "low-grade, diffuse astrocytoma" (AII) refer to tumors of astrocytic origin with relatively low proliferative activity and without obvious anaplastic features on histologic examination [2]. The category comprises three histologic variants,

including *fibrillary astrocytoma*, *protoplasmic astrocytoma*, and *gemistocytic astrocytoma* (sometimes described as "variants") [1, 3]. Overall these tumors represent approximately 1.6 % of all gliomas and 2.1 % of astrocytomas and account for 2,700–4,600 new brain tumor diagnoses per year in the USA [2]. They occur with peak incidence in the young adult population (ages 20–34), where they represent approximately 10.2 % of primary CNS tumors, 30.0 % of all gliomas, and 25.2 % of all malignant brain tumors [4]. In this age group their survival rates at 1, 5, and 10 years are 91.6, 58.5, and 40.7 %, respectively [4]. However, these tumors are observed across all age groups and are associated with relatively longer survival times in the pediatric population and with relatively shorter survival times in older adults [4].

In the adult population, most AIIs will ultimately progress to anaplastic astrocytomas and then to "secondary" glioblastomas [1, 5, 6]. This tendency suggests that AIIs represent an early stage in the evolution of secondary glioblastoma, and many of the molecular characteristics described in AIIs are likely to be early steps along the path to full-scale malignant transformation of the astrocyte. For this reason it is difficult to describe a set of genomic and epigenomic features that are unique to this grade of glioma, and descriptions of the molecular biology of AIIs should be viewed through this lens.

Many molecular investigations include a small number of AIIs as one part of larger experimental samples containing various grades of glioma. These studies tend to identify genomic and epigenomic changes that occur with relatively low frequency in AIIs and become more prevalent as gliomas progress to higher grades. Reporting the relative frequency of such changes in AIIs adds little to a focused discussion of AII-specific molecular biology, and interested readers should refer to any of a number of texts on high-grade gliomas that place these findings in the context of the molecular pathogenesis and evolution of glioblastoma [7, 8]. Instead, in this section we summarize those molecular features that appear common to a large proportion of AIIs. These molecular features may logically be assumed to represent at least some of the functionally significant, early subcellular changes involved in the process of malignant astrocytic transformation, and understanding these features may be the most clinically relevant approach to interpreting the molecular biology of AIIs.

Oligodendroglioma

The synonymous terms "oligodendroglioma" and "low-grade oligodendroglioma" (OII) refer to tumors of oligodendroglial histology with low proliferative activity and without obvious anaplastic features on microscopic examination [2]. There are no specific histologic variants of OII [1]. Among all grades of glioma (excluding glioblastoma), oligodendroglioma histology is outnumbered by astrocytic histology by a factor of 3 [1, 8]. They occur with peak incidence in the third to fifth decades [1, 8], and the 1-, 5-, and 10-year survival rates for OIIs in adults are 94.2, 79.5, and 63.6 %, respectively [4]. OIIs are less common in pediatric patients [1], but when they do occur in this age group, they are associated with better survival rates than those for OIIs in adults [4].

OIIs have recently become the subject of considerable attention in translational neuro-oncology research because they represent the first primary brain tumor that can be routinely and consistently stratified by molecular features into two clinically distinct subgroups. OIIs with "deletions" of chromosome 1p ± 19q are associated with a relatively longer survival and may exhibit improved response to adjuvant therapy, whereas those in which chromosome 1p ± 19q is intact behave more aggressively [1]. This finding supports the long-standing concerns of many neuro-oncologists that histologic subtypes of glioma may not adequately capture all clinically relevant variability among these tumors [9, 10] and serves as important proof of principle for ongoing investigations for molecular subclassification of gliomas.

Chromosomal Abnormalities

Diffuse Astrocytoma

The most common chromosomal abnormalities in AII are trisomies or polysomies of chromosome 7 [1, 3], with gains of 7 or 7q observed in approximately 50 % of these tumors [11, 12]. Gains in 8q have also been reported to occur with some consistency in AIIs [13], and gains of 5p, 9, and 19p have also been inconsistently observed [3, 8, 14]. Chromosomal losses in AIIs have been reported most commonly involving chromosome 17p [8, 13, 15] and less frequently on chromosomes 6q [16], 10p, 13q, 19q, and 22q and the sex chromosomes [3, 8, 14].

Oligodendroglioma

The most common chromosomal abnormality in OIIs, occurring in approximately 50 % of these tumors (although some report 80+ %) [2, 17–24], is a combined "loss" of the short arm of chromosome 1 (1p) and the long arm of chromosome 19 (19q) [1, 8, 17]. These tumors demonstrate *loss* of one entire copy of these chromosomal arms due to an unbalanced t(1;19)(q10;p10) translocation [25, 26], and this finding is commonly (although technically inaccurately) described as "1p/19q codeletion". Conversely, partial deletions of these loci [1] or isolated loss of 1p [8] are rare. Of the two chromosomal losses, 1p has the greater specificity, as 19q losses have been observed in other histologic types and grades of glioma [27]. Notwithstanding, 1p/19q codeletion is not completely specific to OIIs, as it has also been occasionally reported in astrocytomas, oligoastrocytomas [8], and glioblastomas [28]. Combined losses of 1p/19q appear to be mutually exclusive of several other molecular abnormalities commonly associated with gliomas, including loss of heterozygosity (LOH) on 17p and *TP53* mutation [29–32]. This suggests that the molecular pathway leading to the 1p/19q codeleted OII may be distinct from those involved in other forms of glioma pathogenesis [14].

The exact molecular mechanisms associated with the development of the unique t(1;19)(q10;p10) translocation in OIIs are not yet fully understood. Recent evidence suggests that the centromeric regions of chromosomes 1 and 19 show a high degree of sequence homology [33]. This has been hypothesized to result in centromeric co-localization of chromosomes 1 and 19, which might promote centromeric instability and thus favor the translocation [26, 33]. Additional investigations regarding the specifics of this process and the clinical and molecular significance of this finding are ongoing.

Additional chromosomal abnormalities have also been reported in OIIs, although less frequently than 1p/19q codeletions. These include deletions involving chromosomes 4, 6, 11p, 14, and 22q [18, 20] and occasional losses of chromosomes 9 and 10 [1]. Array-based comparative genomic hybridization has also suggested submegabase deletions associated with OIIs, including 300–550 kb regions on 11q13 and 13q12 [34]. The validity and consistency of these focal deletions remains to be determined.

Genomic Abnormalities

Diffuse Astrocytoma

TP53

The *TP53* gene localizes to chromosome 17p13.1 and its protein product (p53) is involved in several cellular processes, including cell cycle regulation, response of cells to DNA damage, cell differentiation, and cell death [35]. Activated p53 induces transcription of $p21^{Waf1/Cip1}$, which regulates cell cycle progression at G_1 via its activity on cyclin-CDK complexes [15, 16]. The activity of p53 is modulated by MDM4 (MDMX) as well as by MDM2, the latter of which is modulated [36] by $p14^{ARF}$.

Sixty (60 %) to 80 % of AIIs have allelic loss on 17p that includes the *TP53* locus [8, 14, 15], and most AIIs with the retained locus exhibit *TP53* mutations [8, 37–39]. This makes complete absence of wild-type p53 the most common

genomic abnormality in AIIs [8, 14]. The incidence of *TP53* mutations is higher in secondary than in primary glioblastoma [40, 41] but does not increase appreciably between AIIs and glioblastoma [42–45], lending genome-level support to the hypothesis that AIIs represent an early stage in the evolution of secondary glioblastoma [1, 2, 36]. This hypothesis is further supported by the findings that common *TP53* mutations both in AIIs [46] and in secondary glioblastomas [41] occur at codons 248 or 273 (while the *TP53* mutations observed in primary glioblastomas are more broadly distributed) and that G:C A:T mutation in CpG islands are more frequent in secondary than in primary glioblastoma. The latter observation suggests that different mechanisms may lead to the acquisitions of the *TP53* mutations seen in these two glioblastoma subtypes [8, 41].

Isocitrate Dehydrogenase

The enzyme isocitrate dehydrogenase (IDH) catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate in the citric acid cycle and uses $NADP^+$ as a proton acceptor [47]. A total of five IDH isozymes have been described, although IDH1 and IDH2 are currently believed to be the most relevant to glioma biology. The IDH1 enzyme localizes to the cytosol and peroxisome [47], while the IDH2 enzyme assumes the more classic, mitochondrial localization [48]. A genome-wide analysis of glioblastoma identified *IDH1* (2q33) [48, 49] gene mutations in 12 % of these tumors [50], prompting additional investigations into the potential role of *IDH* mutation in glioma biology. Subsequent studies demonstrated that *IDH* mutations are most common in WHO grade II and III gliomas as well as in secondary (but not primary) glioblastomas [51, 52]. Approximately 80 % of AIIs have been shown to harbor *IDH1* gene mutations, and *IDH2* (15q26.1) gene mutations are often present in the residual fraction [51]. This finding makes *IDH* gene mutations the most common and consistent genetic abnormality in AIIs reported to date. Notably, there does not appear to be a statistical association between *IDH* mutations and *TP53* mutations in AIIs [53], although these data remain inconsistent [36, 54].

The specific IDH1 mutation observed in low-grade gliomas is almost always (>90 %) [55] a point mutation at position 132, where wild-type arginine is replaced by histidine in the mutant form (R132H) [53]. Other rare mutations at this position include substitutions of arginine with cysteine (R132C), serine (R132S), leucine (R132L), glycine (R132G), or valine (R132V) [51, 53]. These mutations are all heterozygous, and no truncation or frame shift mutants have yet been described [56]. Position 132 belongs to an evolutionarily conserved region representing the binding site of the isocitrate substrate [53], and the R132 mutations result in reduced enzymatic activity toward isocitrate [51, 52, 57]. Recent kinetic studies have demonstrated that R132 mutations alter the relative affinity of the IDH1 active site, favoring α-ketoglutarate over isocitrate and resulting in increased production of α-hydroxyglutarate in cells harboring the mutation [58]. Structural investigations have suggested a mechanistic explanation for this observation related to its effects on subunit dimerization [59], and a "dominant inhibition" model whereby concurrent underproduction of α-ketoglutarate and overproduction of α-hydroxyglutarate may favor oncogenesis has been proposed [56]. Supplementary hypotheses include contributions to oncogenesis through induction of the HIF-α pathway [57], while others suggest that IDH mutations may not be oncogenic but may instead represent protective mechanisms that interfere with the metabolism of tumor cells [60].

IDH2 is the only human protein homologue of IDH1 that uses $NDAP^+$ as a proton acceptor [51], and its arginine at position 172 (R172) is exactly analogous to R132 in IDH1. Five point mutations have been identified in IDH2, resulting in replacements of R172 with glycine (R172G), methionine (R172M), lysine (R172K), serine (R172S), and tyrosine (R172Y) [51, 61, 62]. Kinetic and structural studies of IDH2 have not been as extensive as those for IDH1, but the strong similarities between these isozymes and the involved mutations suggest comparable underlying biology.

PDGFR

The platelet-derived growth factor receptor (PDGFR) is a tyrosine kinase receptor that interacts with the RAS pathway (and thus the PI3K/PTEN/AKT/mTOR pathway) [36] via the SOS-Grb2 intermediary [63, 64]. As downstream pathways also modulated by the epidermal growth factor receptor (EGFR), PDGFR-associated pathways have been of considerable interest in glioma research [36]. This has the potential to lead to some degree of confusion regarding the relative importance of these pathways in AII versus glioblastoma, and it is therefore important to clarify the current molecular evidence regarding PDGFR pathways in AIIs.

A number of preclinical and translational studies have reported putative roles for various components of the PDGF/PDGFR proteins in the biology of glioblastoma [36, 65, 66]. However, despite being perpetuated throughout the glioma genomics literature [1, 36] as being overexpressed in up to 60 % of AIIs [1, 14], firm evidence for PDGFR overexpression in AII is sparse. Two small studies from the early 1990s [67, 68], each including only five AIIs in their analyses, reported that PDGFR-α appeared overexpressed in gliomas of all grades, including AIIs. Attempts to validate this finding have been inconsistent [69, 70], and ascribing an important, functional role to PDGFR-α in AII on the basis of current evidence appears premature. This distinction is even more important given numerous reports suggesting a role for the overlapping EGFR/RAS/PI3K/PTEN/AKT/mTOR pathway in the biology of primary but not secondary glioblastoma [36] and the possible mutual exclusivity between p53 mutations and EGFR overexpression [43]. Moreover, EGFR overexpression is currently considered to be one factor that distinguishes primary from secondary glioblastoma, as it is observed in approximately 40 % of the former but is rare in the latter [36, 41, 43, 71, 72]. Given these data, it appears that the tyrosine kinase receptor pathways may be of much greater significance to primary glioblastoma biology than to the biology that defines the AII-secondary glioblastoma spectrum.

Other Genomic Abnormalities

A comprehensive meta-analysis [73] of studies specifically reporting on gene expression in low-grade gliomas performed through 2006 identified only 11 studies [69, 74–83] describing specific patterns of gene expression in grade I and/or grade II gliomas. The investigators summarized these results and then verified the most commonly reported gene expression patterns using RT-PCR [73]. With regard to gene expression in AIIs, the authors reported data from six studies [69, 74, 75, 77, 80, 83] comparing expression in AIIs versus normal controls. They found consistent evidence for underexpression of the *TYRO3* gene and for overexpression of the genes, *CD9*, *TIMP3*, *CSPG2*, *EGFR*, *PDGRFA*, and *NTF3*, as well as a single report of overexpression of *KCNN3* [73]. Comparison between AIIs and glioblastoma revealed no instances of specific gene overexpression in AIIs relative to glioblastoma but found consistent evidence for relative underexpression of *NCAM1*, *FN*, *EGFR*, *VEGF*, *IGFBP2*, *IGFBP3*, and *IGFBP5* as well as an isolated report of underexpression of *MMP16* [73].

In light of the previous comments regarding PDGFRA and EGFR, additional clarification regarding some of these genomic findings [73] is necessary. Review of the source publications in which *PDGFA* and *EGFR* expression differences were noted [69, 80, 83] demonstrates relatively small sample numbers, and two of the three [69, 83] studies were reported by the same research group. One of these studies [69] reported >2-fold overexpression in *PDGFRA* to be present in only two of ten AIIs analyzed. Accordingly, we caution against drawing firm conclusions from these data regarding the actual role of *EGFR* and *PDGFRA* in AIIs, as considerable evidence (described above) suggests that these genomic features are more consistently associated with higher-grade gliomas.

Additional reports involving AII genomics include those that characterize expression and propose potential roles for human herpesvirus-6 variants [84], the *LGI1* [85] and *BR-3* [86] gene products, and the *SoxD* and *SoxE* gene families [87] in AII biology and in malignant progression

of gliomas. Additional research is necessary before definitive conclusions can be made regarding the putative roles and overall significance of these candidate molecules.

Oligodendroglioma

1p/19q Candidate Genes

Despite consistent and convincing evidence for 1p/19q deletions in OII, the specific gene(s) whose loss is associated with the unique clinical phenotype of codeleted OIIs (see below) remains unclear. Proposed candidate genes on 1p include *Notch2* (1p13-p11) [88], *DIRAS3* (1p31) [89], *CDKN2C* (1p32) [90], *RAD54* (1p32) [91], *CITED4* (1p34.2) [92], *CAMTA1* (1p36) [93], *DFFB* (1p36) [94], *TP73* (1p36.3) [95], and *SHREW1* (1p36.32) [96]. Because 19q is completely lost in the OII translocation, mapping studies for identification of candidate gene regions on this chromosome have focused on brain tumors of other histologic types with partial deletions of 19q [27, 97–100]. These studies have suggested a potential role for several genes on the 19q3 region [27, 98–100], but additional investigations have not demonstrated consistent mutations of these genes [101]. Epigenomic studies (see below) suggest potential roles for *ZNF342* (19q13) [102], *p190RhoGAP* (19q13.3) [103], *EMP3* (19q13.3) [104], and *PEG3* (19q13.4) [105, 106], but definitive evidence for any of these candidate genes has yet to be demonstrated [1, 14, 107].

Isocitrate Dehydrogenase

As in AIIs, IDH1 (and/or IDH2) mutations are common in OIIs [36, 51, 53, 61, 62] and have been observed in >80 % of these tumors [51]. Many of the studies regarding the specific mutations and their functional significance have been conducted on mixed populations of AIIs and OIIs, and thus, the IDH1 R132 and the IDH2 R172 mutations are believed to be the relevant abnormalities in both tumor types. Although the high rate of IDH mutations in both AIIs and OIIs initially suggested that these mutations were independent of other molecular features that differentiated these tumor types [36, 51, 53, 61, 62], more recent evidence suggests that there may be a high degree of correlation between IDH mutations and chromosome 1p/19q codeletions [62]. Many of these investigations are conducted with populations containing a mixture of OIIs and AIIs [53, 62] and do not stratify independently by 1p/19q status and WHO grade, limiting the ability to study the relationship in detail. One investigation where stratification was performed, however, demonstrated 1p/19q codeletions in 85 % of tumors with IDH mutations, while no tumors with wild-type IDH were found to be 1p/19q codeleted [51]. The pathophysiologic significance of this finding remains to be determined.

Other Abnormalities

EGFR amplification has been reported in approximately 50 % of OIIs, although this represents older data from small studies of relatively few tumors [108]. PDGFA and PDGFB as well as their receptors (PDGFR-α and PDGFR-β) appear to be overexpressed in a large percentage of OIIs [109], making this finding more common among these tumors than in AIIs. More recently, overexpression of *rPTPβ/γ* has been reported to distinguish OIIs from AIIs [110].

Epigenomic Abnormalities

Diffuse Astrocytoma

Epigenomic investigations represent a relatively recent area of research in the molecular biology of AIIs. The most robust epigenomic data involves the *ARF* gene [111, 112], which localizes to the *CDKN2A* (*INK4/ARF*) locus on chromosome 9p21 [111, 113]. Its gene product, $p14^{ARF}$, binds to MDM2 and stabilizes both MDM2 and p53 [111, 113–115]. Accordingly, methylation of the $p14^{ARF}$ promoter results in decreased production of the p14 gene product and relative destabilization of MDM2 and p53. In a single study, *ARF* ($p14^{ARF}$) promoter hypermethylation has been documented in 26 % of AIIs, which was frequently observed in AIIs without primary p53

mutations [112]. All AIIs in this study harboring *ARF* ($p14^{ARF}$) promoter methylation ultimately progressed to secondary glioblastomas. Similarly, promoter hypermethylation of the DNA-repair gene *O^6-methylguanine-DNA methyltransferase* (*MGMT*) has also been observed in 63 % of AIIs [112]. Interestingly, limited data suggests that *MGMT* hypermethylation is associated with p53 mutation but is mutually exclusive to *ARF* ($p14^{ARF}$) gene hypermethylation [14, 112]. Additional reports suggest epigenomic silencing of the *PCDH-γ-A11* (5q31) [116], *PTEN* (10q23.31) [117], and *EMP3* (19q13) [118] genes in AII, and further investigations are likely to reveal additional instances of epigenomic abnormalities in these tumors [119, 120].

Oligodendroglioma

OIIs demonstrate lower levels of MGMT expression than AIIs [2, 121]. Some evidence suggests that up to 60–80 % of OIIs may exhibit hypermethylation of the *MGMT* promoter [122–124] (more common than in AIIs) and that this hypermethylation correlates with chromosome 1p/19q loss [122, 125], while others have not observed these effects [126, 127]. Additional genes that have been found to be hypermethylated in some OIIs include *CDKN2A* (9p21), *CDKN2B* (9p21), *ARF* (9p21), *RB1* (13q14), *TP73* (1p36.3), *DAPK1* (9q34.1), *ESR1* (6q25.1), *TIMP3* (22q12.3), *THBS* (15q15), and *GSTP1* (11q13) [20, 124].

Clinical Correlations

Diffuse Astrocytoma

Few molecular markers have demonstrated prognostic significance in AIIs. The evidence is most comprehensive for the putative relationship between p53 status and clinical outcomes, but even here the results remain unclear. Early investigations demonstrated no apparent relationship between p53 expression levels and overall survival [128]. The literature presents conflicting evidence regarding a potential relationship between abnormalities in p53 and malignant progression, with data arguing both for [129] and against [44] a potential association. Several studies agree, however, that p53 mutation does appear to be associated with an increased likelihood of tumor recurrence [44, 46, 129]. One possible explanation for these nebulous findings may be that the relationship between p53 status and clinical outcomes varies between subtypes of AII. For instance, some investigators have suggested that much of the overall prognostic impact of p53 status may be related to its disproportionate association with the gemistocytic AII subtype [46]. Another possible explanation may be that specific p53 mutations are associated with unique prognostic profiles. This is exemplified by the apparent correlation between codon 175 *TP53* mutation and an increased risk of progression and malignant transformation [46].

Other genomic and epigenomic changes may also have prognostic implications. *IDH1* and *IDH2* gene mutations have been suggested as markers of more favorable survival phenotypes [61, 62, 130], although many of the studies in which this has been demonstrated do not necessarily separate AIIs from oligodendrogliomas. It therefore remains possible that disproportionate overrepresentation of oligodendroglioma in the experimental samples of these studies influenced the results, and the ultimate generalizability of these potential prognostic biomarkers specifically to AIIs remains to be determined. EGFR [70, 72] (although uncommon in AIIs) and PDGFR [70] overexpression may be associated with shorter survival times in patients with AIIs. Additionally, *MGMT* promoter methylation has been associated with response to chemotherapy and thus to improved survival in AII patients [131].

Oligodendroglioma

Perhaps the most widely reported molecular finding with a clinical correlation is the relationship between the combined loss of chromosomes 1p/19q and improvements in survival [24, 132–135] and response to chemo- [136–139] and radiotherapy

[140]. Data regarding the prognostic significance of *TP53* mutation status and/or LOH 17p13 specifically in OIIs is limited, but some evidence suggests that these may be independent, unfavorable predictors of overall and progression-free survival [141, 142]. Gains on chromosome 8q may also be associated with poor outcomes in OIIs, but this data is derived from a relatively small study on a population of oligodendrogliomas of mixed WHO grades [143]. While other correlations between molecular markers and survival or response-to-therapy phenotypes have been reported [14, 20], these have almost always been studied primarily in OIIIs, making their generalizability specifically to OIIs unclear.

Oligoastrocytoma

Oligoastrocytomas (OAII), also called "mixed gliomas" [1], represent a unique WHO class of grade II glioma that is characterized by tumors exhibiting a mixture of astrocytic and oligodendroglial histologic morphology. Molecular evidence suggests that this histologic class may comprise an unbalanced mixture of two primary tumor genotypes, AII and OII [1, 20, 29]. This is supported by the observation that 30–50 % of OAIIs exhibit chromosome 1p/19q codeletions [17, 19, 23, 29] (OII-like), while approximately 30 % carry *TP53* gene mutations [17, 19, 29, 31] (AII-like). Moreover, OAIIs with 1p/19q codeletions have been observed to exhibit more prominent oligodendroglioma-like features on microscopic examination, whereas those with *TP53* mutations are more histologically similar to astrocytoma [29].

One study has proposed that chromosomal data may be useful for subdividing OAIIs into four subclasses [144]. This approach may be reasonable if OAII is a genotypically distinct tumor type but may introduce unnecessary complexity if it is nothing more than a mixture of AII and OII genotypes. This proposed scheme has not been further validated, but it underscores the translational relevance of determining the true genotypic nature of OAII. Without such data only broad correlations of genotype with phenotype are possible for this WHO class, such as recent investigations suggesting that 1p/19q codeletions may be a generally favorable prognostic factor in OAIIs [145].

While addressing this issue is important, it remains difficult to draw from current data firm conclusions regarding the degree to which OAII biology is novel versus the extent to which the biological observations in OAII can be explained simply as a mixture of AII and OII genotypes. One directly related but seldom-discussed factor that should be considered when interpreting molecular analyses of OAIIs is the method of extraction of molecular material from the tissue samples. Experimental protocols that homogenize tissue blocks are likely to extract biological samples for analysis that are heterogeneous mixtures of the molecular constituents of both the oligodendroglia-like and astrocytoma-like tumor regions, while those that use microdissection of specific regions may be more likely to isolate molecular material that is biased toward one of the two constituent cell types. Studies employing the latter methodology are presently lacking, but such investigations are necessary if comprehensive, comparative molecular analyses of the fundamental similarities and differences between tumors classified as OAII, AII, and OII, as well as careful investigations of the clonal origins of OAIIs, are to be performed.

Pediatric Grade II Infiltrative Gliomas

Clinical evidence shows that WHO grade II infiltrative astrocytomas in pediatric patients have a lower rate of malignant transformation than those in adults (10 % vs. 90 %) [146]. These findings suggest that, despite identical WHO classification, pediatric grade II infiltrative gliomas may represent a unique disease process that could be expected to harbor a novel genotype. Current evidence regarding this hypothesis is nebulous, and it is difficult to draw definitive conclusions regarding the molecular comparability of adult and pediatric grade II infiltrative gliomas. While a complete discussion of the molecular differences between adult and pediatric glioma genomics is outside the scope of this

chapter, a brief overview of the current status of this data is beneficial in order to draw attention to this persistent ambiguity.

Most investigations of specific molecular differences between pediatric and adult low-grade gliomas have thus far been conducted at the chromosomal level. While 50 % or more of adult infiltrating gliomas may have some form of chromosomal abnormality [11, 12, 17–23], rates for comparable abnormalities in pediatric patients have been reported to be relatively lower [147–154]. Notwithstanding, chromosomal abnormalities in these pediatric tumors are not rare [154]. For example, rates of chromosome 1p and 19q loss in pediatric populations may be similar to [155] or greater than [156] those in adults, although they do not appear to be associated with the same prognostic significance in children [155].

Definitive conclusions regarding the actual rate of chromosomal abnormalities in pediatric diffuse infiltrating grade II gliomas, as well as the clinical significance of these findings, are difficult to determine definitively based upon current data. Most relevant studies combine (often disproportionately) grade II gliomas with gliomas of other grades for aggregate analyses of "low-grade gliomas". Aggregation with either pilocytic astrocytomas, in which chromosomal abnormalities are known to be uncommon, or with anaplastic (grade III) gliomas, in which prognosis may differ, may significantly bias results [147–156]. When the primary data are presented such that infiltrating glioma karyotypes can be examined independently [147, 148, 153, 154], the rates of chromosomal abnormalities generally appear higher in the grade II subgroup than is reported for the aggregate data set. This suggests that disproportionate inclusion of pilocytic astrocytomas may artifactually dilute the commonly reported rates of chromosomal abnormalities in pediatric infiltrative low-grade gliomas and that these may, in fact, approach those of the adult population. Similarly, conclusions regarding the prognostic implications of 1p/19q status in grade II gliomas may not be generalizable from the population of predominantly grade III patients in which it was studied [155]. Data interpretation is further complicated by the relatively low absolute number of infiltrating gliomas included in many of these studies.

Genomic profiling studies comparing adult and pediatric gliomas suggest that, in general, transcriptome-level differences may exist between these entities [157], but data on differential rates of expression of specific genes is currently limited. Some evidence suggests that EGFR overexpression may be relatively more common in pediatric tumors [158]. Conversely, OLIG2 expression may be relatively less common [159]. The clinical significance of these findings remains to be determined.

Molecular Classification of Low-Grade Gliomas

This chapter highlights a number of molecular characteristics of low-grade glioma subtypes that may have prognostic and therapeutic relevance. However, because the current WHO system relies solely on histologic features for classification [1], there is currently no formal mechanism by which molecular data can be used to improve the accuracy of glioma classification. Additionally, ambiguous WHO criteria can make classification of some low-grade gliomas challenging and can introduce subjectivity that may limit the reproducibility of glioma classification [160]. Accordingly, several investigators have suggested that molecular strategies for glioma classification be considered, and numerous efforts have been made toward developing these strategies for low-grade gliomas.

While a comprehensive review of the topic of molecular classification of low-grade gliomas is outside the scope of this chapter, an overview of the proposed general approaches to such classification is appropriate. Several proof-of-principle studies have demonstrated the ability to use molecular data to stratify low-grade gliomas into classes that overlap with the WHO scheme [9, 161]. From here, a number of specific strategies have been applied to the task of molecular classification of these tumors. Approaches based on the expression of single genes or gene products have been successful at resolving some of the difficulties associated with purely histologic differentiating between AII, OII, and OAII [110], and strategies employing various combinations of genomic and chromosomal data have

demonstrated similar success in this task [31, 162]. Classification techniques based solely on genomic data for a small subset of genes have also been successfully applied to the task of molecular stratification of various categories of low-grade gliomas [163], as have schemes that use more comprehensive sets of gene expression profiles [9, 82, 164]. Recently, epigenomic profiles involving patterns of CpG island methylation have also been used to define subsets of grade II gliomas with apparent differences in survival phenotype [165]. The actual methods for classification using molecular data vary from simple algorithms based on one or a few markers [31, 110, 163] to more complex mathematical models based on aggregate molecular data sets [9, 161, 162].

Issues regarding the practicality of implementation and utilization of molecular classification schemes for low-grade gliomas, the accuracy of putative molecular class discriminators, and the optimal approach for maximizing research, diagnostic, and clinical utility of molecular classification strategies are yet to be fully resolved [160]. Nevertheless, there is considerable optimism in the translational neuro-oncology community that molecular data will ultimately prove to be a useful adjunct for classification of low-grade gliomas.

Conclusion

Molecular and translational research in WHO grade II diffuse gliomas remains an area of active research through which several, practical discoveries have already been made. Future investigations in this arena will include attempts to clarify the relative importance of potentially clinically relevant molecular markers, including p53, chromosomes 1p and 19q, and IDH1 and IDH2; endeavors to expand upon preclinical discoveries of novel potential markers; and efforts to incorporate molecular markers into tumor classification strategies. The translational neuro-oncology community remains optimistic that significant progress to further understand the pathophysiology, clinical behavior, and optimal management of "diffuse low-grade gliomas" will continue to be made in the coming years.

References

1. Louis D, Ohgaki H, Wiestler O, Cavenee W, editors. WHO classification of tumors of the central nervous system. Lyon: IARC; 2007.
2. Schiff D, Brown PD, Giannini C. Outcome in adult low-grade glioma: the impact of prognostic factors and treatment. Neurology. 2007;69:1366–73.
3. Reifenberger G, Collins VP. Pathology and molecular genetics of astrocytic gliomas. J Mol Med. 2004;82:656–70.
4. (CBTRUS) CBTRotUS. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2004–2007. 2011. Central Brain Tumor Registry of the United States, Hinsdale. 2011. Available at website: http://www.cbtrus.org/2011-NPCR-SEER/WEB-0407-Report-3-3-2011.pdf. Accessed 03 Mars 2011.
5. Inoue R, Isono M, Abe M, Abe T, Kobayashi H. A genotype of the polymorphic DNA repair gene MGMT is associated with de novo glioblastoma. Neurol Res. 2003;25:875–9.
6. Bethke L, Webb E, Murray A, Schoemaker M, Johansen C, Christensen HC, et al. Comprehensive analysis of the role of DNA repair gene polymorphisms on risk of glioma. Hum Mol Genet. 2008;17:800–5.
7. Barnett GH. High-grade gliomas: diagnosis and treatment. Totowa: Humana Press; 2007.
8. Rees J, Wen P, editors. Neuro-oncology. Philadelphia: Elsevier; 2010.
9. Marko NF, Prayson RA, Barnett GH, Weil RJ. Integrated molecular analysis suggests a three-class model for low-grade gliomas: a proof-of-concept study. Genomics. 2010;95:16–24.
10. Marko NF, Toms SA, Barnett GH, Weil R. Genomic expression patterns distinguish long-term from short-term glioblastoma survivors: a preliminary feasibility study. Genomics. 2008;91:395–406.
11. Schrock E, Blume C, Meffert MC, du Manoir S, Bersch W, Kiessling M, et al. Recurrent gain of chromosome arm 7q in low-grade astrocytic tumors studied by comparative genomic hybridization. Genes Chromosomes Cancer. 1996;15:199–205.
12. Wessels PH, Twijnstra A, Kessels AG, Krijne-Kubat B, Theunissen PH, Ummelen MI, et al. Gain of chromosome 7, as detected by in situ hybridization, strongly correlates with shorter survival in astrocytoma grade 2. Genes Chromosomes Cancer. 2002;33:279–84.
13. Nishizaki T, Ozaki S, Harada K, Ito H, Arai H, Beppu T, et al. Investigation of genetic alterations associated with the grade of astrocytic tumor by comparative genomic hybridization. Genes Chromosomes Cancer. 1998;21:340–6.
14. von Deimling A, editor. Gliomas. Heidelberg: Springer; 2009.
15. Watanabe K, Peraud A, Gratas C, Wakai S, Kleihues P, Ohgaki H. p53 and PTEN gene mutations in gemistocytic astrocytomas. Acta Neuropathol. 1998;95:559–64.

16. Miyakawa A, Ichimura K, Schmidt EE, Varmeh-Ziaie S, Collins VP. Multiple deleted regions on the long arm of chromosome 6 in astrocytic tumours. Br J Cancer. 2000;82:543–9.
17. Reifenberger J, Reifenberger G, Liu L, James CD, Wechsler W, Collins VP. Molecular genetic analysis of oligodendroglial tumors shows preferential allelic deletions on 19q and 1p. Am J Pathol. 1994;145:1175–90.
18. Jeuken JW, von Deimling A, Wesseling P. Molecular pathogenesis of oligodendroglial tumors. J Neurooncol. 2004;70:161–81.
19. Okamoto Y, Di Patre PL, Burkhard C, Horstmann S, Jourde B, Fahey M, et al. Population-based study on incidence, survival rates, and genetic alterations of low-grade diffuse astrocytomas and oligodendrogliomas. Acta Neuropathol. 2004;108:49–56.
20. Reifenberger G, Louis DN. Oligodendroglioma: toward molecular definitions in diagnostic neuro-oncology. J Neuropathol Exp Neurol. 2003;62: 111–26.
21. von Deimling A, Louis DN, von Ammon K, Petersen I, Wiestler OD, Seizinger BR. Evidence for a tumor suppressor gene on chromosome 19q associated with human astrocytomas, oligodendrogliomas, and mixed gliomas. Cancer Res. 1992;52:4277–9.
22. Bello MJ, Vaquero J, de Campos JM, Kusak ME, Sarasa JL, Saez-Castresana J, et al. Molecular analysis of chromosome 1 abnormalities in human gliomas reveals frequent loss of 1p in oligodendroglial tumors. Int J Cancer. 1994;57:172–5.
23. Kraus JA, Koopmann J, Kaskel P, Maintz D, Brandner S, Schramm J, et al. Shared allelic losses on chromosomes 1p and 19q suggest a common origin of oligodendroglioma and oligoastrocytoma. J Neuropathol Exp Neurol. 1995;54:91–5.
24. Kanner AA, Staugaitis SM, Castilla EA, Chernova O, Prayson RA, Vogelbaum MA, et al. The impact of genotype on outcome in oligodendroglioma: validation of the loss of chromosome arm 1p as an important factor in clinical decision making. J Neurosurg. 2006;104:542–50.
25. Griffin CA, Burger P, Morsberger L, Yonescu R, Swierczynski S, Weingart JD, et al. Identification of der(1;19)(q10;p10) in five oligodendrogliomas suggests mechanism of concurrent 1p and 19q loss. J Neuropathol Exp Neurol. 2006;65:988–94.
26. Jenkins RB, Blair H, Ballman KV, Giannini C, Arusell RM, Law M, et al. A t(1;19)(q10;p10) mediates the combined deletions of 1p and 19q and predicts a better prognosis of patients with oligodendroglioma. Cancer Res. 2006;66:9852–61.
27. Smith JS, Alderete B, Minn Y, Borell TJ, Perry A, Mohapatra G, et al. Localization of common deletion regions on 1p and 19q in human gliomas and their association with histological subtype. Oncogene. 1999;18:4144–52.
28. Houillier C, Lejeune J, Benouaich-Amiel A, Laigle-Donadey F, Criniere E, Mokhtari K, et al. Prognostic impact of molecular markers in a series of 220 primary glioblastomas. Cancer. 2006;106:2218–23.
29. Maintz D, Fiedler K, Koopmann J, Rollbrocker B, Nechev S, Lenartz D, et al. Molecular genetic evidence for subtypes of oligoastrocytomas. J Neuropathol Exp Neurol. 1997;56:1098–104.
30. von Deimling A, Fimmers R, Schmidt MC, Bender B, Fassbender F, Nagel J, et al. Comprehensive allelotype and genetic analysis of 466 human nervous system tumors. J Neuropathol Exp Neurol. 2000;59: 544–58.
31. Mueller W, Hartmann C, Hoffmann A, Lanksch W, Kiwit J, Tonn J, et al. Genetic signature of oligoastrocytomas correlates with tumor location and denotes distinct molecular subsets. Am J Pathol. 2002;161: 313–9.
32. Ueki K, Nishikawa R, Nakazato Y, Hirose T, Hirato J, Funada N, et al. Correlation of histology and molecular genetic analysis of 1p, 19q, 10q, TP53, EGFR, CDK4, and CDKN2A in 91 astrocytic and oligodendroglial tumors. Clin Cancer Res. 2002;8: 196–201.
33. Vogazianou AP, Chan R, Backlund LM, Pearson DM, Liu L, Langford CF, et al. Distinct patterns of 1p and 19q alterations identify subtypes of human gliomas that have different prognoses. Neuro Oncol. 2010; 12:664–78.
34. Rossi MR, Gaile D, Laduca J, Matsui S, Conroy J, McQuaid D, et al. Identification of consistent novel submegabase deletions in low-grade oligodendrogliomas using array-based comparative genomic hybridization. Genes Chromosomes Cancer. 2005;44:85–96.
35. Bogler O, Huang HJ, Kleihues P, Cavenee WK. The p53 gene and its role in human brain tumors. Glia. 1995;15:308–27.
36. Ohgaki H, Kleihues P. Genetic alterations and signaling pathways in the evolution of gliomas. Cancer Sci. 2009;100:2235–41.
37. Ichimura K, Bolin MB, Goike HM, Schmidt EE, Moshref A, Collins VP. Deregulation of the p14ARF/MDM2/p53 pathway is a prerequisite for human astrocytic gliomas with G1-S transition control gene abnormalities. Cancer Res. 2000;60:417–24.
38. Rasheed BK, McLendon RE, Herndon JE, Friedman HS, Friedman AH, Bigner DD, et al. Alterations of the TP53 gene in human gliomas. Cancer Res. 1994; 54:1324–30.
39. James CD, Carlbom E, Nordenskjold M, Collins VP, Cavenee WK. Mitotic recombination of chromosome 17 in astrocytomas. Proc Natl Acad Sci USA. 1989;86:2858–62.
40. Ohgaki H, Kleihues P. Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas. J Neuropathol Exp Neurol. 2005;64:479–89.
41. Ohgaki H, Dessen P, Jourde B, Horstmann S, Nishikawa T, Di Patre PL, et al. Genetic pathways to glioblastoma: a population-based study. Cancer Res. 2004;64:6892–9.

42. Sidransky D, Mikkelsen T, Schwechheimer K, Rosenblum ML, Cavanee W, Vogelstein B. Clonal expansion of p53 mutant cells is associated with brain tumour progression. Nature. 1992;355:846–7.
43. Watanabe K, Tachibana O, Sata K, Yonekawa Y, Kleihues P, Ohgaki H. Overexpression of the EGF receptor and p53 mutations are mutually exclusive in the evolution of primary and secondary glioblastomas. Brain Pathol. 1996;6:217–23; discussion 223–14.
44. Watanabe K, Sato K, Biernat W, Tachibana O, von Ammon K, Ogata N, et al. Incidence and timing of p53 mutations during astrocytoma progression in patients with multiple biopsies. Clin Cancer Res. 1997;3:523–30.
45. von Deimling A, Eibl RH, Ohgaki H, Louis DN, von Ammon K, Petersen I, et al. p53 mutations are associated with 17p allelic loss in grade II and grade III astrocytoma. Cancer Res. 1992;52:2987–90.
46. Peraud A, Kreth FW, Wiestler OD, Kleihues P, Reulen HJ. Prognostic impact of TP53 mutations and P53 protein overexpression in supratentorial WHO grade II astrocytomas and oligoastrocytomas. Clin Cancer Res. 2002;8:1117–24.
47. Geisbrecht BV, Gould SJ. The human PICD gene encodes a cytoplasmic and peroxisomal NADP(+)-dependent isocitrate dehydrogenase. J Biol Chem. 1999;274:30527–33.
48. RefSEQ. Isocitrate dehydrogenase. In: National Center for Biotechnology Information. 2008. www.ghr.nlm.nih.gov/gene/IDH1.
49. Narahara K, Kimura S, Kikkawa K, Takahashi Y, Wakita Y, Kasai R, et al. Probable assignment of soluble isocitrate dehydrogenase (IDH1) to 2q33.3. Hum Genet. 1985;71:37–40.
50. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, et al. An integrated genomic analysis of human glioblastoma multiforme. Science. 2008;321:1807–12.
51. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, et al. IDH1 and IDH2 mutations in gliomas. N Engl J Med. 2009;360:765–73.
52. Ichimura K, Pearson DM, Kocialkowski S, Backlund LM, Chan R, Jones DT, et al. IDH1 mutations are present in the majority of common adult gliomas but rare in primary glioblastomas. Neuro Oncol. 2009;11:341–7.
53. Balss J, Meyer J, Mueller W, Korshunov A, Hartmann C, von Deimling A. Analysis of the IDH1 codon 132 mutation in brain tumors. Acta Neuropathol. 2008;116:597–602.
54. Watanabe T, Nobusawa S, Kleihues P, Ohgaki H. IDH1 mutations are early events in the development of astrocytomas and oligodendrogliomas. Am J Pathol. 2009;174:1149–53.
55. Gravendeel LA, Kloosterhof NK, Bralten LB, van Marion R, Dubbink HJ, Dinjens W, et al. Segregation of non-p.R132H mutations in IDH1 in distinct molecular subtypes of glioma. Hum Mutat. 2010;31:E1186–99.
56. Zhao S, Guan KL. IDH1 mutant structures reveal a mechanism of dominant inhibition. Cell Res. 2010;20:1279–81.
57. Zhao S, Lin Y, Xu W, Jiang W, Zha Z, Wang P, et al. Glioma-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1alpha. Science. 2009;324:261–5.
58. Pietrak B, Zhao H, Qi H, Quinn C, Gao E, Boyer JG, et al. A tale of two subunits: how the neomorphic R132H IDH1 mutation enhances production of alphaHG. Biochemistry. 2011;50:4804–12.
59. Yang B, Zhong C, Peng Y, Lai Z, Ding J. Molecular mechanisms of "off-on switch" of activities of human IDH1 by tumor-associated mutation R132H. Cell Res. 2010;20:1188–200.
60. Zhu J, Zuo J, Xu Q, Wang X, Wang Z, Zhou D. Isocitrate dehydrogenase mutations may be a protective mechanism in glioma patients. Med Hypotheses. 2011;76:602–3.
61. Metellus P, Coulibaly B, Colin C, de Paula AM, Vasiljevic A, Taieb D, et al. Absence of IDH mutation identifies a novel radiologic and molecular subtype of WHO grade II gliomas with dismal prognosis. Acta Neuropathol. 2010;120:719–29.
62. Labussiere M, Idbaih A, Wang XW, Marie Y, Boisselier B, Falet C, et al. All the 1p19q codeleted gliomas are mutated on IDH1 or IDH2. Neurology. 2010;74:1886–90.
63. Williams LT. Signal transduction by the platelet-derived growth factor receptor. Science. 1989;243:1564–70.
64. Schlessinger J. SH2/SH3 signaling proteins. Curr Opin Genet Dev. 1994;4:25–30.
65. di Tomaso E, London N, Fuja D, Logie J, Tyrrell JA, Kamoun W, et al. PDGF-C induces maturation of blood vessels in a model of glioblastoma and attenuates the response to anti-VEGF treatment. PLoS One. 2009;4:e5123.
66. Lokker NA, Sullivan CM, Hollenbach SJ, Israel MA, Giese NA. Platelet-derived growth factor (PDGF) autocrine signaling regulates survival and mitogenic pathways in glioblastoma cells: evidence that the novel PDGF-C and PDGF-D ligands may play a role in the development of brain tumors. Cancer Res. 2002;62:3729–35.
67. Hermanson M, Funa K, Hartman M, Claesson-Welsh L, Heldin CH, Westermark B, et al. Platelet-derived growth factor and its receptors in human glioma tissue: expression of messenger RNA and protein suggests the presence of autocrine and paracrine loops. Cancer Res. 1992;52:3213–9.
68. Guha A, Glowacka D, Carroll R, Dashner K, Black PM, Stiles CD. Expression of platelet derived growth factor and platelet derived growth factor receptor mRNA in a glioblastoma from a patient with Li-Fraumeni syndrome. J Neurol Neurosurg Psychiatry. 1995;58:711–4.
69. Huang H, Colella S, Kurrer M, Yonekawa Y, Kleihues P, Ohgaki H. Gene expression profiling of low-grade

diffuse astrocytomas by cDNA arrays. Cancer Res. 2000;60:6868–74.
70. Varela M, Ranuncolo SM, Morand A, Lastiri J, De Kier Joffe EB, Puricelli LI, et al. EGF-R and PDGF-R, but not bcl-2, overexpression predict overall survival in patients with low-grade astrocytomas. J Surg Oncol. 2004;86:34–40.
71. Ekstrand AJ, Sugawa N, James CD, Collins VP. Amplified and rearranged epidermal growth factor receptor genes in human glioblastomas reveal deletions of sequences encoding portions of the N- and/or C-terminal tails. Proc Natl Acad Sci USA. 1992;89: 4309–13.
72. Liu L, Backlund LM, Nilsson BR, Grander D, Ichimura K, Goike HM, et al. Clinical significance of EGFR amplification and the aberrant EGFRvIII transcript in conventionally treated astrocytic gliomas. J Mol Med. 2005;83:917–26.
73. Rorive S, Maris C, Debeir O, Sandras F, Vidaud M, Bieche I, et al. Exploring the distinctive biological characteristics of pilocytic and low-grade diffuse astrocytomas using microarray gene expression profiles. J Neuropathol Exp Neurol. 2006;65: 794–807.
74. Sallinen SL, Sallinen PK, Haapasalo HK, Helin HJ, Helen PT, Schraml P, et al. Identification of differentially expressed genes in human gliomas by DNA microarray and tissue chip techniques. Cancer Res. 2000;60:6617–22.
75. Godard S, Getz G, Delorenzi M, Farmer P, Kobayashi H, Desbaillets I, et al. Classification of human astrocytic gliomas on the basis of gene expression: a correlated group of genes with angiogenic activity emerges as a strong predictor of subtypes. Cancer Res. 2003;63:6613–25.
76. Hunter S, Young A, Olson J, Brat DJ, Bowers G, Wilcox JN, et al. Differential expression between pilocytic and anaplastic astrocytomas: identification of apolipoprotein D as a marker for low-grade, non-infiltrating primary CNS neoplasms. J Neuropathol Exp Neurol. 2002;61:275–81.
77. Rickman DS, Bobek MP, Misek DE, Kuick R, Blaivas M, Kurnit DM, et al. Distinctive molecular profiles of high-grade and low-grade gliomas based on oligonucleotide microarray analysis. Cancer Res. 2001;61:6885–91.
78. Gutmann DH, Hedrick NM, Li J, Nagarajan R, Perry A, Watson MA. Comparative gene expression profile analysis of neurofibromatosis 1-associated and sporadic pilocytic astrocytomas. Cancer Res. 2002;62: 2085–91.
79. Khatua S, Peterson KM, Brown KM, Lawlor C, Santi MR, LaFleur B, et al. Overexpression of the EGFR/FKBP12/HIF-2alpha pathway identified in childhood astrocytomas by angiogenesis gene profiling. Cancer Res. 2003;63:1865–70.
80. Ljubimova JY, Lakhter AJ, Loksh A, Yong WH, Riedinger MS, Miner JH, et al. Overexpression of alpha4 chain-containing laminins in human glial tumors identified by gene microarray analysis. Cancer Res. 2001;61:5601–10.
81. van den Boom J, Wolter M, Kuick R, Misek DE, Youkilis AS, Wechsler DS, et al. Characterization of gene expression profiles associated with glioma progression using oligonucleotide-based microarray analysis and real-time reverse transcription-polymerase chain reaction. Am J Pathol. 2003;163: 1033–43.
82. Wong KK, Chang YM, Tsang YT, Perlaky L, Su J, Adesina A, et al. Expression analysis of juvenile pilocytic astrocytomas by oligonucleotide microarray reveals two potential subgroups. Cancer Res. 2005;65:76–84.
83. Huang H, Hara A, Homma T, Yonekawa Y, Ohgaki H. Altered expression of immune defense genes in pilocytic astrocytomas. J Neuropathol Exp Neurol. 2005; 64:891–901.
84. Crawford JR, Santi MR, Thorarinsdottir HK, Cornelison R, Rushing EJ, Zhang H, et al. Detection of human herpesvirus-6 variants in pediatric brain tumors: association of viral antigen in low grade gliomas. J Clin Virol. 2009;46:37–42.
85. Besleaga R, Montesinos-Rongen M, Perez-Tur J, Siebert R, Deckert M. Expression of the LGI1 gene product in astrocytic gliomas: downregulation with malignant progression. Virchows Arch. 2003;443: 561–4.
86. Weil KC, Berge MS, Sehgal A. Molecular characterization of a novel human brain tumor-associated gene BR-3. Anticancer Res. 2002;22:1467–74.
87. Schlierf B, Friedrich RP, Roerig P, Felsberg J, Reifenberger G, Wegner M. Expression of SoxE and SoxD genes in human gliomas. Neuropathol Appl Neurobiol. 2007;33:621–30.
88. Boulay JL, Miserez AR, Zweifel C, Sivasankaran B, Kana V, Ghaffari A, et al. Loss of NOTCH2 positively predicts survival in subgroups of human glial brain tumors. PLoS One. 2007;2:e576.
89. Yu Y, Xu F, Peng H, Fang X, Zhao S, Li Y, et al. NOEY2 (ARHI), an imprinted putative tumor suppressor gene in ovarian and breast carcinomas. Proc Natl Acad Sci USA. 1999;96:214–9.
90. Husemann K, Wolter M, Buschges R, Bostrom J, Sabel M, Reifenberger G. Identification of two distinct deleted regions on the short arm of chromosome 1 and rare mutation of the CDKN2C gene from 1p32 in oligodendroglial tumors. J Neuropathol Exp Neurol. 1999;58:1041–50.
91. Bello MJ, de Campos JM, Vaquero J, Ruiz-Barnes P, Kusak ME, Sarasa JL, et al. hRAD54 gene and 1p high-resolution deletion-mapping analyses in oligodendrogliomas. Cancer Genet Cytogenet. 2000; 116:142–7.
92. Tews B, Roerig P, Hartmann C, Hahn M, Felsberg J, Blaschke B, et al. Hypermethylation and transcriptional downregulation of the CITED4 gene at 1p34.2 in oligodendroglial tumours with allelic losses on 1p and 19q. Oncogene. 2007;26:5010–6.
93. Barbashina V, Salazar P, Holland EC, Rosenblum MK, Ladanyi M. Allelic losses at 1p36 and 19q13 in gliomas: correlation with histologic classification, definition of a 150-kb minimal deleted region on

1p36, and evaluation of CAMTA1 as a candidate tumor suppressor gene. Clin Cancer Res. 2005; 11:1119–28.
94. McDonald JM, Dunmire V, Taylor E, Sawaya R, Bruner J, Fuller GN, et al. Attenuated expression of DFFB is a hallmark of oligodendrogliomas with 1p-allelic loss. Mol Cancer. 2005;4:35.
95. Dong S, Pang JC, Hu J, Zhou LF, Ng HK. Transcriptional inactivation of TP73 expression in oligodendroglial tumors. Int J Cancer. 2002;98: 370–5.
96. McDonald JM, Dunlap S, Cogdell D, Dunmire V, Wei Q, Starzinski-Powitz A, et al. The SHREW1 gene, frequently deleted in oligodendrogliomas, functions to inhibit cell adhesion and migration. Cancer Biol Ther. 2006;5:300–4.
97. Hartmann C, Johnk L, Kitange G, Wu Y, Ashworth LK, Jenkins RB, et al. Transcript map of the 3.7-Mb D19S112–S246 candidate tumor suppressor region on the long arm of chromosome 19. Cancer Res. 2002;62:4100–8.
98. Rosenberg JE, Lisle DK, Burwick JA, Ueki K, von Deimling A, Mohrenweiser HW, et al. Refined deletion mapping of the chromosome 19q glioma tumor suppressor gene to the D19S412-STD interval. Oncogene. 1996;13:2483–5.
99. Smith JS, Tachibana I, Pohl U, Lee HK, Thanarajasingam U, Portier BP, et al. A transcript map of the chromosome 19q-arm glioma tumor suppressor region. Genomics. 2000;64:44–50.
100. Mora J, Cheung NK, Chen L, Qin J, Gerald W. Loss of heterozygosity at 19q13.3 is associated with locally aggressive neuroblastoma. Clin Cancer Res. 2001;7:1358–61.
101. Hartmann C, Mueller W, von Deimling A. Pathology and molecular genetics of oligodendroglial tumors. J Mol Med. 2004;82:638–55.
102. Hong C, Bollen AW, Costello JF. The contribution of genetic and epigenetic mechanisms to gene silencing in oligodendrogliomas. Cancer Res. 2003;63: 7600–5.
103. Wolf RM, Draghi N, Liang X, Dai C, Uhrbom L, Eklof C, et al. p190RhoGAP can act to inhibit PDGF-induced gliomas in mice: a putative tumor suppressor encoded on human chromosome 19q13.3. Genes Dev. 2003;17:476–87.
104. Tews B, Felsberg J, Hartmann C, Kunitz A, Hahn M, Toedt G, et al. Identification of novel oligodendroglioma-associated candidate tumor suppressor genes in 1p36 and 19q13 using microarray-based expression profiling. Int J Cancer. 2006;119:792–800.
105. Trouillard O, Aguirre-Cruz L, Hoang-Xuan K, Marie Y, Delattre JY, Sanson M. Parental 19q loss and PEG3 expression in oligodendrogliomas. Cancer Genet Cytogenet. 2004;151:182–3.
106. Jiang X, Yu Y, Yang HW, Agar NY, Frado L, Johnson MD. The imprinted gene PEG3 inhibits Wnt signaling and regulates glioma growth. J Biol Chem. 2010;285:8472–80.
107. Schramm J, editor. Low-grade gliomas, vol. 35. New York: Springer; 2010.
108. Reifenberger J, Reifenberger G, Ichimura K, Schmidt EE, Wechsler W, Collins VP. Epidermal growth factor receptor expression in oligodendroglial tumors. Am J Pathol. 1996;149:29–35.
109. Di Rocco F, Carroll RS, Zhang J, Black PM. Platelet-derived growth factor and its receptor expression in human oligodendrogliomas. Neurosurgery. 1998;42: 341–6.
110. Hagerstrand D, Smits A, Eriksson A, Sigurdardottir S, Olofsson T, Hartman M, et al. Gene expression analyses of grade II gliomas and identification of rPTPbeta/zeta as a candidate oligodendroglioma marker. Neuro Oncol. 2008;10:2–9.
111. Nakamura M, Watanabe T, Klangby U, Asker C, Wiman K, Yonekawa Y, et al. p14ARF deletion and methylation in genetic pathways to glioblastomas. Brain Pathol. 2001;11:159–68.
112. Watanabe T, Katayama Y, Yoshino A, Yachi K, Ohta T, Ogino A, et al. Aberrant hypermethylation of p14ARF and O6-methylguanine-DNA methyltransferase genes in astrocytoma progression. Brain Pathol. 2007;17:5–10.
113. Sherr CJ. Divorcing ARF and p53: an unsettled case. Nat Rev Cancer. 2006;6:663–73.
114. Stott FJ, Bates S, James MC, McConnell BB, Starborg M, Brookes S, et al. The alternative product from the human CDKN2A locus, p14(ARF), participates in a regulatory feedback loop with p53 and MDM2. EMBO J. 1998;17:5001–14.
115. Kamijo T, Weber JD, Zambetti G, Zindy F, Roussel MF, Sherr CJ. Functional and physical interactions of the ARF tumor suppressor with p53 and Mdm2. Proc Natl Acad Sci USA. 1998;95:8292–7.
116. Waha A, Guntner S, Huang TH, Yan PS, Arslan B, Pietsch T, et al. Epigenetic silencing of the protocadherin family member PCDH-gamma-A11 in astrocytomas. Neoplasia. 2005;7:193–9.
117. Wiencke JK, Zheng S, Jelluma N, Tihan T, Vandenberg S, Tamguney T, et al. Methylation of the PTEN promoter defines low-grade gliomas and secondary glioblastoma. Neuro Oncol. 2007;9: 271–9.
118. Kunitz A, Wolter M, van den Boom J, Felsberg J, Tews B, Hahn M, et al. DNA hypermethylation and aberrant expression of the EMP3 gene at 19q13.3 in human gliomas. Brain Pathol. 2007;17:363–70.
119. Costello JF, Plass C, Cavenee WK. Aberrant methylation of genes in low-grade astrocytomas. Brain Tumor Pathol. 2000;17:49–56.
120. Yu J, Zhang H, Gu J, Lin S, Li J, Lu W, et al. Methylation profiles of thirty four promoter-CpG islands and concordant methylation behaviours of sixteen genes that may contribute to carcinogenesis of astrocytoma. BMC Cancer. 2004;4:65.
121. Silber JR, Bobola MS, Ghatan S, Blank A, Kolstoe DD, Berger MS. O6-methylguanine-DNA methyltransferase activity in adult gliomas: relation to patient and tumor characteristics. Cancer Res. 1998;58:1068–73.
122. Dong SM, Pang JC, Poon WS, Hu J, To KF, Chang AR, et al. Concurrent hypermethylation of multiple

genes is associated with grade of oligodendroglial tumors. J Neuropathol Exp Neurol. 2001;60:808–16.
123. Mollemann M, Wolter M, Felsberg J, Collins VP, Reifenberger G. Frequent promoter hypermethylation and low expression of the MGMT gene in oligodendroglial tumors. Int J Cancer. 2005;113:379–85.
124. Alonso ME, Bello MJ, Gonzalez-Gomez P, Arjona D, Lomas J, de Campos JM, et al. Aberrant promoter methylation of multiple genes in oligodendrogliomas and ependymomas. Cancer Genet Cytogenet. 2003; 144:134–42.
125. Huang L, Jiang T, Yuan F, Li GL, Cui Y, Liu EZ, et al. Correlation of chromosomes 1p and 19q status and expressions of O6-methylguanine DNA methyltransferase (MGMT), p53 and Ki-67 in diffuse gliomas of World Health Organization (WHO) grades II and III: a clinicopathological study. Neuropathol Appl Neurobiol. 2009;35:367–79.
126. Watanabe T, Nakamura M, Kros JM, Burkhard C, Yonekawa Y, Kleihues P, et al. Phenotype versus genotype correlation in oligodendrogliomas and low-grade diffuse astrocytomas. Acta Neuropathol. 2002;103:267–75.
127. Levin N, Lavon I, Zelikovitsh B, Fuchs D, Bokstein F, Fellig Y, et al. Progressive low-grade oligodendrogliomas: response to temozolomide and correlation between genetic profile and O6-methylguanine DNA methyltransferase protein expression. Cancer. 2006;106:1759–65.
128. Hilton DA, Love S, Barber R, Ellison D, Sandeman DR. Accumulation of p53 and Ki-67 expression do not predict survival in patients with fibrillary astrocytomas or the response of these tumors to radiotherapy. Neurosurgery. 1998;42:724–9.
129. Ishii N, Tada M, Hamou MF, Janzer RC, Meagher-Villemure K, Wiestler OD, et al. Cells with TP53 mutations in low grade astrocytic tumors evolve clonally to malignancy and are an unfavorable prognostic factor. Oncogene. 1999;18:5870–8.
130. Sanson M, Marie Y, Paris S, Idbaih A, Laffaire J, Ducray F, et al. Isocitrate dehydrogenase 1 codon 132 mutation is an important prognostic biomarker in gliomas. J Clin Oncol. 2009;27:4150–4.
131. Everhard S, Kaloshi G, Criniere E, Benouaich-Amiel A, Lejeune J, Marie Y, et al. MGMT methylation: a marker of response to temozolomide in low-grade gliomas. Ann Neurol. 2006;60:740–3.
132. Felsberg J, Erkwoh A, Sabel MC, Kirsch L, Fimmers R, Blaschke B, et al. Oligodendroglial tumors: refinement of candidate regions on chromosome arm 1p and correlation of 1p/19q status with survival. Brain Pathol. 2004;14:121–30.
133. Kujas M, Lejeune J, Benouaich-Amiel A, Criniere E, Laigle-Donadey F, Marie Y, et al. Chromosome 1p loss: a favorable prognostic factor in low-grade gliomas. Ann Neurol. 2005;58:322–6.
134. Sasaki H, Zlatescu MC, Betensky RA, Johnk LB, Cutone AN, Cairncross JG, et al. Histopathological-molecular genetic correlations in referral pathologist-diagnosed low-grade "oligodendroglioma". J Neuropathol Exp Neurol. 2002;61:58–63.
135. Smith JS, Tachibana I, Passe SM, Huntley BK, Borell TJ, Iturria N, et al. PTEN mutation, EGFR amplification, and outcome in patients with anaplastic astrocytoma and glioblastoma multiforme. J Natl Cancer Inst. 2001;93:1246–56.
136. Ino Y, Zlatescu MC, Sasaki H, Macdonald DR, Stemmer-Rachamimov AO, Jhung S, et al. Long survival and therapeutic responses in patients with histologically disparate high-grade gliomas demonstrating chromosome 1p loss. J Neurosurg. 2000;92: 983–90.
137. Cairncross JG, Ueki K, Zlatescu MC, Lisle DK, Finkelstein DM, Hammond RR, et al. Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. J Natl Cancer Inst. 1998;90:1473–9.
138. Kaloshi G, Benouaich-Amiel A, Diakite F, Taillibert S, Lejeune J, Laigle-Donadey F, et al. Temozolomide for low-grade gliomas: predictive impact of 1p/19q loss on response and outcome. Neurology. 2007;68:1831–6.
139. Hoang-Xuan K, Capelle L, Kujas M, Taillibert S, Duffau H, Lejeune J, et al. Temozolomide as initial treatment for adults with low-grade oligodendrogliomas or oligoastrocytomas and correlation with chromosome 1p deletions. J Clin Oncol. 2004;22:3133–8.
140. Bauman GS, Ino Y, Ueki K, Zlatescu MC, Fisher BJ, Macdonald DR, et al. Allelic loss of chromosome 1p and radiotherapy plus chemotherapy in patients with oligodendrogliomas. Int J Radiat Oncol Biol Phys. 2000;48:825–30.
141. McLendon RE, Herndon 2nd JE, West B, Reardon D, Wiltshire R, Rasheed BK, et al. Survival analysis of presumptive prognostic markers among oligodendrogliomas. Cancer. 2005;104:1693–9.
142. Walker C, du Plessis DG, Joyce KA, Fildes D, Gee A, Haylock B, et al. Molecular pathology and clinical characteristics of oligodendroglial neoplasms. Ann Neurol. 2005;57:855–65.
143. Kitange G, Misra A, Law M, Passe S, Kollmeyer TM, Maurer M, et al. Chromosomal imbalances detected by array comparative genomic hybridization in human oligodendrogliomas and mixed oligoastrocytomas. Genes Chromosomes Cancer. 2005;42:68–77.
144. Jeuken JW, Sprenger SH, Boerman RH, von Deimling A, Teepen HL, van Overbeeke JJ, et al. Subtyping of oligo-astrocytic tumours by comparative genomic hybridization. J Pathol. 2001;194:81–7.
145. Eoli M, Bissola L, Bruzzone MG, Pollo B, Maccagnano C, De Simone T, et al. Reclassification of oligoastrocytomas by loss of heterozygosity studies. Int J Cancer. 2006;119:84–90.
146. Broniscer A, Baker SJ, West AN, Fraser MM, Proko E, Kocak M, et al. Clinical and molecular characteristics of malignant transformation of low-grade glioma in children. J Clin Oncol. 2007;25:682–9.
147. Bhattacharjee MB, Armstrong DD, Vogel H, Cooley LD. Cytogenetic analysis of 120 primary pediatric brain tumors and literature review. Cancer Genet Cytogenet. 1997;97:39–53.
148. Ward SJ, Karakoula K, Phipps KP, Harkness W, Hayward R, Thompson D, et al. Cytogenetic analysis

of paediatric astrocytoma using comparative genomic hybridisation and fluorescence in-situ hybridisation. J Neurooncol. 2010;98:305–18.
149. Roberts P, Chumas PD, Picton S, Bridges L, Livingstone JH, Sheridan E. A review of the cytogenetics of 58 pediatric brain tumors. Cancer Genet Cytogenet. 2001;131:1–12.
150. Orr LC, Fleitz J, McGavran L, Wyatt-Ashmead J, Handler M, Foreman NK. Cytogenetics in pediatric low-grade astrocytomas. Med Pediatr Oncol. 2002; 38:173–7.
151. Orellana C, Hernandez-Marti M, Martinez F, Castel V, Millan JM, Alvarez-Garijo JA, et al. Pediatric brain tumors: loss of heterozygosity at 17p and TP53 gene mutations. Cancer Genet Cytogenet. 1998;102:93–9.
152. Wong KK, Tsang YT, Chang YM, Su J, Di Francesco AM, Meco D, et al. Genome-wide allelic imbalance analysis of pediatric gliomas by single nucleotide polymorphic allele array. Cancer Res. 2006;66:11172–8.
153. Nakamura M, Shimada K, Ishida E, Higuchi T, Nakase H, Sakaki T, et al. Molecular pathogenesis of pediatric astrocytic tumors. Neuro Oncol. 2007;9:113–23.
154. Miwa T, Hirose Y, Sasaki H, Ezaki T, Yoshida K, Kawase T. Single-copy gain of chromosome 1q is a negative prognostic marker in pediatric nonependymal, nonpilocytic gliomas. Neurosurgery. 2011;68: 206–12.
155. Pollack IF, Finkelstein SD, Burnham J, Hamilton RL, Yates AJ, Holmes EJ, et al. Association between chromosome 1p and 19q loss and outcome in pediatric malignant gliomas: results from the CCG-945 cohort. Pediatr Neurosurg. 2003;39:114–21.
156. Myal Y, Del Bigio MR, Rhodes RH. Age-related differences in 1p and 19q deletions in oligodendrogliomas. BMC Clin Pathol. 2003;3:6.
157. Zhang JG, Kruse CA, Driggers L, Hoa N, Wisoff J, Allen JC, et al. Tumor antigen precursor protein profiles of adult and pediatric brain tumors identify potential targets for immunotherapy. J Neurooncol. 2008;88:65–76.
158. Tabori U, Rienstein S, Dromi Y, Leider-Trejo L, Constantini S, Burstein Y, et al. Epidermal growth factor receptor gene amplification and expression in disseminated pediatric low-grade gliomas. J Neurosurg. 2005;103:357–61.
159. Otero JJ, Rowitch D, Vandenberg S. OLIG2 is differentially expressed in pediatric astrocytic and in ependymal neoplasms. J Neurooncol. 2011;104: 423–38.
160. Gupta M, Djalilvand A, Brat DJ. Clarifying the diffuse gliomas: an update on the morphologic features and markers that discriminate oligodendroglioma from astrocytoma. Am J Clin Pathol. 2005;124: 755–68.
161. Fuller GN, Hess KR, Rhee CH, Yung WK, Sawaya RA, Bruner JM, et al. Molecular classification of human diffuse gliomas by multidimensional scaling analysis of gene expression profiles parallels morphology-based classification, correlates with survival, and reveals clinically-relevant novel glioma subsets. Brain Pathol. 2002;12:108–16.
162. Kim YH, Nobusawa S, Mittelbronn M, Paulus W, Brokinkel B, Keyvani K, et al. Molecular classification of low-grade diffuse gliomas. Am J Pathol. 2010;177: 2708–14.
163. Korshunov A, Meyer J, Capper D, Christians A, Remke M, Witt H, et al. Combined molecular analysis of BRAF and IDH1 distinguishes pilocytic astrocytoma from diffuse astrocytoma. Acta Neuropathol. 2009;118:401–5.
164. Cooper LA, Gutman DA, Long Q, Johnson BA, Cholleti SR, Kurc T, et al. The proneural molecular signature is enriched in oligodendrogliomas and predicts improved survival among diffuse gliomas. PLoS One. 2010;5:e12548.
165. Noushmehr H, Weisenberger DJ, Diefes K, Phillips HS, Pujara K, Berman BP, et al. Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. Cancer Cell. 2010;17: 510–22.