Grade II of the WHO grading scheme for glial neoplasms comprises 5 distinct diagnoses for tumors of astrocytic or oligodendroglial lineage: diffuse astrocytoma, oligodendroglioma, oligoastrocytoma, pleomorphic xanthoastrocytoma, and pilomyxoid astrocytoma. The WHO system classifies solely based upon histological appearance, and so aggregation of these tumors into a single grade reflects their individual, perceived positions along various spectrums of histological aggressiveness rather than any fundamental genotypic or phenotypic similarities. In fact, these 5 tumors represent molecularly and clinically unique entities, and, for this reason, collectivization of these tumors (along with WHO Grade I gliomas) under the heading of “low-grade gliomas” is rapidly falling out of favor. Nonetheless, Grade II tumors differ considerably from WHO Grade I, III, and IV gliomas, and so the 5 Grade II gliomas are often discussed together.

Managing and predicting the course of these tumors has historically proven challenging, so basic and translational research in Grade II 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 gliomas remains nebulous. The authors’ goal for this review was to present a comprehensive discussion of current knowledge regarding the molecular characteristics of these 5 WHO Grade II tumors on the chromosomal, genomic, and epigenomic levels. Additionally, they discuss the emerging evidence suggesting molecular differences between adult and pediatric Grade II gliomas. Finally, they present an overview of current strategies for using molecular data to classify low-grade gliomas into clinically relevant categories based on tumor biology.

Key Words • astrocytoma • oligodendroglioma • oligoastrocytoma • pilomyxoid • pleomorphic • xanthoastrocytoma • molecular • genetics • brain tumor • classification • pediatric • translational • neurooncology

Abbreviations used in this paper: AII = WHO Grade II astrocytoma; EGFR = epidermal growth factor receptor; IDH = isocitrate dehydrogenase; LOH = loss of heterozygosity; OAI = WHO Grade II oligoastrocytoma; OII = WHO Grade II oligodendroglioma; PDGF = platelet-derived growth factor; PDGFR = PDGF receptor; PXA = pleomorphic xanthoastrocytoma (WHO Grade II).
investigated. Additionally, we have attempted to organize the data into a logical and organized framework through which it can be more readily understood. A summary of the chromosomal, genomic, and epigenomic changes associated with low-grade gliomas is presented in Table 1.

We have specifically excluded the WHO Grade II tumor of ependymal origin (ependymoma) from this discussion. Despite technically being a "WHO Grade II glioma," the biology and clinical characteristics of the ependymomas are considerably different from those of the 5 entities that we discuss in this review. We therefore believe that Grade II ependymomas are better treated within the context of a comprehensive discussion of the molecular biology of ependymoma in general.

### Diffuse Astrocytoma

**Overview**

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 histological examination. The category comprises 3 histological variants, including fibrillary astrocytoma, protoplasmic astrocytoma, and gemistocytic astrocytoma (sometimes described as “variants”). 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 US. They occur with peak incidence in the young adult population (age 20–34 years), where they represent approximately 10.2% of primary CNS tumors, 30.0% of all gliomas, and 25.2% of all malignant brain tumors. In this age group their survival rates at 1, 5, and 10 years are 91.6%, 58.5%, and 40.7%, respectively. 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.

In the adult population, most AIs will ultimately progress to anaplastic astrocytomas and then to “secondary” glioblastomas. This tendency suggests that AIs represent an early stage in the evolution of secondary glioblastoma, and many of the molecular characteristics described in AIs 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 AIs should be viewed through this lens.

Many molecular investigations include a small number of AIs as one part of a larger experimental sample containing various grades of glioma. These studies tend to identify genomic and epigenomic changes that occur with relatively low frequency in AIs and become more prevalent as gliomas progress to higher grades. Reporting the relative frequency of such changes in AIs 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. Instead, in this section we summarize...
Molecular biology of Grade II gliomas

those molecular features that appear common to a large proportion of AIIIs. 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 AIIIs.

Chromosomal Abnormalities

The most common chromosomal abnormalities in AII are trisomies or polysomies of chromosome 7,91,134 with gains of 7 or 7q observed in approximately 50% of these tumors.15,135,136 Gains in 8q have also been reported to occur with some consistency in AIIIs,16 and gains of 5p, 9, and 19p have also been inconsistently observed.133,134,180 Chromosomal losses in AIIIs have been reported most commonly involving 17p16,13,188 and less frequently on 6q,106 10p, 13q, 19q, 22q, and the sex chromosomes.133,134,177

Genomic Abnormalities

TP53. The TP53 gene localizes to 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.15 Activated p53 induces transcription of p21[^1]^{13,15} which regulates cell cycle progression at G1 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 being modulated by14,18 TP53. Sixty (60%) to 80% of AIIIs have allelic loss on 17p that includes the TP53 locus,133,187,185 and most AIIIs with the retained locus exhibit TP53 mutations.6,132,133 This makes complete absence of wild-type p53 the most common genomic abnormality in AIIIs.133,177 The incidence of TP53 mutations is higher in secondary than in primary glioblastoma17,19 but does not increase appreciably between AIIIs and glioblastoma,6,132,133 lending genomе-level support to the hypothesis that AIIIs represent an early stage in the evolution of secondary glioblastoma.91,138,153 This hypothesis is further supported by the findings that common TP53 mutations both in AIIIs27 and in secondary glioblastomas17 occur at codons 248 or 273 (while the TP53 mutations observed in primary glioblastomas are more broadly distributed) and that GC→AT mutation in CpG islands are more frequent in secondary than in primary glioblastoma.191,138 The latter observation suggests that different mechanisms may lead to the acquisitions of the TP53 mutations seen in these 2 glioblastoma subtypes.17,133

Isocitrate Dehydrogenase. The enzyme isocitrate dehydrogenase (IDH) catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate in the citric acid cycle and uses NADP[^2] as a proton acceptor.24 A total of 5 IDH isozenzymes 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,24 while the IDH2 enzyme assumes the more classic, mitochondrial localization (http://www.ncbi.nlm.nih.gov/gene/3418). A genome-wide analysis of glioblastoma identified IDH1 (2q33)[15] (http://www.ncbi.nlm.nih.gov/gene/3418) gene mutations in 12% of these tumors,125 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.51,190 Approximately 80% of AIIIs have been shown to harbor IDH1 gene mutations, and IDH2 (15q26.1) gene mutations are often present in the residual fraction.190 This finding makes IDH gene mutations the most common and consistent genetic abnormality in AIIIs reported to date. Notably, there does not appear to be a statistical association between IDH mutations and TP53 mutations in AIIIs,6 although these data remain inconsistent.118,190

The specific IDH1 mutation observed in low-grade gliomas is almost always (> 90%)99 a point mutation at position 132, where wild-type arginine is replaced by histidine in the mutant form (R132H).6 This mutation is commonly referred to as the canonical IDH1 mutation. Other, rare mutations at this position include substitutions of arginine with cysteine (R132C), serine (R132S), leucine (R132 L), glycine (R132G), or valine (R132 V),6,199 all of which can be described as noncanonical IDH1 mutations. These mutations are all heterozygous, and no truncation or frame shift mutants have yet been described.4 Position 132 belongs to an evolutionarily conserved region representing the binding site of the isocitrate substrate,6 and the R132 mutations result in reduced enzymatic activity toward isocitrate.64,199,207 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.128 Structural investigations have suggested a mechanistic explanation for this observation related to its effects on subunit dimerization,208 and a “dominant inhibition” model, whereby concurrent underproduction of α-ketoglutarate and overproduction of α-hydroxyglutarate may favor oncogenesis, has been proposed.209 Supplementary hypotheses include contributions to oncogenesis through induction of the HIF-α pathway,207 while others suggest that IDH mutations may not be oncogenic but may instead represent protective mechanisms that interfere with the metabolism of tumor cells.209

IDH2 is the only human protein homolog of IDH1 that uses NADP[^2] as a proton acceptor,199 and its arginine at position 172 (R172) is exactly analogous to R132 in TP53. This has led to the hypothesis that both IDH1 and IDH2 may be used in glioma biology as a potential oncoenzyme.157,206,207 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)18 via the SOS-Grb2 intermediary.205,195
As downstream pathways also modulated by the epidermal growth factor receptor (EGFR), PDGFR-associated pathways have been of considerable interest in glioma research. 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 AIs.

A number of preclinical and translational studies have reported putative roles for various components of the PDGF/PDGFR proteins in the biology of glioblastoma. However, despite being described throughout the glioma genomics literature, as being overexpressed in up to 60% of AIs, firm evidence for PDGFR overexpression in AII is sparse. Two small studies from the early 1990s, each including only 5 AIs in their analyses, reported that PDGFR-α appeared overexpressed in gliomas of all grades, including AIs. Attempts to validate this finding have been inconsistent, 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/AKT/mTOR pathway in the biology of primary but not secondary glioblastoma and the possible mutual exclusivity between p53 mutations and EGFR overexpression.

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. Given these data, it appears that the tyrosine kinase receptor pathways may play a 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 of studies specifically reporting on gene expression in low-grade gliomas performed through 2006 identified only 11 studies describing specific patterns of gene expression in Grade I and/or Grade II gliomas. The investigators summarized these results, then verified the most commonly reported gene expression differences were noted in AIIs without primary p53 mutations. One possible explanation for these nebulous findings may be that the relationship between p53 and clinically significant manifestations of various genomic features is more consistently associated with higher-grade gliomas.

Epigenetic Abnormalities

Epigenomic investigations represent a relatively recent area of research in the molecular biology of AIs. The most robust epigenomic data involves the ARF gene, which localizes to the CDKN2A (INK4/ARF) locus on chromosome 9 (9p21). Its gene product, p14ARF, binds to MDM2 and stabilizes both MDM2 and p53. Accordingly, methylation of the p14ARF promoter results in decreased production of the p14 gene product and relative destabilization of MDM2 and p53. In a single study, ARF (p14ARF) promoter hypermethylation has been documented in 26% of AIs, which was frequently observed in AIs without primary p53 mutations. All AIs in this study harboring ARF (p14ARF) promoter methylation ultimately progressed to secondary glioblastomas.

Similarly, promoter hypermethylation of the DNA-repair gene O6-methylguanine-DNA methyltransferase (MGMT) has also been observed in 63% of AIs. Interestingly, limited data suggest that MGMT hypermethylation is associated with p53 mutation but is mutually exclusive to ARF (p14ARF) promoter hypermethylation. Additional reports suggest epigenomic silencing of the PCDH-γ-AI1 (5q31), PTEN (10q23.31), and EMPI3 (19q13) genes in AII, and further investigations are likely to reveal additional instances of epigenomic abnormalities in these tumors.

Clinical Correlations

Few molecular markers have demonstrated prognostic significance in AIs. 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. The literature presents conflicting evidence regarding a potential relationship between abnormalities in p53 and malignant progression, with data arguing both for and against a potential association. Several studies agree, however, that p53 mutation does appear to be associated with an increased likelihood of tumor recurrence. One possible explanation for these nebulous findings may be that the relationship between p53 and
Molecular biology of Grade II gliomas

status and clinical outcomes varies between subtypes of AI. 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 AI subtype. 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.

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, although many of the studies in which this has been demonstrated do not necessarily separate AIs from oligodendroglomas. It therefore remains possible that disproportionate overrepresentation of oligodendrogloma in the experimental samples of these studies influenced the results, and the ultimate generalizability of these potential prognostic biomarkers specifically to AIs remains to be determined. Overexpression of EGFR and PDGFRβ (although uncommon in AIs) and MGMT promoter methylation has been associated with response to chemotherapy and thus with improved survival in patients with AIs.

Oligodendrogloma

Overview

The synonymous terms “oligodendrogloma” and “low-grade oligodendrogloma” (OII) refer to tumors of oligodendroglial histology with low proliferative activity and without obvious anaplastic features on microscopic examination. There are no specific histological variants of OII. Among all grades of glioma (excluding glioblastoma), astrocytic histology is 3 times more common than oligodendroglial histology. Oligodendroglomas occur with peak incidence in the 3rd to 5th decades, and the 1, 5, and 10-year survival rates for OIIs in adults are 94.2%, 79.5%, and 63.6%, respectively. OIIs are less common in pediatric patients, but when they do occur in this age group they are associated with better survival rates than those for OIIs in adults.

OIIs have recently become the subject of considerable attention in translational neurooncology 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 1p, with or without deletion of 19q, respond well to chemotherapy and are associated with a relatively longer survival, whereas those in which 1p is intact, with or without 19q, behave more aggressively. This finding supports the longstanding concerns of many neurooncologists that histological subtypes of glioma may not adequately capture all clinically relevant variability among these tumors and serves as important proof of principle for ongoing investigations for molecular subclassification of gliomas.

Chromosomal Abnormalities

The most common chromosomal abnormality in OIIs, occurring in approximately 50% of these tumors (although some report it in more than 80%), is a combined “loss” of the short arm of chromosome 1 (1p) and the long arm of chromosome 19 (19q). This and the 1p/19q translocation, and this finding is commonly referred to as “1p/19q codeletion.” Conversely, partial deletions of these loci or isolated loss of 1p or 19q are rare. Of the 2 chromosomal losses, 1p has the greater specificity, as 19q losses have been observed in other histological types and grades of glioma. Notwithstanding, 1p/19q codeletion is not completely specific to OIIs, as it has also been occasionally reported in astrocytomas, oligoastrocytomas, and glioblastomas. Combined losses of 1p and 19q appear to be mutually exclusive of several other molecular abnormalities commonly associated with gliomas, including 1p LOH and TP53 mutation. 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.

The exact molecular mechanisms associated with the development of the unique (1p;19q)(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. This has been hypothesized to result in centromeric colocalization of chromosomes 1 and 19, which might promote centromeric instability and thus favor the translocation. 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 chromosome 4, chromosome 6, the short arm of chromosome 11 (11p), chromosome 14, and the long arm of chromosome 22 (22q). In addition to these chromosomes, deletions have been observed in other chromosomal regions including 3p, 9p, 9q, 10p, 10q, 11q, 13q, 15q, 16q, and 22q. The validity and consistency of these deletion breakpoints remain to be determined.

Genomic Abnormalities

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), DIRAS3 (1p31), CITED4 (1p34.2), CAMTA1 (1p36.2), FFB (1p36.3), and SHREW1 (1p36.3). Because 1q 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 histological types with partial deletions of...
These studies have suggested a potential role for several genes on the 19q3 region, but additional investigations have not demonstrated consistent mutations of these genes. Epigenomic studies (see below) suggest potential roles for ZNF342 (19q13), pi90RhoGAP (19q13.3), EMP3 (19q13.3), and PEG3 (19q13.4), but definitive evidence for any of these candidate genes has yet to be demonstrated.

Isocitrate Dehydrogenase. As in AIs, IDH1 (and/or IDH2) mutations are common in OIs, and have been observed in more than 80% of these tumors. Many of the studies regarding the specific mutations and their functional significance have been conducted on mixed populations of AIs and OIs, 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 AIs and OIs initially suggested that these mutations were independent of other molecular features that distinguished these tumor types, more recent evidence suggests that there may be a high degree of correlation between IDH mutations and chromosome 1p/19q codeletions. Many of these investigations are conducted in populations with a mixture of OIs and AIs 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. The pathophysiological significance of this finding remains to be determined.

Other Abnormalities. EGFR amplification has been reported in approximately 50% of OIs, although this represents older data from small studies of relatively few tumors. PDGFα and PDGFB, as well as their receptors (PDGFR-α and PDGFR-β) appear to be overexpressed in a large percentage of OIs, making this finding more common among these tumors than in AIs. More recently, overexpression of rPTPβγ has been reported to distinguish OIs from AIs.

The role of OLIG1 and OLIG2 bHLH-family transcription factors in oligodendroglialoma identification merits specific mention. Data from murine models suggests that the OLIG gene products are essential regulators of ventral neuroectodermal progenitor cell fate and oligodendrocyte development. This led to initial enthusiasm that identification of these markers may assist in the diagnosis of oligodendroglialoma. This enthusiasm has since been tempered by the demonstration that the OLIG transcription factors are overexpressed in most neuroectodermal tumors at both the RNA and protein levels. Accordingly, the diagnostic role of OLIG1 and OLIG2 is currently relegated to that of a molecular marker for general neuroectodermal lineage in brain tumors.

Epigenomic Abnormalities

OIs demonstrate lower levels of MGMT expression than AIs. Some evidence suggests that up to 60%–80% of OIs may exhibit hypermethylation of the MGMT promoter (more common than in AIs) and that this hypermethylation correlates with 1p/19q loss, while others have not observed these effects. Additional genes that have been found to be hypermethylated in some OIs include CDKN2A (9p21), CDKN2B (9p21), ARF (9p21), RB1 (13q14), TP73 (1p36.3), DAPK1 (9q34.1), ESR1 (6q25.1), TIMP3 (22q12.3), THBS3 (15q15), and GSTP1 (11q13).

Clinical Correlations

Perhaps the most widely reported molecular finding with a clinical correlation is the relationship between the combined loss of 1p and 19q and improvements in survival and response to chemotherapy and radiotherapy. Data regarding the prognostic significance of TP53 mutation status and/or LOH at 17p13 specifically in OIs is limited, but some evidence suggests that these may be independent, unfavorable predictors of overall and progression-free survival.202 Gains on the long arm of chromosome 8 (8q) may also be associated with poor outcomes in OIs, but these data are derived from a relatively small study on a population of oligodendrogliomas of mixed WHO grades. While other correlations between molecular markers and survival or response-to-therapy phenotypes have been reported, these have almost always been studied primarily in OIs, making their generalizability specifically to OIs unclear.

Oligoastrocytoma

Oligoastrocytomas (OAIs), also called “mixed gliomas,” represent a unique WHO class of Grade II glioma that is characterized by tumors exhibiting a mixture of astrocytic and oligodendroglial histological morphology. Molecular evidence suggests that this histological class may comprise an unbalanced mixture of 2 primary tumor genotypes—AII and OII. This is supported by the observation that 30%–50% of OAIs exhibit chromosome 1p/19q codeletions (OII-like), while approximately 30% carry TP53 gene mutations (AII-like). Moreover, OAIs with 1p/19q codeletions have been observed to exhibit more prominent oligodendroglialoma-like features on microscopic examination, whereas those with TP53 mutations are more histologically similar to astrocytomas.

One study has proposed that chromosomal data may be useful for subdividing OAIs into 4 subclasses. 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 in recent investigations suggesting that 1p/19q codeletions may be a generally favorable prognostic factor in OAIs. 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 ex-
Molecular biology of Grade II gliomas

A Comment on Pediatric Grade II Infiltrating 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%).17 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. Although a complete discussion of the molecular differences between adult and pediatric glioma genomics is outside the scope of this review, a brief overview of the current status of these data is beneficial 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,11,70,81,121,136,138,180,193 rates for comparable abnormalities in pediatric patients have been reported to be relatively lower.14,105,113,122,123,141,183

Notwithstanding, chromosomal abnormalities in these pediatric tumors are not rare.105 For example, rates of 1p and 19q loss in pediatric populations may be similar to29 or greater than114 those in adults, although they do not appear to be associated with the same prognostic significance in children.129

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 on the basis of 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.14,105,113,122,123,129

The primary data are presented in a manner that allows independent examination of infiltrating glioma karyotypes,14,105,113,183 the rates of chromosomal abnormalities generally appear higher in the Grade II subgroup than the rates reported for the aggregate data set. This suggests that disproportionate inclusion of pilocytic astrocytomas may artifically 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 patients with predominantly Grade III tumors in which it was studied.129 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,205 but data on differential rates of expression of specific genes are currently limited. Some evidence suggests that EGFR overexpression may be relatively more common in pediatric tumors.106 Conversely, OLIG2 expression may be relatively less common.124 The clinical significance of these findings remains to be determined.

Pleomorphic Xanthoastrocytoma

Pleomorphic xanthoastrocytoma (PXA) was first described as a unique histopathological entity in 1979 and was characterized in subsequent years as an astrocytic tumor unique in its superficial cortical location, histological variability (pleomorphism), prominent xanthomatous cells, predilection toward occurring in the young, distinct clinical course, and favorable response to surgical resection.32,91,131 Literature regarding the molecular biology of PXAs is scarce and incomplete, attributable primarily to the rarity of this lesion. Notwithstanding, a few immunohistochemical, cytological, and molecular studies have made significant contributions to the present understanding of PXA as a unique pathological process that is genotypically distinct from other WHO Grade II gliomas.

One distinguishing feature of PXAs, supported by molecular and subcellular findings, is the tendency of these tumors to exhibit a dichotomous astrocytic/neuronal genotype and phenotype. Immunopositivity for glial markers, including glial fibrillary acidic protein (GFAP) and S-100 protein, is consistently present, suggesting a primarily astrocytic phenotype.36,62,130 However, neuronal markers, including class-III β-tubulin, synaptophysin, neurofilament proteins, SM1-31, and MAP2 immunopositivity have also been reported in 8%–73% of PXAs, suggesting neuronal differentiation.36,62,97,130 Ultrastructural analyses support this dichotomy, demonstrating features consistent with both astrocytic (intermediate filaments, lipid droplets, lysosomes) and neuronal (microtubules, dense core granules) differentiation.36,49,53

PXA may have diploid or polyploid karyotypes,33,131,138 and chromosomal studies have reported gains of chromosomes 3 and 5 and losses of 20 and 22.22 DNA loss on chromosome 9 appears to be the most
common regional chromosomal abnormality in PXA, occurring in ~50% of these tumors. Other, less common subtelomeric and regional losses involve chromosome 17 (10%), as well as qter, 6pter, 8pter, 9pter, 10p, chromosome 13, 17pter, 18pter, 21qter, and chromosome 22. Subtelomeric duplications have been variably reported at 3pter, 8pter, 12pter, 14qter, 16qter, 19pter, and 20pter, and regional subtelomeric gains have been documented on chromosome X (16%), 2p, 4pter, 5q, 7, 9q, 11qter, 12, 15q, 19, and 20. Translocations have been described involving chromosome 1, 15, 20, and 22.

Many molecular features that are commonly observed in other WHO Grade II gliomas are generally absent in PXAs. TP53 mutations are uncommon, EGFR overexpression is absent, ETV1 LOH on chromosome 1, 8p, 9p, 10, 17, 19q, and 22q are only rarely observed, and deletions or epigenomic inactivation involving CDKN2A or CDKN2B are inconsistent (although potentially functionally significant). Conversely, BRAF mutations (particularly at the V600E “hot spot”) are common in PXA, a molecular characteristic that they share with pilocytic astrocytomas. PXAs are also unique in their expression of wild-type or truncated CD34 in their expression of MDM2 without gene amplification, and in their immunophenotypic heterogeneity. Together, these findings suggest that PXAs may share more molecular similarities with pilocytic astrocytomas than with other WHO Grade II gliomas, and several authors have suggested that PXAs represent a pathophysiological entity distinct from other tumors that nonetheless share the same WHO grade.

It is also important to note that a small subgroup of PXAs is characterized by more aggressive histological features. These are generally designated as “anaplastic PXA” and assigned to WHO Grade III. The clinical behavior of these lesions is more aggressive and the prognosis is worse for these lesions than for their WHO Grade II counterparts. Because of their rarity, relatively little is known regarding the unique molecular biology of anaplastic PXAs; moreover, a detailed discussion of the molecular biology of these Grade III tumors is outside the scope of this review.

Pilomyxoid Astrocytoma

Pilomyxoid astrocytomas are WHO Grade II tumors that are closely related to Grade I pilocytic astrocytomas. They are generally hypothalamic/chiasmatic tumors of the very young (<1 year old) that may have a more aggressive clinical course than traditional pilocytic astrocytomas. As a rare tumor and a relatively recent addition to the WHO scheme, few molecular investigations of pilomyxoid astrocytomas have been performed to date. The most comprehensive is a comparison of copy number changes between pilomyxoid and pilocytic astrocytomas. This study suggested that both tumors share similar chromosomal changes but that pilomyxoid astrocytomas have relatively more regional chromosomal loss on 2p, 2q, 3q with additional, occasional differential loss on 7p, 9q, 15q, and 20q. Infrequent differential gains were also noted on 8q. Specific genomic differences include a high frequency of underexpression of the ALDH1L1 gene (89%), a finding shared with phenotypically aggressive pilocytic astrocytomas that may be of phenotypic significance. Mutations of p53 may be a feature of pilomyxoid astrocytomas, although these data are based on an analysis of only 3 samples and are therefore difficult to generalize. A single case of BCR gene disruption occurring in a pilomyxoid astrocytoma and variable activation of the hedgehog pathway in 4 pilomyxoid astrocytomas have also been reported.

Molecular Classification of Low-Grade Gliomas

This review 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 histological features for classification, 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. 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 review, 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. 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 histological differentiation between AII, OII, and OAI, and strategies employing various combinations of genomic and chromosomal data have demonstrated similar success in this task. 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, as have schemes that use more comprehensive sets of gene expression profiles. 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. The actual methods for classification using molecular data vary from simple algorithms based on one or a few markers to more complex mathematical models based on aggregate molecular data sets.

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 maxi-
Molecular biology of Grade II gliomas

mizing research, diagnostic, and clinical utility of molecular classification strategies are yet to be fully resolved. Nevertheless, there is considerable optimism in the translational neurooncology community that molecular data will ultimately prove to be a useful adjunct for classification of low-grade gliomas.

Conclusions

Molecular and translational research in WHO Grade II 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, lp and/or 19q deletion, and IDH1 and IDH2, endeavors to expand upon preclinical discoveries of novel potential markers, and efforts to incorporate molecular markers into tumor classification strategies. We remain optimistic that significant progress toward further understanding of the pathophysiology, the clinical behavior, and the optimal management of these tumors will continue to be made in the coming years.

Disclosure

Dr. Marco is supported in part by a grant from the American Association of Neurological Surgeons’ William P. VanWagenen Fellowship program and by an education and training grant from the Neurosurgery Research and Education Foundation. Dr. Weil is supported in part by the Melvin Burkhart chair in neurosurgical oncology and by the Karen Colina Wilson research endowment within the Brain Tumor and Neuro-Oncology Center at the Cleveland Clinic Foundation.

Author contributions to the study and manuscript preparation include the following. Conception and design: both authors. Acquisition of data: Marko. Analysis and interpretation of data: both authors. Drafting the article: Marko. Critically revising the article: all authors. Review and critique of the manuscript: both authors. Approved the final version of the manuscript on behalf of both authors: Marko. Administrative/technical/material support: Weil. Study supervision: Weil.

References


57. Huang L, Jiang T, Yuan F, Li GL, Cui Y, Liu EZ, et al: Cor-
Molecular biology of Grade II gliomas


86. Li YS, Ramsay DA, Fan YS, Armstrong RF, Del Maestro RF: Cytogenetic evidence that a tumor suppressor gene in the long arm of chromosome 1 contributes to glioma growth. Cancer Genet Cyto genetics 84:46–50, 1995


Molecular biology of Grade II gliomas

pression in supratentorial WHO grade II astrocytomas and oligoastrocytomas. *Clin Cancer Res* 8:1117–1124, 2002


Yin XL, Hui AB, Li, Da Chang, Ding M, Chang AR, Ng HK: Genetic imbalances in pleomorphic xanthoastrocytoma detected by comparative genomic hybridization and literature review. *Cancer Genet Cytofgenet* 132:14–19, 2002

N. F. Marko and R. J. Weil
Molecular biology of Grade II gliomas


Accepted December 6, 2012.

Address correspondence to: Nicholas F. Marko, M.D., The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, FC7, Houston, Texas 77030. email: nfmarko@mdanderson.org.