Pathological and Molecular Advances in Pediatric Low-Grade Astrocytoma

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Abstract
Pediatric low-grade astrocytomas are the most common brain tumors in children. They can have similar microscopic and clinical features, making accurate diagnosis difficult. For patients whose tumors are in locations that do not permit full resection, or those with an intrinsically aggressive biology, more effective therapies are required. Until recently, little was known about the molecular changes that drive the initiation and growth of pilocytic and other low-grade astrocytomas beyond the association of a minority of cases, primarily in the optic nerve, with neurofibromatosis type 1. Over the past several years, a wide range of studies have implicated the BRAF oncogene and other members of this signaling cascade in the pathobiology of pediatric low-grade astrocytoma. In this review, we attempt to summarize this rapidly developing field and discuss the potential for translating our growing molecular knowledge into improved diagnostic and prognostic biomarkers and new targeted therapies.
CLINICAL AND DEMOGRAPHIC FEATURES

Pediatric low-grade astrocytomas (PLGAs) arise throughout the central nervous system (CNS) but are found most often in the cerebellum, followed by the cerebrum and deep midline structures, optic pathways, brain stem, and spinal cord (1). According to 2012 Central Brain Tumor Registry of the United States (CBTRUS) data, pilocytic astrocytomas (PAs) are the second most common brain tumor (after embryonal neoplasms) in the 0–4-year age group, the most common tumors in the 5–14-year age group, and the second most common tumors (after pituitary neoplasms) in the 15–19-year age group (http://www.cbtrus.org).

Figure 1 depicts primary pediatric brain tumor diagnoses based on current CBTRUS data. Most noninfiltrative PLGAs (i.e., pilocytic and pilomyxoid astrocytomas, pleomorphic xanthoastrocytomas, and subependymal giant cell astrocytomas) show variable contrast enhancement on imaging studies, whereas infiltrating diffuse low-grade astrocytomas are generally nonenhancing.

Outcomes for this group of tumors are good overall but vary depending on the extent of surgical resection and histopathological classification. In one large institutional series of children with low-grade astrocytomas, 5-year overall survival (OS) was 96% for PA patients and 48% for diffuse astrocytoma patients (2). Gross total resection has consistently been strongly prognostic of long-term survival, but can often be achieved only when tumors are localized to the cerebellum or superficial cerebrum (3–5). Also, PAs may behave more aggressively when arising in adults (6).

HISTOLOGICAL CLASSIFICATION

Pilocytic Astrocytomas (Grade I)

PAs are generally characterized by a biphasic growth pattern that includes both compacted bipolar cells (Figure 2a) and regions with microcysts and more loosely textured cells (Figure 2b). Characteristic elements include Rosenthal fibers and eosinophilic granular bodies (Figure 2a,b). Oligodendroglial-appearing cells are often encountered in PAs, and in some cases they can constitute a significant portion of the tumor (7), making diagnosis difficult in small samples. Most lesions are cytologically bland, although some atypia can be present. Mitotic figures are present only in a minority of cases, and the lack of infiltration, as well as the presence of more specific features such as eosinophilic granular bodies, allows most cases to be differentiated from high-grade astrocytomas (8).

Numerous potential histological and immunohistochemical prognostic markers have been investigated in PAs. Tumors with an elevated Ki67 proliferation index may be associated with worse progression-free survival (9, 10), although in other studies proliferation was not prognostic (11–13). Anaplastic features, including cytological atypia, hypercellularity, high mitotic activity, and necrosis, can also portend worse outcomes (14). Morphological or immunohistochemical evidence of oligodendroglial differentiation may also predict aggressive behavior in PA patients (7, 11).
Figure 2
Histopathological features of pediatric low-grade astrocytomas. (a) Pilocytic astrocytomas (PAs) with compacted glial cells and Rosenthal fibers (arrow). (b) Microcysts (asterisk) and eosinophilic granular bodies (arrow) in a looser region of a PA. (c) Pilomyxoid astrocytoma composed of monomorphic cells with a pronounced perivascular growth pattern. (d) Spindled, pleomorphic cells in a pleomorphic xanthoastrocytoma. (e) Enlarged ganglion cell–like elements in a subependymal giant cell astrocytoma. (f) Diffuse astrocytomas are only modestly hypercellular, with scattered atypical cells (arrow).

Pilomyxoid Astrocytomas (Grade II)

Pilomyxoid astrocytomas are composed of monomorphic bipolar cells, often arrayed around vessels, with a mucoid background matrix; they lack Rosenthal fibers and eosinophilic granular bodies (Figure 2c). Referred to in early reports as infantile PAs, these tumors’ distinct microscopic features and more aggressive clinical behavior were highlighted in 1999 by Tihan et al. (15), leading to their recognition as a potentially distinct variant in the 2007 World Health Organization (WHO) classification (8). These tumors are often found in hypothalamus, in optic chiasm, and around the third ventricle, although other sites can also be involved. Recently, transitional tumors with mixed pilocytic and pilomyxoid features have been described, as well as cases in which a
pilomyxoid astrocytoma “matured” into a classic PA over time (16). These observations suggest that these lesions represent part of a spectrum rather than completely distinct entities.

**Pleomorphic Xanthoastrocytomas (Grade II)**

Pleomorphic xanthoastrocytomas (PXAs) generally show greater cellularity and atypia than do other entities discussed in this review, and they may be misdiagnosed as high-grade gliomas. They occur most frequently in teenagers and young adults, often involve superficial cortex and meninges, and can contain lipidized xanthomatous astrocytes (8). Eosinophilic granular bodies as well as spindled and pleomorphic glial elements are all common, and these tumors frequently attract a lymphocytic infiltrate and contain abundant extracellular reticulin (Figure 2d). CD34 expression has been proposed as a useful immunohistochemical feature (17).

**Subependymal Giant Cell Astrocytomas (Grade I)**

Subependymal giant cell astrocytomas (SEGAs) are benign tumors that are tightly associated with the autosomal dominant inherited condition tuberous sclerosis, and arise almost exclusively in the walls of the lateral ventricles (8). Spindled, gemistocytic, and ganglion cell–like elements can be present; the latter cells are the most characteristic finding (Figure 2e). Immunohistochemical and ultrastructural studies have shown mixed glial and neuronal differentiation in tumor cells (18–20), and despite their designation as astrocytomas, these tumors are probably best regarded as mixed glial-neuronal neoplasms.

**Diffuse Astrocytomas (Grade II)**

Infiltrating fibrillary astrocytomas in children are morphologically similar to their WHO grade II counterparts in adults. They are characterized by modest cellularity, diffuse infiltration of normal brain elements; and a lack of significant mitotic activity, vascular proliferation, or necrosis (Figure 2f) (8). Because of their ability to spread diffusely, these tumors are difficult to completely resect and have worse outcomes than do PAs in children (2). As in adults, they can progress to high-grade gliomas, although such progression often does not occur. Emerging data suggest that diffuse astrocytomas in the pediatric population are molecularly distinct from their morphologically similar adult counterparts.

**Diagnostic Difficulties in Pediatric Low-Grade Astrocytomas**

Tumors that are difficult to classify are unfortunately all too often encountered among the spectrum of PLGAs. In one institutional study of 278 consecutive PLGAs resected between 1965 and 1996, 75 cases (27%) did not clearly fit into a WHO diagnostic category. A more recent review of 1,670 pediatric brain tumors of all types, diagnosed at our institution between 2003 and 2008, identified 302 cases that could not be classified, and 7 of the 10 most common problems with diagnosis involved low-grade gliomas (P. Burger, personal communication). In some cases, diagnostic dilemmas arise due to small biopsies, but in others, they reflect an inability of the current scheme to fit a heterogeneous spectrum of lesions. Molecular studies could help with these problematic issues and, as described below, are beginning to shed light on some diagnostic difficulties.

**MOLECULAR ADVANCES**

Aside from PAs arising in neurofibromatosis type 1 (NF1) patients, and SEGAs in children and young adults with tuberous sclerosis, for many years little was known about the molecular underpinnings of PLGAs. Sporadic PAs do not inactivate NF1 and generally lack changes to the oncogenes and tumor suppressors altered in adult diffuse astrocytomas (8, 21). Early cytogenetic studies of PAs were notable for
a lack of detectable chromosomal alterations, with largely normal karyotypes in the more than 100 cases initially studied (8). Mutations in IDH1 or IDH2 have been identified in most low-grade gliomas in adults; interestingly, they are almost never detected in pediatric low- or high-grade gliomas and, when present, have been reported mostly in children who are at least 14 years old (22–24). This finding suggests that adolescents with IDH1- or IDH2-mutant tumors may represent the youngest patients with “adult” gliomas. Over the past few years, however, it has become clear that most nonsyndromic PLGAs harbor genomic alterations that affect the function of BRAF and that mitogen-activated protein kinase (MAPK) represents the dominant genetically altered pathway in these tumors (Figure 3).

**BRAF Fusions and MAPK Activation in Pediatric Low-Grade Astrocytomas**

In 2008, five different groups identified ∼2-Mb gains at chromosome 7q34 in most PLGAs by using array-based comparative genomic hybridization (array CGH) (Figure 4a) (25–29). Fluorescence in situ hybridization (FISH) and other molecular analyses showed that these gains represented segmental duplications in the region (Figure 4b) (25, 28). In these initial studies, between 53% and 77% of the PLGAs examined (mostly PAs) contained the duplication at 7q34, and relatively few other chromosomal alterations were detected, suggesting that this duplication represents the dominant change in PAs.

Two groups demonstrated that 7q34 duplication resulted in the expression of a novel fusion transcript between the KIAA1549 locus and BRAF that included the BRAF kinase domain but lacked the inhibitory N-terminal regulatory region of this oncogene (Figure 4c) (28, 29). The fusion showed constitutive kinase activity and transformed NIH 3T3 cells (29). BRAF induced signaling in the MAPK pathway, and activated targets, including phosphorylated MEK (pMEK) and/or phosphorylated ERK (pERK) (Figure 4d), were identified in most of the PLGA specimens examined in these initial studies (25, 27, 28).

Many additional reports have confirmed these exciting findings and have firmly established KIAA1549:BRAF duplication/fusion as the most common genetic change in PAs (30–39). Specific breakpoints between KIAA1549 and BRAF can vary, but all lead to loss of the BRAF autoregulatory domain (Figure 5). In a recent study of 106 pediatric low-grade brain tumors, five types of KIAA1549:BRAF gene fusions were identified; they involved exons 1–16/9–18 (49%), 1–15/9–18 (35%), 1–16/11–18 (8%), 1–15/11–18 (6%), and 1–17/10–18 (1%) (39). In this study, the 1–16/11–18 fusion was limited to infratentorial sites, and the 1–15/11–18 fusion to supratentorial locations, but it will be necessary to examine larger numbers of cases before firm conclusions about associations between fusion genotype and tumor phenotype can be drawn. Genetic mapping of the breakpoints involved have highlighted enrichment for microhomologous DNA sequences,
Molecular alterations involving \textit{BRAF}. (\textit{a}) Segmental gains at chromosome 7q34 approximately 2 Mb in size are commonly identified in pilocytic astrocytomas (PAs) by use of array-based comparative genomic hybridization. (\textit{b}) Fluorescence in situ hybridization reveals duplication in this region, with an extra copy of the region encoding BRAF. (Inset) A normal control. (\textit{c}) Sequencing of a \textit{KIAA1549:BRAF} fusion product between exons 16 and 9 of the two genes. (\textit{d}) PAs contain abundant active phosphorylated ERK. Normal vessels serve as an internal negative control (asterisks).

suggesting that microhomology-mediated break-induced replication may be a mechanism underlying the rearrangements (40).

The \textit{NF1} gene product inhibits RAS and BRAF activity, and the discovery of fusions that activate BRAF therefore links syndromic and sporadic PAs to the same oncogenic signaling cascade (Figure 3). Some patients with Noonan syndrome, in which MAPK signaling is activated by mutations in \textit{PTPN11, SOS1}, and \textit{KRAS}, have also been reported to have PAs (41–43); this observation provides further support for the central role of this pathway. A rare rosette-forming glioneuronal tumor of the posterior fossa that demonstrates strong pERK immunoreactivity has also been reported in a case of Noonan syndrome (44).

Novel genetic mechanisms that drive the activation of the MAPK pathway in PLGAs continue to be discovered. Two groups identified oncogenic fusions between \textit{SRGAP3} and \textit{RAF1} that were predicted to give rise to unregulated kinase activity, similar to that observed with \textit{KIAA1549:BRAF} (Figure 5) (30, 45). Rare 3-bp insertions at position 599 have also been described in PAs (32, 45–47). This alteration (\textit{BRAFinsT}) results in duplication of a threonine residue and causes more-than-sixfold increases in kinase activity in vitro (47). An interstitial deletion that causes fusions between \textit{FAM131B} and \textit{BRAF} has also been identified (37). However, both the \textit{SRGAP3} and \textit{FAM131B} fusions and \textit{BRAFinsT} are much less common than \textit{KIAA1549:BRAF} fusions in PLGAs.
Point Mutations in **BRAF**, **RAF**, and **RAS**

MAPK signaling is also sometimes activated in PLGAs by point mutations in various pathway members. Indeed, prior to the discovery of **BRAF** gene duplications, rare oncogenic mutations in **KRAS** had been described (48, 49). Subsequent studies have confirmed the presence of occasional **KRAS** mutations in PAs. Forshew et al. (30) sequenced **HRAS**, **KRAS**, and **NRAS** in 50 PLGAs and found an activating **KRASG12A** mutation in one cerebellar PA that lacked **BRAF** and **RAF1** fusions. Cin et al. (37) examined **HRAS**, **KRAS**, **NRAS**, **PTP11**, and **RAF1** in 125 primary PA samples and identified oncogenic **KRAS** mutations in 2 tumors. Thus, approximately 2% of PAs have mutations that activate **KRAS**. Point mutations in **RAF1** may not be selected for, due to its lower basal activity compared with that of **BRAF** (45, 50), but it is not clear why mutations in **HRAS** and **NRAS** are not present in PLGAs.

The most common point mutation in PLGAs occurs in **BRAF** itself, at codon 600, and results in the substitution of valine by glutamic acid. The **BRAFV600E** mutation was first described in extra-CNS tumor cell lines; it is most frequent in melanoma but can also be found in a range of other neoplasms (51). In PLGAs, **BRAFV600E** mutations were identified in 4 of 66 (6%) of the tumors examined by Pfister et al. (25), including 3 PAs and 1 diffuse astrocytoma. Subsequent studies have also readily identified **BRAFV600E** mutations in PLGAs (28, 30, 32, 37–39, 46, 47), although as discussed below, these mutations are most common in tumors other than PAs.

**BRAF** and **RAF1** fusions are generally mutually exclusive with other genetic alterations activating MAPK signaling, but some exceptions have been reported. Cin et al. (37) identified two PA patients with concomitant **BRAFV600E** mutation and **BRAF** fusion, one of whom also had NFI syndrome. In another study, 6 tumors out of 198 had both **BRAF** fusion and **BRAFV600E** mutation (13).

**Genetic Alterations and Tumor Site**

Tandem duplications involving **BRAF** do not occur uniformly in PLGAs at all sites in the CNS (Figure 6). The percentage of cerebellar/posterior fossa PAs with molecular alterations at 7q34 is particularly high, ranging from 63% to 94% in various reports (27, 28, 30, 31, 37, 52). In one study of 32 posterior fossa PAs, 30 had **KIAA1549:BRAF** fusions, 1 had a **SRGAP3:RAF1** fusion, and 1 had a mutation in **KRAS** (30). In contrast, the frequency of **BRAF** duplication or fusion in PAs arising in the cerebral cortex is quite low, ranging from 0% to 50% (27, 29, 31–33, 37, 32–54). A total of 72 cerebral cases were reported in these studies; 18 (25%) showed **BRAF** duplication or fusion (Figure 6). Our review of published cases yielded percentages of **BRAF** duplications at other sites that were higher than the percentage found in cerebral cortex but lower than that in posterior fossa. These sites included the optic pathways (42 of 83, 51%), deep gray matter (17 of 41, 41%), brain stem (56 of 95, 59%), and spinal cord (6 of 11, 55%).
Localization of \( \text{BRAF} \) duplication or fusion in pilocytic astrocytomas. Summary of tumor localization from published cases, including patients with neurofibromatosis type 1.

The limited number of other molecular changes in PAs makes it harder to definitively assess their spatial distribution. However, it seems that \( \text{BRAF}^{\text{V600E}} \) and \( \text{KRAS} \) mutations are more common in PAs arising outside the posterior fossa. In one series, 10 out of 49 (20%) noncerebellar PAs had point mutations in one of these two oncogenes, versus 3 of 76 (4%) cerebellar lesions—a statistically significant difference (37). Also, Schindler et al. (46) found a statistically significant association between \( \text{BRAF}^{\text{V600E}} \) and the extracerebellar location of PAs (\( p = 0.009 \)). In contrast, the few \( \text{SRGAP3:RAF1} \) and \( \text{FAM131B:BRAF} \) fusions reported to date have largely been in PAs arising in the cerebellum (30, 37).

The cause of the increased incidence of \( \text{BRAF} \) duplication in the posterior fossa is not clear, but it may reflect increased susceptibility of the tumor cell(s) of origin at this site. Also, \( \text{BRAF} \) rearrangements in PAs may be less common in adult patients (55), which could reflect changes in tumor histogenesis as patients age. Regional differences in expression of the fusions may also play a role. Although the \( \text{KIAA1549}, \text{SRGAP3}, \) and \( \text{FAM131B} \) genes are all expressed in the CNS, little is known about their precise levels in various regions or cell types. Investigators recently demonstrated that \( \text{KIAA1549:BRAF} \) fusions are expressed in PAs at levels that are roughly equivalent to those of the endogenous \( \text{KIAA1549} \) gene (39).

**Senescence in Pediatric Low-Grade Astrocytomas**

An interesting feature of PLGAs is the propensity of some tumors, particularly PAs, to occasionally spontaneously stop growing or even regress (56, 57). A similar pattern of initial neoplastic proliferation followed by growth arrest is often observed in benign melanocytic nevi of the skin, which also commonly contain genetic alterations in \( \text{BRAF} \) (58). This process has been...
termed oncogene-induced senescence (OIS); senescence is defined as an irreversible growth arrest. OIS arises from induction of the p16/Rb, p14ARF/p53, and/or DNA damage-response pathways by \( \text{BRAF} \) and other oncogenes (59, 60). Markers of OIS include enlargement and flattening of cells, expression of p16 and p53, and activation of acidic senescence-associated \( \beta \)-galactosidase (SA-\( \beta \)-Gal). These markers are frequently found in premalignant lesions but are essentially absent in malignant tumor cells, suggesting the latter have found ways to bypass or escape senescence. One such mechanism is deletion of p16, which is frequently identified in malignant melanoma (59, 60).

Given the importance of BRAF activation in PAs, and these tumors’ often indolent growth pattern, it is not surprising that several groups have examined the potential role of OIS. Raabe et al. (61) and Jacob et al. (62) have shown that OIS markers, including p16, p53, and SA-\( \beta \)-Gal, are expressed in both primary PA samples and low-passage tumor cultures. These groups also found that introducing \( \text{BRAF}^{V600E} \) into human neural stem cells, hTERT (human telomerase reverse transcriptase)-immortalized astrocytes, or fetal astrocytes causes growth arrest and the induction of p16 and SA-\( \beta \)-Gal. OIS has also been documented in benign cutaneous neurofibromas driven by \( \text{NF1} \) loss (63).

The role of p16 in escape from OIS and aggressive tumor growth has attracted particular attention. Jacob et al. found that loss of p16 was required for isolation of astrocyte clones that stably expressed \( \text{BRAF}^{V600E} \). Raabe et al. examined p16 expression in 66 PA cases by using immunohistochemistry and found that the 9 patients whose tumors were p16 negative had significantly shorter OS. Additional support for a prognostic clinical role comes from two groups. Rodriguez et al. (52) selectively identified homozygous p16 deletions in more aggressive anaplastic PAs. Horbinski et al. (13) examined a large cohort of 198 PLGAs and found that p16 deletion was the second strongest predictor of adverse outcome (after midline location) in the group overall and that it also correlated with significantly shorter progression-free survival in tumors with \( \text{BRAF} \) rearrangement. Taken together, these data strongly support the concept that OIS contributes to the sometimes indolent behavior of PAs and that p16 loss can contribute to escape from senescence and clinically aggressive tumor growth (Figure 7). It remains to be determined whether OIS also plays a role in the pathobiology of other PLGAs.

Another potential contributor to the slowing or arrest of growth in PLGAs is replicative senescence mediated by the shortening of telomeres. By using the polymerase chain reaction (PCR)-based telomeric repeat amplification assay, Chong et al. (64) documented telomerase activity in many high-grade gliomas but not in the 16 PAs and 2 PXAs examined. Tabori et al. (65) subsequently confirmed a lack of telomerase activity in 11 pediatric low-grade gliomas, as well as a significant decrease in telomere length over time. These observations led them to propose that the lack of telomere maintenance may contribute to growth arrest or regression of PLGAs.
These authors also found that longer telomere length is inversely correlated with survival, which further supported this concept. A telomerase-independent process known as alternative lengthening of telomeres (ALT) is active in almost half of pediatric glioblastomas. However, ALT is very rare or absent in PAs, although ALT-associated promyelocytic leukemia bodies have been identified in some cases.

**Neurofibromatosis Type 1**

NF1 is caused by germ-line mutations in the gene at 17q11.2 that encodes for neurofibromin, a tumor suppressor that works as a GTPase-activating protein to deactivate RAS. PAs are the most frequent NF1-associated CNS tumors, and approximately 15% of pediatric NF1 patients develop optic pathway gliomas. Most molecular studies have demonstrated BRAF alterations to be mutually exclusive with NF1 clinical status. Conversely, the neurofibromin gene is almost never altered in sporadic PAs, and NF1-associated and sporadic PAs have distinct global gene expression patterns. However, in rare instances NF1-associated tumors have had additional activating mutations in BRAF. These cases include KIAA1549:BRAF fusion in a NF1-associated pilomyxoid astrocytoma; a BRAFV600E point mutation in a NF1-associated PA; and an interesting “triple hit” with concomitant NF1 syndrome, BRAFV600E, and KIAA1159-BRAF fusion.

Although a major molecular consequence of neurofibromin loss is MAPK pathway activation, additional signaling nodes, including mTOR pathway activation, contribute to tumorigenesis in NF1. Indeed, activation of the PI3K/AKT/mTOR signaling axis is a prominent feature of the rare PAs that develop anaplastic change, 24% of which are associated with NF1. Recent studies have also highlighted a role for the nonneoplastic stromal microenvironment in optic glioma development in NF1 model systems. NF1 heterozygous microglia are required for glioma formation in these models, in addition to Nf1 homozygous loss in neoplastic astrocytes. Recent evidence suggests that stroma-derived factors (e.g., CXCL12) lead to altered cyclic AMP levels and facilitate tumor formation in this setting.

**AKT/mTOR**

Another important signaling pathway operating downstream from neurofibromin and RAS is AKT/mTOR, which leads to increased protein translation, cell growth, and survival through two multiprotein complexes (mTORC1 and mTORC2) that vary in their sensitivity to rapamycin. The prototypical low-grade glioma in which mTOR activation is an intrinsic molecular property is the tuberous sclerosis–associated SEGA. Tuberous sclerosis is characterized by germ-line mutations in the tumor suppressor genes TSC1 and TSC2, which regulate AKT activation through RHEB, and there has been recent clinical success with mTOR pathway inhibitors. Some studies have also highlighted a role for mTOR signaling in NF1-associated tumors, in particular PAs. Examination of Nf1-deficient mouse models has suggested that there is anatomical variation in neuroglial progenitor proliferation through AKT activation. Recent studies have also suggested a possible role for differential mTOR activation in subsets of NF1-associated low-grade gliomas that are difficult to classify by traditional criteria.

Outside of the syndrome-associated low-grade gliomas, little is known about mTOR pathway activation in tumorigenesis or progression in PLGAs. However, immunohistochemical studies suggest that this pathway is active in at least some tumors. Interestingly, mutations in PIK3CA have been reported in three of four rosette-forming glioneuronal tumors, rare low-grade neoplasms with a frequent PA-like component.

**Gene Expression Analysis**

Early studies suggested that PAs have expression profiles that are distinct from those of...
high-grade gliomas (85–87) and that PAs can also be differentiated from diffuse astrocytomas (88), oligodendrogliomas, and normal white matter (89). Additional studies identified MBP (myelin basic protein) and Matrilin as potential markers of poor outcome (90, 91). Gene expression analysis has also been used to suggest potential cells of origin for PAs, which may be region specific (73, 92). However, unlike the situation for other childhood brain tumors, such as medulloblastomas and ependymomas, large-scale studies integrating pathology, clinical factors, and mutations or copy number change with messenger RNA and microRNA expression have not yet been published. For pediatric medulloblastomas and adult glioblastomas, such correlations have been critical for parsing clinically and genetically meaningful tumor subgroups and potential cells of origin and for generating molecularly relevant classification schemes (93). Hopefully, similarly integrated data will soon be available for PLGAs.

**CLINICAL AND THERAPEUTIC IMPLICATIONS**

**Diagnostic Utility of BRAF Alterations**

Distinguishing between the various types of PLGAs can be difficult; therefore, testing for BRAF alterations could be of great use diagnostically. In the first report in which KIAA1549:BRAF fusions were identified, Jones et al. (29) noted that almost all the cases were PAs. A few PLGAs that were not originally diagnosed as PAs contained the alteration, but because they were all cerebellar and associated with survival of greater than 12 years, the authors suggested that they might represent PAs that had been misclassified. Several subsequent investigations have confirmed a strong association between PA histology and BRAF duplication or fusion (31, 34, 54), but some exceptions exist. In a study including 27 pediatric diffuse astrocytomas, Jacob et al. (31) did not detect duplication of 7q34, but the same group later found some diffuse astrocytomas with KIAA1549:BRAF fusions (53). Forshew et al. (30) reported a KIAA1549:BRAF fusion in 1 of 11 pediatric diffuse astrocytomas in their study, whereas Sievert et al. (28) identified the duplication in 3 of 6 of these tumors.

BRAF duplication or fusion events occur fairly frequently in pilomyxoid astrocytomas (30, 39, 53), which supports a recent study suggesting that pilocytic and pilomyxoid astrocytomas may be part of a single disease spectrum (16). However, the few PXAs examined to date have not shown these alterations (30, 39). Also, BRAF fusions have not been identified in high-grade pediatric gliomas. Finally, BRAF fusions were recently reported in a few pediatric low-grade glioneuronal tumors (39).

Taken together, these reports of rare fusions in diffuse astrocytomas and other nonpilocytic lesions suggest that such molecular changes involving BRAF are highly enriched in PAs but are not absolutely specific for this diagnosis.

In contrast to BRAF duplication or fusion, point mutations in BRAF are most common in low-grade pediatric brain tumors other than PAs and are also found in higher-grade gliomas (54). Dougherty et al. (94) identified BRAFV600E mutations in 9 of 18 (50%) of gangliogliomas as well as in several PXAs. A study of 1,320 nervous system tumors found that BRAFV600E was most common in PXAs (57 of 87, 66%) and WHO grade I gangliogliomas (14 of 77, 18%) (46). Another group also identified common BRAFV600E mutations in PXAs (12 of 20, 60%) (95).

**Prognostic Utility of BRAF Alterations in Pediatric Low-Grade Astrocytomas**

Another potential clinical application of molecular testing for alterations in BRAF and other MAPK pathway members is to determine which PLGAs are more aggressive, thereby allowing more precise delivery of therapy. Neither our group nor Cin et al. found better outcomes in PLGA patients whose tumors contained BRAF fusions (37, 39). Hawkins et al. (53) focused on a “clinically relevant” subgroup of PLGA cases, defined as non-NF1 patients...
with noncerebellar tumor location and subtotal resection. They reported that \textit{KLAA1549:BRAF} fusions were significantly associated with better outcome in a cohort of 70 PLGA patients who met these criteria (53). However, when we examined an analogous subgroup within our cohort, we did not identify any trend toward better outcome with \textit{BRAF} fusion (39).

Horbinski et al. (35) examined \textit{BRAF} status in 118 unselected PAs with outcome data, but they did not identify significantly different outcomes in cases with and without the duplication in their initial study. In a subsequent, larger study of 198 cases, of which 143 were PAs, the same group found a trend toward improved progression-free survival in patients with low-grade gliomas whose tumors had \textit{BRAF} rearrangements ($p = 0.06$) (13). They also noted that the only patients with \textit{BRAF} rearrangements who died had midline tumors. In contrast, patients in their cohort with \textit{BRAFV600E} had a trend toward increased risk of progression ($p = 0.07$) (13). Given the somewhat conflicting data in these various studies, it seems that although \textit{BRAF} fusions may portend better outcomes, examination of a larger cohort, preferably from a controlled clinical trial, will be necessary to definitively determine the prognostic role of this alteration.

**Clinical Testing for \textit{BRAF} Alterations**

To date, no standard approach to testing for the various alterations affecting MAPK signaling in PLGAs has been developed. Because fresh tissue is not always available, assays that can work with formalin-fixed paraffin-embedded (FFPE) specimens are of the greatest practical utility. Given the frequent presence of \textit{BRAFV600E} point mutations in cutaneous melanoma and other common neoplasms arising outside the CNS, many clinical labs have standard tests for this alteration that are based on sequencing or hybridization (96). A monoclonal antibody that is specific for the \textit{BRAFV600E} protein has also recently been developed and will be useful in small specimens (97).

A range of approaches to identify \textit{BRAF} fusions have also been reported. Many laboratories have used duplication of the 7q34 region as a marker for this change. This duplication can be assessed by FISH, which has the advantages of working well in FFPE tissues, spatially localizing the change, and detecting it in small groups of cells. As costs have dropped, array CGH has become more widely used in a diagnostic capacity in both fresh and FFPE samples, and this technology can detect changes across the genome rather than only those at 7q34, including loss of the \textit{p16} locus. Direct identification of fusion transcripts by use of reverse transcription PCR is highly specific and yields information about fusion breakpoints, but only predetermined regions can be assessed (39). Although this method has traditionally been performed with RNA extracted from frozen tumors, investigators have recently demonstrated that 97% sensitivity and 91% specificity can be achieved with fusion transcripts isolated from FFPE tissue (38). Finally, pyrosequencing has been used to detect fusions and changes in \textit{BRAF} gene dosage in FFPE specimens, and this approach may identify some alterations that are not found with PCR primers specific for various fusions (98).

**Preclinical Testing Models**

The development of effective new therapies for PLGAs would be greatly assisted by cell- or animal-based models that accurately reflect the molecular biology and pathology of these tumors. Unfortunately, PLGA models are not as advanced overall as high-grade gliomas, although transgenic mice with NF1-associated optic gliomas have been generated and used for preclinical testing (99, 100). Another genetically engineered mouse model that may prove useful in preclinical PLGA testing was recently reported by Gronych et al. (101). These authors found that BRAF activation alone in nestin-positive murine neural progenitors was sufficient to induce the formation of cerebral low-grade gliomas, but this tumor induction was achieved by a combination of V600E
mutation and deletion of the negative regulatory region. Therefore, it remains to be determined whether fusions of the BRAF kinase domain with KIAA1549 or other partners analogous to those in humans will be sufficient to drive PLGA tumorigenesis.

Cultures of human PLGAs represent an additional potential platform for preclinical testing. It has been difficult to develop useful lines from PAs and other indolent PLGAs, but some have been reported (25, 102). The recent description of OIS in low-passage cultures may at least partly account for these problems (61, 62). It may be possible to develop more robust cultures by maintaining them as neurospheres in serum-free media or by manipulating the expression of p16 and other OIS factors. Now that signature genetic defects in BRAF have been discovered, it will be critical to demonstrate that any cell lines developed carry the molecular markers of the tumors from which they are derived.

Some preclinical testing has been done with currently available models. Using their Nf1 mutant optic glioma model, Banerjee et al. (74) explored various rapamycin doses that affect mTOR signaling and showed that not all pathway biomarkers accurately reflect effective pathway response or changes in tumor growth. Proliferation of one human PA culture was inhibited by pharmacological MAPK blockade (25). Finally, murine neurospheres transduced with active BRAF are responsive to sorafenib (101), and engineered human neural stem and progenitor cell systems (61, 62) can also be used in this fashion.

**Therapeutic Possibilities**

Given the high frequency of BRAF activation via duplication or fusion and mutation in pilocytic and pilomyxoid astrocytomas, there is considerable interest in targeted inhibition of the MAPK pathway as therapy for these tumors (Figure 8). Sorafenib (Nexavar®, Bayer and Onyx Pharmaceuticals) is an inhibitor of BRAF that has less potency against BRAFV600E. Sorafenib is in Phase II studies against recurrent and chemotherapy-refractory PLGAs (ClinicalTrials.gov trial number NCT01338857). AZD6244 (selumetinib, AstraZeneca and Array BioPharma) is a potent inhibitor of MEK that is in Phase I trials against PLGAs (ClinicalTrials.gov trial numbers NCT01386450 and NCT01089101).

*BRAFV600E* mutations are rare among PLGAs overall but are relatively common in PXAs, gangliogliomas, and a subset of extracerebellar PAs (46, 54, 94, 95). Vemurafenib is a competitive small molecule that was designed to bind to and inhibit the ATP binding domain of the *BRAFV600E* mutant, but not other forms of BRAF (103, 104). Following impressive, albeit transient responses of recurrent melanoma to vemurafenib (104), the US Food and Drug Administration (FDA) has approved it for the treatment of *BRAFV600E* mutation–positive, inoperable, or metastatic melanomas. It is anticipated that there will be a clinical trial of vemurafenib against *BRAFV600E* mutant low-grade gliomas in the near future.

The AKT/mTOR pathway has been implicated in several types of PLGAs, including SEGAs and PAs. Clinical trials have demonstrated that mTOR inhibitors,

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**Figure 8**

Therapeutic agents targeting the BRAF/RAF1 and AKT/mTOR pathways.
including sirolimus and everolimus (RAD-001 or Afinitor®, Novartis Pharmaceuticals), have activity against SEGAs (105), and everolimus has received approval by the FDA for the treatment of SEGAs that cannot be surgically resected. A recently reported Phase I/II study of sirolimus (Rapamune®, Pfizer) and erlotinib (Tarceva®, Genentech) examined 16 patients with recurrent PLGA (106). Of the 7 children with NF1 in this clinical trial, all of them had either stable disease or tumor responses. A Phase II study of everolimus against recurrent and chemotherapy-refractory PLGAs has recently been completed (ClinicalTrials.gov trial number NCT00782626); results are pending. As agents targeting the MAPK and AKT/mTOR pathways are tested, it will be critical to also search for molecular biomarkers that are predictive of response.

CONCLUSIONS

It is now clear that alterations affecting the BRAF oncogene and other members of the MAPK cascade represent the main genetic defects in PLGAs. Segmental duplications that cause fusions between KIAA1549 and BRAF are the most common, and they result in a novel protein with constitutive kinase activity. Tumors lacking KIAA1549:BRAF fusions often undergo other changes with similar functional effects, including SRGAP3:RAF1 and FAM131B: BRAF fusions, as well as mutations and insertions that activate BRAF. These findings link syndromic and sporadic PAs and suggest that MAPK is the dominant signaling pathway to target therapeutically. The detection of BRAF activation as a cardinal feature of PLGAs has also led to the discovery that OIS may account for the spontaneous growth arrest or regression of some tumors.

The challenges now are to translate these new discoveries into improved diagnostic, prognostic, and predictive markers and to develop targeted therapies for patients with clinically aggressive tumors. PLGAs are a heterogeneous group of lesions, and although BRAF fusions are not entirely specific for one entity, they are almost always encountered in PAs. Therefore, molecular BRAF testing may help us to distinguish these tumors from other pediatric gliomas. The prognostic role of BRAF alteration is still not clear, and analyses of large, uniformly treated cohorts will probably be required to definitively assess associations with outcome. Finally, it will be critical to determine whether specific genetic changes predict response to therapies targeting BRAF or other pathway members. The recent initiation of several clinical trials of MAPK inhibitors in PLGA patients should provide some initial insights into this key issue.

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