Molecular Insights into Pediatric Brain Tumors Have the Potential to Transform Therapy

Amar Gajjar1, Stefan M. Pfister2, Michael D. Taylor3, and Richard J. Gilbertson1,4

Abstract
High-throughput genomic technologies have shed light on the biologic heterogeneity of several pediatric brain tumors. The biology of the four common pediatric brain tumors—namely medulloblastoma; ependymoma; high-grade glioma (HGG), including diffuse intrinsic pontine glioma; and low-grade glioma—is highlighted in this CCR Focus article. The discovery that medulloblastoma consists of four different subgroups, namely WNT, SHH, Group 3, and Group 4, each with distinct clinical and molecular features, has affected the treatment of children with medulloblastoma. Prospective studies have documented the efficacy of SMO inhibitors in a subgroup of patients with SHH medulloblastoma. Efforts are ongoing to develop specific therapies for each of the subgroups of medulloblastoma. Similar efforts are being pursued for ependymoma, HGG, and diffuse intrinsic pontine glioma where the disease outcome for the latter two tumors has not changed over the past three decades despite several prospective clinical trials. Developing and testing targeted therapies based on this new understanding remains a major challenge to the pediatric neuro-oncology community. The focus of this review is to summarize the rapidly evolving understanding of the common pediatric brain tumors based on genome-wide analysis. These novel insights will add impetus to translating these laboratory-based discoveries to newer therapies for children diagnosed with these tumors.

See all articles in this CCR Focus section, "Discoveries, Challenges, and Progress in Primary Brain Tumors."

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Introduction
The ability to analyze tissues on a genome-wide scale has transformed our understanding of childhood brain tumors. Tumors once regarded as single entities have been shown to include multiple subgroups, each with distinct patterns of gene mutation and expression, clinical behavior, and in some cases, cellular origin (1–4). These advances have been made possible by technologies capable of cataloging the sequence, copy number, and expression of all genes. In addition to advancing understanding of the molecular basis of pediatric brain tumors, the use of these technologies across species has pinpointed cells in the developing nervous system that generate brain tumors (5). These studies have unmasked a common theme in pediatric brain tumors in which molecular discrete subtypes of each tumor likely arise from topographically discrete neural progenitor cells that are selectively susceptible to specific transforming mutations (2). This article highlights how these new approaches and concepts are informing understanding and treatment of the common types of pediatric brain tumors, which differ from therapeutic approaches in adult gliomas discussed in the other CCR Focus articles (6–8).

Medulloblastoma
The term “medulloblastoma” was originally used to describe all small round blue cell tumors of the cerebellum. Although histologic variants of medulloblastoma were recognized, for example, classic and nodular desmoplastic, the molecular basis of these variants was not known and all patients with the disease received the same treatment (9). The discovery of hSNF5/INI1 mutations in atypical teratoid/rhabdoid tumors (ATRT), provided the first firm evidence that not all tumors treated as medulloblastoma were the same disease (10). Indeed, infants with ATRT emerged as those with especially poor prognosis among large cohorts of patients previously regarded as having medulloblastoma (11). The development of high-quality antibodies to detect ATRTs by routine IHC equipped clinicians with a tool to reliably segregate ATRTs from medulloblastoma in routine clinical practice (12). Similar analyses have separated other small round blue cell tumors from medulloblastoma, including ETANTR (embryonal...
tumor with abnormal neuropil and true rosettes), medulloepithelioma, and ependymoblastoma. ETANTR has been shown to harbor a recurrent amplified fusion between the embryonal gene TTHY1 and a primate-specific microRNA cluster on chromosome 19 (C19MC; ref. 13).

In addition to resolving medulloblastoma from other small round blue cell tumors, studies conducted over the past 10 years have identified distinct subtypes of medulloblastoma. These discoveries were made possible by genomic technologies, particularly those that measure gene expression. These studies have identified four main subgroups of medulloblastoma: WNT, SHH, Group 3, and Group 4 (Table 1; Fig. 1; ref. 14). Alternative approaches to genomics to diagnose medulloblastoma subgroups are in development, and are likely to prove increasingly important to clinical management in light of the significant prognostic and treatment differences among subgroups (15).

WNT medulloblastomas are typically diagnosed in older children and teenagers, rarely metastasize, and have an excellent prognosis with >95% event-free survival rates at 10 years in recent clinical trials (16, 17). About 80% of WNT medulloblastomas have mutations in the gene encoding β-catenin, and about 80% have a deletion of one copy of chromosome 6 (monosomy 6; ref. 1). The first mouse model of WNT medulloblastoma together with analyses of human MRI demonstrated that these tumors arise outside of the cerebellum from the embryonic lower rhombic lip (5). Subsequent studies of large numbers of human MRIs have confirmed this finding. Thus, WNT medulloblastoma represents a distinct disease form with excellent prognosis. Therefore, clinical trials enrolling these patients

Table 1. Subgroups of common pediatric brain tumors with distinct molecular and clinical features

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Molecular subgroup</th>
<th>Molecular and clinical features</th>
</tr>
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<tbody>
<tr>
<td>Medulloblastoma</td>
<td>WNT</td>
<td>Monosomy 6; nuclear β-catenin staining; CTNNBI mutations in exon 3; 10% of patients; older age group; midline tumor location; excellent clinical outcome</td>
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<tr>
<td></td>
<td>SHH</td>
<td>Heterogeneous molecular features depending on age of presentation; PTCH1, SMO, and SUFU mutations, GLI2 and MYCN amplification; germline TP53 mutations; 30% of patients; cerebellar hemispheric location; intermediate prognosis except in infants who have a good prognosis</td>
</tr>
<tr>
<td></td>
<td>Group 3</td>
<td>MYC amplification; high incidence of metastatic disease; 25% of patients; male predominance; younger age group; poor prognosis</td>
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<tr>
<td></td>
<td>Group 4</td>
<td>i(17)q; MYCN amplification; 35% of patients; male predominance; intermediate prognosis</td>
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<tr>
<td>Ependymoma</td>
<td>C11orf95–RELA + RELA fusion transcripts; 70% supratentorial tumors</td>
<td></td>
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<tr>
<td></td>
<td>RELA fusion negative</td>
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<td></td>
<td>CIMP⁺</td>
<td>CPG island methylator phenotype; chr 1 q gain; 80% of posterior fossa tumors; younger age group; male predominance; anaplastic histology; intermediate prognosis</td>
</tr>
<tr>
<td></td>
<td>CIMP⁻</td>
<td>Multiple chromosomal abnormalities; older age group; good prognosis</td>
</tr>
<tr>
<td>Spinal cord tumors</td>
<td>&lt;10% of pediatric tumors; NF2 mutation; myxopapillary histology; good prognosis</td>
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<td>HGG</td>
<td>K27</td>
<td>H3.1 and H3.3 K27 mutation; PDGFRα focal amplification; TP53 mutation; high proportion of pediatric patients; midline location; poor outcome</td>
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<td></td>
<td>G34</td>
<td>H3.3 G34 mutation; TP53 mutation; hemispheric location; poor outcome</td>
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<tr>
<td></td>
<td>IDH1</td>
<td>IDH1 and TP53 mutation; frontal lobe location; rare in pediatric patients; intermediate outcome</td>
</tr>
<tr>
<td></td>
<td>RTK1</td>
<td>PDGFRα and EGFR focal amplification; CDKN2A and CDKN2B homozygous deletion; rare in pediatric patients; poor outcome</td>
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<tr>
<td>Mesenchymal</td>
<td>NFI mutation; PDGFRα and EGFR focal amplification; CDKN2A and CDKN2B homozygous deletion</td>
<td></td>
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<tr>
<td>LGG</td>
<td>BRAF V600E</td>
<td>~70% of pleomorphic xanthoastrocytoma (PXA); ganglioglioma (GG); diffuse astrocytoma</td>
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<td></td>
<td>KIAA–BRAF fusion; BRAF duplication</td>
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<td></td>
<td>MYBL1 rearrangement</td>
<td>High proportion of angiogenic glioma</td>
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<tr>
<td></td>
<td>FGFR1 duplication</td>
<td>PA; diffuse astrocytoma; dysembryoplastic neuroepithelial tumor (DNET)</td>
</tr>
</tbody>
</table>

Abbreviations: HGG, high-grade glioma; LGG, low-grade glioma.
are focused on deescalating radiotherapy and/or chemotherapy with the goal of maintaining excellent survival while diminishing long-term side effects. Ongoing efforts in the laboratory are seeking to generate additional new, relatively nontoxic treatments for these patients.

SHH medulloblastomas are emerging as an especially heterogeneous subgroup of medulloblastomas that affects patients from infancy to adulthood (18). The availability of drugs that target Smoothened, the main upstream activator of the SHH pathway, positioned this subtype as the first in which targeted therapies have been translated to the clinic (19, 20). Some forms of SHH medulloblastoma are especially challenging to treat, for example, teenagers with SHH tumors have a particularly poor prognosis. SHH medulloblastomas also appear to arise in patients with germline mutations in TP53 and evidence of shattering of the chromosomes (chromothripsis) in their tumors (21, 22).

Group 3 medulloblastoma arises exclusively in children, is frequently metastatic, and has the worst prognosis of all the subgroups (14). Consequently, there is considerable interest in developing new curative treatments for this disease. The development of a new mouse model of the disease has enabled the first high-throughput drug screens (HTDS) to identify new treatments of this subgroup (23). The FDA-approved drugs gemcitabine and pemetrexed are among the most promising first candidates and are now being tested prospectively in the SJMB12 clinical trial (NCT01878617; ref. 24). Developing molecular-based therapies of Group 3 medulloblastoma will be more challenging because these tumors contain genetic alterations that are difficult to target, for example, amplification of MYC, or the associated fusion gene PVT1–MYC (25). Group 4 medulloblastomas are the most common, but perhaps the least understood of the subgroups. The only subgroup-specific genetic event identified to date in this subgroup is tandem duplications of the Parkinson disease–associated gene SNCAIP on chromosome 5 (26–28). Mouse models of Group 4 medulloblastoma have not yet been developed, further hindering understanding of this common subtype.

Beyond the broad divisions provided by gene expression profiles, further studies have unmasked additional heterogeneity within subgroups, particularly high-risk SHH patients and an interesting group of relatively low-risk Group 3 patients. Next-generation sequencing and copy number studies of large numbers of patients have identified a disappointing number of highly recurrent events that could serve as therapeutic targets. However, patterns of gene mutation have emerged that may inform new treatment approaches, and in particular a convergence of mutations on genes controlling epigenetic processes (29). As epigenetic events are by definition reversible, this may offer a therapeutic window for the treatment of patients with medulloblastoma.

In addition to inter-tumoral heterogeneity, medulloblastomas display considerable intra-tumoral heterogeneity that may present even more of a challenge to those seeking to develop new treatments. Medulloblastoma metastases are genetically and biologically very different from their parent primary tumors (30). This observation has important implications for treatment because the vast majority of research and preclinical development focuses on the primary tumor. The fact that most Group 3 and Group 4 medulloblastomas recur metastatically rather than at the primary site strongly suggests that our drug...
development strategies need to take into account differences between primary and secondary disease. If metastases are different from the primary tumor, novel therapies against the primary tumor may not increase survival rates.

**Ependymoma**

Ependymomas are tumors of the brain and spinal cord. Surgery and irradiation remain the mainstay of treatment of this disease because chemotherapy is ineffective in most patients (31). Consequently, ependymoma is incurable in up to 40% of cases. Histologic similarities among ependymomas have led investigators to treat these tumors as a single entity; however, recent genomic studies of gene expression and DNA copy number alterations have shown that ependymomas from different regions of the central nervous system (CNS) include discrete subtypes that display disparate prognoses, transcriptional profiles, and genetic

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**Figure 2.** Schematic presentation showing the role of epigenetic changes to the histone tails that influence gene expression. A, addition or removal of methyl group by methyltransferases leads to hyper- or hypomethylation of DNA, leading to gene repression or activation, respectively. B, a chromosome segment composed of compact DNA wrapped around octamers of core histones (nucleosomes) in which the DNA is inaccessible. C, epigenetic modification of the histone tails leads to unwinding of the DNA, rendering genes accessible for transcription. D, common posttranslational modifications occurring in the histone H3.3 tail that regulates gene transcription. In pediatric high-grade glioma, K27 and G34 are often mutated.
alterations, suggesting that they are different diseases. Recent data have documented that ependymoma comprises five distinct tumor subtypes—C11orf95-RELA-positive and -negative disease in the supratentorial compartment; CIMP(CPG island methylator phenotype)-positive and CIMP-negative disease in the posterior fossa; and spinal cord tumors (Table 1; Fig. 2; refs. 32, 33). Spinal cord ependymomas in patients with NF2 are clinically distinct as they often have an indolent course following surgical resection alone and can be observed following resection of the tumor (34, 35). Thus, contemporary efforts to cure all patients with ependymoma must be concerned with understanding the biologic basis of these disease subtypes, and where necessary, developing subtype-specific therapies.

Initial cross-species genomic studies showed that ependymomas from different regions of the CNS share the same gene expression profiles of neural stem cells (NSC) in the corresponding region of the developing brain and spine (2). These data provided the first explanation for the regional heterogeneity of ependymoma and identified regionally discrete NSCs as candidate cells of origin of the different disease subtypes. Support for this notion was provided by subsequent studies that demonstrated that EPHB2, a putative oncogene of supratentorial ependymoma, transformed forebrain, but not hindbrain or spinal NSCs, to generate ependymomas in mice. In addition to providing new insights into the origin and biology or ependymoma, this work established a new paradigm for understanding the molecular and cellular origins of cancer subtypes.

Building on the understanding that ependymoma comprises regionally discrete subtypes, investigators performed whole-genome sequencing of supratentorial and posterior fossa ependymomas with the aim of identifying driver mutations of these tumors (32, 33). Both studies found very few single nucleotide variations, insertions/deletions, or focal (<5 genes) copy number variations in ependymomas. However, Parker and colleagues noted that structural variations (SV) occurred significantly more frequently in supratentorial than other forms of the disease (32). Further analysis showed that these SVs clustered within a highly focal region of chromothripsis on chromosome 11q12.1-13.3. This genomic disruption resulted in a novel translocation that fused a poorly characterized gene, C11orf95, to RELA, the principal effector of canonical NF-kB signaling in 70% of supratentorial, but no posterior fossa or spinal ependymomas, making it the most recurrent genetic alteration in ependymoma. RNA sequencing demonstrated that splicing is required to generate the mature C11orf95-RELA transcript of which there are seven distinct variants. The most frequent includes exons 1–2 of C11orf95 and, except for the first two codons, the entire open reading frame of RELA. C11orf95-RELA–positive ependymomas also expressed high-levels of CCND1, a direct transcriptional target of NF-kB signaling, and L1CAM, which is associated with aberrant cell–cell adhesion, invasion, and NF-kB activation in tumors. Because IHC detected strong CCND1 and L1CAM expression only in C11orf95-RELA–positive formalin-fixed paraffin-embedded supratentorial ependymomas, these markers also afford a potential diagnostic test for translocation positive disease. Functional studies showed that the C11orf95-RELA fusion drives an aberrant NF-kB transcriptional program in mouse NSCs as well as highly penetrant brain tumors that recapitulated the “clear cell” and finely branched vasculature characteristic of “vascular-variant” human supratentorial ependymoma.

In light of the paucity of SNVs in ependymoma, Mack and colleagues studied the concept that posterior fossa ependymomas are driven by a dysregulated epigenome (33). Studying DNA methylation patterns in this group identified two distinct groups of posterior ependymomas: group A posterior fossa ependymomas, which have a much higher extent of CpG island methylation and exhibit a “CpG island methylator” or “CIMP” phenotype (PFA-CIMP+; ependymomas, and PFB CIMP-negative tumors. Interestingly, genes CpG methylated in PFA-CIMP+ ependymoma showed a remarkable convergence on genes documented as silenced in embryonic stem cells by the Polycomb repressive complex 2 (PRC2).

These data suggest that drugs that target DNA CpG methylation, PRC2/EZH2, and/or histone deacetylase inhibitors could represent the first rational strategies for therapy of PFA-CIMP+ ependymoma. Indeed, treatment of PFA-CIMP+ ependymoma, but not supratentorial ependymoma, with the tool compound 3-deazaneplanocin A (DZNep) that targets the PRC2 complex decreased expression of EZH2, decreased trimethylation of H3K27, and increased cleavage of PARP. Furthermore, in vivo treatment of human PFA-CIMP+ ependymoma xenografts with DZNep decreased tumor volume and improved survival.

In an effort to develop new treatments, investigators have performed HTDS of different ependymoma subtypes. One study developed an HTDS campaign that detects compound toxicity in dose response, against ependymoma cells, and NSC with high reproducibility and sensitivity. The screening of the following was conducted: 3,161 bioactive compounds, 1,648 “orphan-kinase inhibitor scaffolds,” 367 inhibitors of specific kinases in GlaxoSmithKline’s Published Kinase Inhibitor Set (GSK-PKIS), and 275 FDA-approved drugs. Of these compounds, 2.6% (n = 140/5,303) displayed anti-ependymoma activity, and pinpointed specific cell functions and pathways that are vulnerable to attack. Of particular note, C11orf95–RELA fusion–negative tumors were shown to be highly sensitive to 5-fluorouracil treatment, significantly prolonging the survival of mice with these tumors relative to control or conventionally treated (i.e., carboplatin, topotecan, or irinotecan) mice (36).

High-Grade Glioma, Including Diffuse Pontine Glioma

Glioblastoma, anaplastic astrocytoma (AA), anaplastic oligodendroglioma (AO), gliomatosis cerebri, and diffuse intrinsic pontine glioma (DIPG) in children have
historically comprised high-grade glioma (HGG) for the purpose of clinical studies. Biopsy results from DIPG and gliomatosis often document the tumor as being a grade 2 astrocytoma, but the clinical behavior of these tumors is similar to aggressive HGGs. As a group, these diseases are the least common malignant brain tumor in children. With a long-term survival rate of less than 10%, HGG remains one of very few incurable pediatric cancers (37). Current treatment protocols include maximal-safe surgical resection while maintaining functional integrity, radiotherapy, and a variety of chemotherapy options. Radiotherapy typically has a temporary effect on HGGs, and no chemotherapy has proven effective against it. The heterogeneity seen, in terms of survival of pediatric patients with HGG, has yet to be explained. However, some ongoing molecular studies suggest that tumors from long-term survivors might not actually be HGGs.

Mutations in the IDH1 gene are a hallmark genetic lesion in adult AA and AO, but they are exceedingly rare in children (38). The same holds true for 1p/19q deletion, the quasidiagnostic cytogenetic lesion in adult oligodendroglioma. In adult neuro-oncology, it has become evident that HGGs lacking either of these two hallmark aberrations have the same aggressive clinical behavior as glioblastoma, even without some morphologic features usually required for this diagnosis (e.g., necrosis and microvascular proliferation; ref. 39). Thus, we must now question whether AA and AO occur at all in the pediatric population. If so, they are so rare that their (presumably different) genetic makeup remains elusive. Gliomatosis cerebri is a particularly invasive and disseminating phenotype of HGG, and whether this HGG has a distinct underlying molecular cause is unknown. We focus here on glioblastoma and DIPG, which comprise the vast majority of pediatric HGGs.

In addition to the absence of hallmark alterations seen in adult AA and AO, common changes in adult glioblastoma (i.e., loss of chromosome 10, gain of chromosome 7, CDKN2A/B deletion, and EGFR amplification) are extremely rare in childhood HGG. However, some genetic alterations, such as PDGFRα or MET amplification and TP53 mutation, are found in both pediatric and adult HGGs. PDGFRα amplification, a potentially druggable lesion, is more common in pediatric HGG cohorts, and it seems to be more frequently observed in secondary glioblastoma after CNS radiotherapy for another indication (or in posttreatment primary HGG samples obtained at postmortem; ref. 40).

Next-generation sequencing studies published over the past 2 years have started to elucidate the unique mutational landscape of pediatric HGG. The most intriguing finding, so far, has been highly recurrent hotspot mutations affecting two distinct residues (K27 and G34) of the noncanonical histone gene H3F3A, as well as the canonical histone genes HIST1H3B and HIST1H3C (3, 41, 42). These studies have demonstrated a striking pattern of spatial heterogeneity, with K27M substitutions almost exclusively occurring in midline tumors (i.e., those in the thalamus, brainstem, or spine) and G34R/V substitutions restricted to hemispheric glioblastomas. Although G34 mutations typically co-occur with ATRX mutations and alternative lengthening of telomeres (a potential point of targeted interference), those mutations are less frequently coexpressed in tumors carrying K27M substitutions (43).

Several groups have shown that K27M mutations functionally inactivate PRC2 by entrapping EZH2, the main enzyme required to establish the repressive chromatin mark K27me3, thus conferring a dominant effect at the level of H3K27me on histones H3 and H3.3 (44). Both histone mutations are thought to arrest the respective cells of origin in a primitive progenitor state and may require additional genetic hits (e.g., TP53 mutation) to drive tumorigenesis and proliferation (Fig. 2). How to molecularly target these constellations of H3, TP53, and ATRX mutations remains to be determined and is the focus of several ongoing HTDS. Four recent DIPG studies have independently identified driver mutations in the ACVR1 gene, mostly in combination with HIST1H3B/C mutations (45–48). The mutated residues are similar to those in the developmental disorder fibrodysplasia ossificans progressiva, and targeted compounds are currently in preclinical development. In a distinct subgroup of midline tumors that mostly occur in the thalamus, investigators have identified recurrent FGFR1 hotspot mutations for which targeted compounds are in clinical trials in other disease entities. Finally, recurrent fusions involving NTRK1, NTRK2, or NTRK3 described in one recent study in a relevant subset of infant (non-brainstem) HGGs might also provide a promising drug target (46).

Attempts to classify HGGs into meaningful molecular subgroups based on gene-expression profiling have proven challenging. More recent work exploiting DNA-methylation fingerprints to guide subgroup affiliations across ages has shown more consistent results. In the pediatric setting, the four major methylation subgroups are highly enriched for the following: H3K27 mutation, H3G34 mutation, PDGFRα amplification (receptor tyrosine kinase subgroup 1), or none of the previous (mesenchymal subgroup; Table 1; Fig. 1). Methylation-based subgrouping can be performed using standard formalin-fixed, paraffin-embedded tumor samples; thus, it is also a feasible test for routine clinical application (49).

In summary, the genetic landscape of pediatric HGG differs greatly from that of the adult disease. Pediatric HGG is also very heterogeneous; the gross split between hemispheric tumors and midline tumors most likely indicates different cells of origin. It remains to be elucidated whether most long-term survivors of childhood HGG simply have a different molecular disease (e.g., low-grade glioma; LGG) and therefore may not require intensive radiotherapy and chemotherapy. The most important translational lesson learned from recent retrospective studies, however, is the value of routine biopsy of all HGGs, including DIPG (for patients enrolled on clinical protocols), the diagnosis of which has been typically based on radiologic findings alone. Without the molecular information gained from analyzing biopsy or autopsy samples (50), these discoveries would not
have been possible and, more importantly for the patients, decisions about novel therapeutic options (conventional as well as molecularly targeted) would remain a trial-and-error approach.

Although genetic mosaicism (i.e., different amplifications in different cells within the same tumor) and intratumoral heterogeneity in general seem to be more common in pediatric HGGs than in other pediatric malignancies, molecularly targeted therapies are worth further exploration. High-quality diagnostic specimens will be required as a basis for treatment stratification, regardless of whether the patient will receive a combination of novel drugs or one of targeted drugs and conventional chemotherapeutic agents. *H3F3A* and *HIST1H3B/C-mutation status and molecular subgrouping should be considered standard diagnostic assays for pediatric HGG; *PDGFRA* and *MET* amplifications, *ATRX* status (by IHC analysis), *FGFR1* and *ACVR1* mutations, and *NTRK2* fusions would be the first candidates to be routinely assessed as potential molecular drug targets. Further work is required to elucidate the molecular features of the remaining proportion of cases of HGG that lack all of the alterations presented here, to establish predictive biomarkers or signatures for the rational use of available targeted drugs, and to understand how to target the epigenetic phenotype conferred by the histone mutations.

**Low-Grade Glioma**

Among the myriad of tumors that can arise in the developing brains of children, LGGs are the most common type. The World Health Organization has recognized two subtypes of childhood LGG, grade 1 and grade 2 tumors. However, using various approaches, independent laboratories have identified three major histologic classes, each comprising several subtypes of the disease: (i) astrocytic tumors, which consist of diffuse astrocytoma, fibrillary astrocytoma, pilocytic astrocytoma, and pleomorphic xanthoastrocytoma; (ii) oligodendrogial tumors, which include oligoastrocytoma and oligodendroglioma; and (iii) neuronal and mixed neoplasms, which consist of ganglioglioma, angiohistiocytic astrocytoma, and pleomorphic xanthoastrocytomas, gangliomas, diffuse astrocytomas, and pilomyxoid astrocytomas and is only rarely detected in pilocytic astrocytomas (63).

Tuberous sclerosis, a hereditary disorder in which benign tumors form in many organs, including the brain, is caused by mutations in two tumor-suppressor genes, *TSC1* and *TSC2*. Patients with tuberous sclerosis are at increased risk of LGG, which develops in 5% to 14% of patients. *TSC1* and *TSC2* are negative regulators of the mTOR pathway, which mediates cell proliferation; thus, mutations in *TSC1* and *TSC2* activate mTOR, thereby increasing a child’s predisposition to LGGs. mTOR inhibitors have demonstrated efficacy in subependymal giant cell astrocytomas and are now the standard of care for these tumors (64–67).

*MYB* gene mutations also occur in multiple types of LGG. *MYB* amplification occurs in diffuse astrocytomas, and focal deletions occur in angiocentric glioma. *MYB* expression is upregulated in the majority (60%) of diffuse LGGs and in a large portion (41%) of pilocytic astrocytomas. *MYBL1*, another member of the *MYB* family of proteins, is mutated in diffuse astrocytomas and angiocentric gliomas (4, 67, 68).

Whole-genome sequencing of large cohorts of pediatric LGGs has identified recurrent alterations in the fibroblast growth factor receptor type 1 gene, *FGFR1*. *FGFR1* N546K and K656E mutations occur in 5% of the supratentorial pilocytic astrocytomas (4, 69). Gene-expression analysis has revealed that *FGF2*, a ligand of *FGFR1*, is overexpressed in pilocytic astrocytomas, compared with the level expressed in other astrocytic tumors. This finding suggests that the *FGF/FGFR* pathway has a crucial function in tumorigenesis of LGGs in children. In addition, *FGFR1* mutations and duplication of its tyrosine kinase domain have been described in pilocytic astrocytomas, diffuse astrocytomas, and dysembryoplastic neuroepithelial tumors (4). Alterations of other MAPK members have also been described in pediatric LGGs. These include genomic alterations affecting the kinase domain of neurotrophic tyrosine kinase type 2 (*NTRK2*), which have been described in pediatric pilocytic astrocytomas (69). Finally, KRAS-activating mutations have been described in 3% to 5% of sporadic pilocytic astrocytomas (70, 71).
Current efforts to advance treatments for children with LGGs include testing of drugs that target mTOR, BRAF, or the MAPK pathway. The PI3K/Akt/mTOR intracellular signaling pathway has been implicated as an important promoter of tumor growth for many LGGs. Activation of the PI3K/Akt/mTOR pathway seems to play a central role in pediatric LGG pathogenesis. Several published studies demonstrate the PI3K/Akt/mTOR pathway activation in approximately 50% of pediatric LGGs based on phosphorylation of the mTORC1 targets S6 and 4E-BP1 and provide the preclinical rationale to test inhibitors of PI3K/AKT/mTOR for the treatment of pediatric LGGs. mTOR, lying downstream of PI3K, is an ideal target for pediatric LGG therapy.

Summary and Future Directions

Biologic insights have triggered a fundamental transformation in the landscape of pediatric brain tumors. This surge of new knowledge has the promise to radically transform the field over the next decade. A recent consensus meeting of neuropathologists has suggested a way to integrate the rapidly emerging molecular information into the diagnostic work up of brain tumors (72). Treatment strategies directed toward specific subtypes of brain tumors defined by
histopathology and molecular diagnostics are being integrated in first-line clinical protocols. With the availability of genetically engineered mouse models and orthotopic xenografts being generated and widely available to research laboratories, there is an added impetus to find newer more effective and less toxic therapies, using high-throughput screening for some of the clinically aggressive tumors. The level of optimism in the pediatric neuro-oncology community is unprecedented, in anticipation that these recent discoveries will lead to a paradigm shift in the diagnosis and treatment of pediatric brain tumors.

**Disclosure of Potential Conflicts of Interest**

A. Gajjar is a consultant/advisory board member for AstraZeneca and Celgene. No potential conflicts of interest were disclosed by the other authors.

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