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The Genetic Signatures of Pediatric High-Grade Glioma: No Longer a One-Act Play

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Advances in understanding pediatric high-grade glioma (pHGG) genetics have revealed key differences between pHGG and adult HGG and have uncovered unique molecular drivers among subgroups within pHGG. The 3 core adult HGG pathways, the receptor tyrosine kinase-Ras-phosphatidylinositide 3-kinase, p53, and retinoblastoma networks, are also disrupted in pHGG, but they exhibit a different spectrum of effectors targeted by mutation. There are also similarities and differences in the genomic landscape of diffuse intrinsic pontine glioma (DIPG) and pediatric nonbrainstem (pNBS)-HGG. In 2012, histone H3 mutations were identified in nearly 80% of DIPGs and ~35% of pNBS-HGG. These were the first reports of histone mutations in human cancer, implicating novel biology in pediatric gliomagenesis. Additionally, DIPG and midline pNBS-HGG vary in the frequency and specific histone H3 amino acid substitution compared with pNBS-HGGs arising in the cerebral hemispheres, demonstrating a molecular difference among pHGG subgroups. The gene expression signatures as well as DNA methylation signatures of these tumors are also distinctive, reflecting a combination of the driving mutations and the developmental context from which they arise. These data collectively highlight unique selective pressures within the developing brainstem and solidify DIPG as a specific molecular and biological entity among pHGGs. Emerging studies continue to identify novel mutations that distinguish subgroups of pHGG. The molecular heterogeneity among pHGGs will undoubtedly have clinical implications moving forward. The discovery of unique oncogenic drivers is a critical first step in providing patients with appropriate, targeted therapies. Despite these insights, our vantage point has been largely limited to an in-depth analysis of protein coding sequences. Given the clear importance of histone mutations in pHGG, it will be interesting to see how aberrant epigenetic regulation contributes to tumorigenesis in the pediatric context. New mechanistic insights may allow for the identification of distinct vulnerabilities in this devastating spectrum of childhood tumors.
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Opening Remarks

The past several years mark a period of tremendous growth in our understanding of pediatric high-grade glioma (pHGG). Advances in genome-wide array-based and sequencing technologies, their precipitous drop in cost, and evaluation of increasingly larger cohorts have all contributed to novel insights into the genetics of these devastating cancers, greatly extending earlier studies that evaluated candidate genes based on their involvement in adult HGG (aHGG). Our aim is to provide context to these studies and highlight their contribution to the current state of pHGG knowledge.

There are a number of biological features to suggest pediatric gliomas differ from those arising in adults. Most adult gliomas are high-grade supratentorial tumors. In contrast, most childhood gliomas are low grade, and both low-grade glioma (LGG) and HGG commonly arise within the posterior fossa, an area seldom affected in adults. Diffuse intrinsic pontine glioma (DIPG) is a brainstem HGG that occurs almost exclusively in children.[1,2] Additionally, the current standard of chemotherapeutic care for aHGG, temozolomide, has not been shown to improve long-term survival in pediatric trials.[3-5] Furthermore,

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malignant transformation, the process whereby a low-grade lesion progresses to a high-grade tumor, is a common event in adults but infrequent in children.[6] Genetic analyses have illuminated molecular differences driving pediatric and adult high-grade gliomagenesis.

The 3 Core aHGG Pathways Show a Different Spectrum of Alteration in pHGG

As the genomic landscape of aHGG came into view, it shaped initial work into the pediatric disease. The first pHGG studies focused primarily on investigating the involvement of high-frequency recurrent events found in adult tumors. For example, epidermal growth factor receptor (EGFR) is the most commonly altered receptor tyrosine kinase (RTK) in aHGG; with the corresponding gene locus undergoing amplification or intragenic deletion or both in ~50% of cases.[7-9] First identified in adult glioblastoma, EGFRvIII is the most common EGFR variant in aHGG and is formed by deletion of exons 2-7 resulting in a constitutively active kinase.[10-12] Accordingly, investigators early on sought to examine the degree of EGFR involvement in pediatric cases of HGG.

A number of studies found that *EGFR* alteration was less frequent in pHGG,[13-19] although gene amplification and EGFRvIII expression were detected in some pHGGs.[20-23] Through genome-wide studies, *PDGFRA*, which encodes platelet-derived growth factor receptor alpha (PDGFRα), was identified as the most commonly targeted RTK in both DIPG and pediatric nonbrainstem (pNBS)-HGG. Alterations in the gene itself include amplification, mutation, or both.[15-19,23-27] Experimentally, overexpression of wild-type (WT) or mutant PDGFRα confer a growth advantage to astrocytes, an effect that is diminished by the introduction of the adenosine triphosphate–competitive inhibitors crenolanib or dasatinib.[27] PDGFRα mutants drive glioma formation in vivo,[27,28] with murine-derived HGGs recapitulating critical features of the human disease such as histopathologic characteristics and expression profiles.[27] In an effort to target PDGFR therapeutically, pediatric trials using dasatinib, crenolanib, or imatinib have been launched.[29-31] Unfortunately, the benefit derived from selective RTK inhibitors may be marginal at best. pHGGs show evidence of intratumoral heterogeneity, with some cells coamplifying multiple RTK genes or discrete cell populations within the same tumor amplifying different genes, suggesting that resistant populations are likely to be present even before treatment with targeted agents.[16,24]

Both PDGFRA and EGFR are part of the RTK-Ras-phosphatidylinositide 3-kinase (PI3K) signaling cascade, which is altered in nearly 90% of aHGGs. Additionally, ~80%-90% of adult tumors show evidence of retinoblastoma (RB) and p53 pathway dysregulation.[7-9,32] For this reason, many of the first genetic pHGG studies focused on these same networks (Fig. 1).

In adults, the most commonly targeted components of the RTK-Ras-PI3K axis downstream of RTKs include activation of PI3K itself, or loss of function of phosphatase and tensin homolog (PTEN), the main negative regulator of PI3K signaling, or NF1, a negative regulator of Ras-mediated signaling.[7-9] Activation of PI3K signaling caused by mutations of *PIK3CA*, encoding the catalytic p110α subunit of PI3K, or *PIK3R1*, encoding the regulatory subunit of PI3K, is usually present in mutually exclusive patterns, occurring in approximately 20% of aHGGs and in a similar frequency of pHGG, including DIPG.[9,25,33-41] The *PTEN* tumor suppressor is located on chromosome 10q. It remains unclear whether all tumors with loss of chromosome 10q are targeting PTEN loss of function when a WT *PTEN* allele is still retained. However, there are examples in experimental systems where *PTEN* haploinsufficiency contributes to tumorigenesis. Loss of heterozygosity (LOH) of chromosome 10q, with or without concurrent *PTEN* mutation, is very frequent in adult glioblastoma, with 10q LOH in approximately 80% and *PTEN* mutation in 25%-40%, whereas the frequency is significantly lower in pHGGs, with 10q LOH in approximately 30% and *PTEN* mutation in less than 5%-15%.[9,13,15-19,23-26,34,35,42-44]

RB pathway dysregulation is common in both pNBS-HGGs and DIPG (Fig. 1). The *CDKN2A* locus codes for 2 tumor suppressors, p16INK4a and ARF.[45] Notably, homozygous deletion of *CDKN2A/B* appears to be almost exclusive to pNBS tumors and largely absent in DIPGs.[15-19,23-26,46] In contrast, amplification of *CDK4/6* or *CCND1/2/3* is found in approximately 30% of DIPG.[16,19,24] *CDK4/6* codes for cyclin D–dependent kinases that phosphorylate the RB protein, facilitating G1-S cell cycle progression. To become active, these kinases must bind to cyclin D family members (encoded by *CCND1/2/3*), which themselves confer substrate specificity.[45] Therapeutic inhibition of this cyclin-CDK complex, using PD-0332991, a highly-selective non–adenosine triphosphate–competitive CDK4/6 inhibitor, significantly increased survival in a murine model of DIPG, both as a single agent or following irradiation.[47]

TP53 mutations occur in up to 35% of pNBS-HGGs (range: 18%-35%) and appear to be more common in DIPGs (40%-50% of cases).[14,25,32,34,35,39-44,48,49]

From the aforementioned data, we can conclude that although the 3 main signaling pathways affected in aHGG are also affected in pHGG, pediatric and adult tumors differ regarding the most frequently mutated effectors.

Copy Number Imbalances and Gene Expression Profiling

Despite some common copy number imbalances such as 13q and 14q loss in approximately one-third of patients with HGG regardless of age or location, aHGG and pHGG also exhibit a unique constellation of gains and losses that distinguish one from the other, and the same can be said for DIPGs and pNBS-HGGs.[15-19,23-26] This suggests that unique combinations of genetic drivers underlie adult and pediatric