Pathology, molecular genetics, and epigenetics of diffuse intrinsic pontine glioma

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Diffuse intrinsic pontine glioma (DIPG) is a devastating pediatric brain cancer with no effective therapy. Histological similarity of DIPG to supratentorial high-grade astrocytomas of adults has led to assumptions that these entities possess similar underlying molecular properties and therefore similar therapeutic responses to standard therapies. The failure of all clinical trials in the last 30 years to improve DIPG patient outcome has suggested otherwise. Recent studies employing next-generation sequencing and microarray technologies have provided a breadth of evidence highlighting the unique molecular genetics and epigenetics of this cancer, distinguishing it from both adult and pediatric cerebral high-grade astrocytomas. This review describes the most common molecular genetic and epigenetic signatures of DIPG in the context of molecular subgroups and histopathological diagnosis, including this tumor entity’s unique mutational landscape, copy number alterations, and structural variants, as well as epigenetic changes on the global DNA and histone levels. The increased knowledge of DIPG biology and histopathology has opened doors to new diagnostic and therapeutic avenues.

Keywords: DIPG, glioma, glioblastoma, pediatric, ACVR1, K27M, histone, H3F3A

Introduction

Diffuse intrinsic pontine gliomas (DIPG), brainstem tumors that diffusely involve the pons, are the most common type of brainstem gliomas (BSG) (1). The mean age of diagnosis for this devastating pediatric neoplasm is 6–7 years (2, 3). The delicate location of these tumors eliminates surgical intervention as a treatment option. Radiation therapy (RT) is the standard of care, although it offers temporary relief of symptoms rather than a real hope of cure (4). Despite 68 clinical trials using various adjuvant chemotherapeutic agents between 1984 and 2014, there has been no improvement in survival compared to radiation alone, and DIPG are currently the number one cause of brain tumor-related death in children (5–18). The median survival of DIPG patients is only 10 months post diagnosis and <10% of patients survive 2 years (19, 20). Since the 1980s, the diagnosis of DIPG was based on clinical findings and diagnostic imaging characteristics on computerized tomography (CT) or magnetic resonance imaging (MRI) (4, 21). The lack of surgical and biopsy material has limited most studies of DIPG biology and histology to post-mortem tissue. Although initial investigation into the safety of incorporating biopsy for BSG showed no surgical mortality and low surgical morbidity (22), the advent of CT and MRI allowed for accurate non-invasive localization of BSG (23). It was noted that patients with DIPG represented the majority of deaths in children with BSG and biopsies, which did not stratify patients into different treatment groups, were abandoned (23).
Recently, the role for biopsy in DIPG diagnosis has been substantially strengthened due to several important discoveries related to the biology and histopathology of this universally fatal tumor (3, 24, 25) and successful use of stereotactic biopsy by several centers with limited morbidity (26–29). Biologically, DIPG is a unique tumor entity which possesses properties that are antithetical when compared to other brain tumors, such as pediatric and adult supratentorial high-grade astrocytomas (HGA), which they most often resemble histologically. This review highlights the unique pathology, genetics, and epigenetics of DIPG.

From Candidate Gene Approaches to Next-Generation Sequencing

Early molecular profiling of pediatric gliomas was limited to candidate gene approaches (30–33) focusing on mutational and copy number profiling of genes known to be frequently implicated in adult HGA, namely EGFR, TP53, IDH1/2, CDKN2A, MGMT, and PTEN (34–36). All early studies of DIPG were performed on small cohorts or case studies, which limited their usefulness in defining the biology of this deadly pediatric cancer. However, conclusions from these studies still highlighted some differences between DIPG and adult HGA. Unfortunately, these candidate gene approaches were limited to what was known about adult HGA and future studies using whole-genome profiling approaches would be required to discover more robust differences between these entities. Whole genome profiling technologies such as array CGH and SNP-genotyping allowed for the first genome-wide copy number analysis of cancers. Although giving a low resolution perspective at first, newer iterations allowed for greater and greater resolution and began to highlight the unique molecular profiles of DIPG when compared to pediatric and adult supratentorial astrocytomas. Copy number signatures at the whole chromosome arm level revealed differences between DIPG and adult HGA (37). Gains of chromosome 1q and losses of 11p, 13q, and 14q were frequently observed in DIPG and less so in supratentorial HGA (37). Subsequent copy number studies, with larger DIPG patient tumor cohorts, revealed frequently altered genes (Table 1) including Pdgfra, TP53, PARP1, PVT-1/MYC, RB1, and PTEN (3, 38, 39). Most recently, next-generation sequencing approaches, including whole-genome sequencing (WGS), whole-exome sequencing (WES), and RNA-seq analysis integrated with histopathology, copy number, gene expression, and methylation profiling, and other molecular techniques have re-defined what we know about DIPG genetics and epigenetics.

Mutational Landscape

The first major breakthrough in defining the DIPG mutational landscape came in 2012, when studies on pediatric brain tumors using whole-genome and WES reported that 70–84% of DIPG possess mutations in histone H3, and that these mutations were predictive of outcome (3, 24, 25). These recurrent mutations, in H3F3A or HIST1H3B/C1 (Figure 1A), result in a p. Lys27Met (K27M) substitution. A subset of other midline astrocytomas, such as those arising in the thalamus, have also been found to harbor K27M histone H3 mutations although at a lower frequency (50). Conversely, pediatric supratentorial HGA rarely possess these mutations, and more frequently have p. Gly34Arg (G34R) or p. Gly34Val (G34V) substitution in histone H3.3. The G34R/V-H3.3 mutations occur in 10–19% of supratentorial HGA cases (24) and are never found in DIPG (3). Furthermore, among supratentorial GBM, these G34R/V-H3.3 mutations are predominantly found in older children and young adults (3). Mutations affecting these two histone residues are extremely rare in adult HGA.

Histones are proteins that form octameric complexes known as nucleosomes around which DNA wraps and condenses into chromatin. The majority of histone mutations in DIPG, 65%, effect histone variant H3.3 (3, 24, 25). Although this histone is coded in two genes, H3F3A and H3F3B, K27M mutations are only found in the former. K27M-H3.1 mutations are found in 12–19% of DIPG and are mutually exclusive from K27M-H3.3 mutations (24, 40). Histone H3.1 is coded by a cluster of 10 genes on chromosome 6 known as the HIST1 cluster (44). Currently, K27M-H3.1 mutations have been detected in HIST1H3B, HIST1H3C, and HIST1H3I.

Histones play a key role in the state of chromatin; however, they themselves do not determine whether chromatin will be in a conformation more permissive to gene expression (euchromatin) or in a conformation less permissive to gene expression (heterochromatin). Several histone marks can be laid on specific residues across the histone tail, including lysine 27, which can be post-translationally modified by either acetylation or mono-, di-, or tri-methylation (51–53). Various cellular machineries are implicated in the reading, writing, and copying of these epigenetic histone marks, including histone acetyltransferases (HATs), histone

| Gene | Alteration type | Frequency in DIPG (%) | Reference |
|------|----------------|----------------------|-----------|
| ACRV1 | Mutation | 20–32 | (40–42) |
| ATRX | Mutation | 9–13 | (3, 24) |
| BRTX | Mutation | 0 | (40–43) |
| CDK6 | Copy number gain | 3–4 | (3, 37, 44) |
| CDKN2A/B | Focal deletion | 3–4 | (37–39) |
| EGFR | Mutation | 0–2 | (40–43, 45) |
| EGFR | Amplification | 0–2 | (40–43) |
| FGFR | Fusion | 0 | (40–43) |
| HSF3A | Mutation | 58–65 | (3, 40–43, 48) |
| HIST1H3B/C | Mutation | 12–19 | (40–43, 46) |
| IDH1/2 | Mutation | 0 | (3, 40–43, 46) |
| MYC/PVT-1 | Copy number gain | 14 | (3, 40) |
| MYCN | Copy number gain | 7 | (3, 40) |
| NF1 | Mutation | 0–3 | (40–43) |
| NF1 | Focal deletion | 7 | (40–43) |
| PDGFA | Mutation | 5–9 | (3, 37, 38, 40, 47, 48) |
| PIK3CA | Mutation | 12–23 | (2, 27) |
| PPP1MD | Mutation | 10–12 | (42, 46) |
| PTEN | Mutation | 42–71 | (2, 3, 32, 40, 49) |
| PTEN | Heterozygous deletion | 36–64 | (2, 3, 32, 40, 49) |
| RB1 | Mutation | 0–3 | (38–43) |
| RB1 | Focal deletion | 14 | (38–43) |
deacetylases (HDACs), histone methyltransferases (HMTs), and histone demethylases (54). The methionine substitution at K27 in DIPG cannot be modified and several in vitro and in vivo studies have documented a global decrease in H3K27me3 in the presence of mutant histone (40, 55–58). This global decrease is attributed to polycomb repressive complex 2 (PRC2) inhibition (59); however, other molecular consequences of this mutation, including its effect on histone marks, global DNA methylation, and gene expression, are still under investigation. Histone H3 mutations are undoubtedly important in DIPG tumorigenesis and/or maintenance but in a majority of cases are found to be partnered with other mutations and copy number alterations (CNAs) (3, 40), suggesting that they are not the sole drivers of DIPG tumorigenesis.

**TP53** mutations and CNAs occur in 42–71% of DIPG (Figure 1B) (3, 40). Hemizygous deletions are often associated with a mutation and strong protein expression by immunohistochemistry (32, 49). Interestingly, DIPG with low-grade astrocytoma histology (grade II), even those harboring H3-mutations, are not mutated for **TP53** (2). Among DIPG with high-grade histology (grade III and IV), both H3-mutant and wild-type DIPG often have mutated **TP53** (2). However, a recent exome sequencing study identified **PPM1D** mutations (Figure 1C) in a subset of H3-mutant but **TP53** wild-type DIPG (46). These mutations account of ~50% of **TP53** wild-type grade II DIPG. **PPM1D** gene codes for WIP1 (wild-type p53-induced protein phosphatase 1D) and has been implicated as an oncogene in other cancers (60–62). Mutations of **PPM1D** have been shown to be functionally equivalent to those of **TP53** (46, 63). Taken together, alterations of the **TP53** signaling pathway in DIPG are only slightly less common than histone H3 mutations.

The third most commonly mutated gene in DIPG codes for the activin A receptor, type I (**ACVR1**), a member of the bone morphogenetic protein (BMP) signaling pathway. Approximately, 20–32% of DIPG harbor mutations in **ACVR1** which significantly overlap with K27M mutations in histone H3.1 (40–42). Previously only reported in a congenital autosomal dominant disease of the connective tissue called fibrodysplasia ossificans progressiva (FOP), **ACVR1** mutations result in ligand-independent constitutive activation of the BMP signaling pathway (64–66). Although seven different **ACVR1** mutations have been reported in DIPG, the most common alteration in this tumor type, p.Gly328Val, has not been reported in FOP patients. Several other residues of **ACVR1** are frequently mutated in DIPG (Figure 1D). These mutations have been shown to increase levels of phosphorylated SMAD1/5.
(40–43) as well as increased gene expression of downstream BMP signaling targets ID1 and ID2 (40). Research into the cooperation between ACVR1 and histone H3.1 mutations, and their effects on tumorigenesis are still ongoing.

**Amplification and Mutations of Receptor Tyrosine Kinases**

Receptor tyrosine kinases (RTKs) are cell surface receptors which are often dysregulated in cancers. The RTK/RAS/PI-3K signaling pathway is the most commonly dysregulated signaling pathway in adult GBM, with 90% of tumors exhibiting CNAs or mutations in pathway members (34). Mutations and CNAs in the platelet-derived growth factor receptor alpha (PDGFRα) have been implicated in both adult and pediatric HGA. Several array-based studies of DIPG revealed amplification of PDGFRα in 28–36% of patient tumors (37, 38). The frequency of these amplifications in pediatric and adult supratentorial HGA was reported in the range of 7–14%, and was preferentially identified in the Proneural subtype of adult GBM (36). In DIPG, PDGFRα amplifications are exclusively found in patients with K27M-H3 mutations and across all astrocytic histologies (grade II-IV) (2, 40). Oncogenic mutations of PDGFRα have also been reported in 5–9% of DIPG (47, 48). Epidermal growth factor receptor (EGFR) copy number gains and mutations are among the most frequent alterations in adult HGA, occurring in 60–85% of GBM (34, 35). In adults, EGFR protein expression correlates with amplifications and EGFRvIII mutations; however in DIPG, EGFR protein expression correlates with amplifications and EGFRvIII PDGFRA of tumors (of DIPG revealed amplification of catedin both adult and pediatric HGA. Several array-based studies of DIPG revealed amplification of PDGFRα in 28–36% of patient tumors (37, 38). The frequency of these amplifications in pediatric and adult supratentorial HGA was reported in the range of 7–14%, and was preferentially identified in the Proneural subtype of adult GBM (36). In DIPG, PDGFRα amplifications are exclusively found in patients with K27M-H3 mutations and across all astrocytic histologies (grade II-IV) (2, 40). Oncogenic mutations of PDGFRα have also been reported in 5–9% of DIPG (47, 48). Epidermal growth factor receptor (EGFR) copy number gains and mutations are among the most frequent alterations in adult HGA, occurring in 60–85% of GBM (34, 35). In adults, EGFR protein expression correlates with amplifications and EGFRvIII mutations; however in DIPG, EGFR CNAs and mutations are extremely rare, found in 0–2% of patient tumors (45), and do not correlate with immunopositivity (67, 68). The RTK/RAS/PI-3K signaling pathway is further dysregulated in DIPG through hemizygous deletions of PTEN (40), as well as mutations of PIK3CA and PIK3R1 (2, 27). Although this pathway has often been targeted in clinical trials, the majority of the CNAs and mutations in the RTK/RAS/PI-3K pathway are clonal events, as determined by fluorescence in situ hybridization (FISH) for PDGFRα amplifications (37) and mutation allele frequency of PIK3CA, suggesting these events likely arise later in tumor development.

**DNA Damage Repair (PARP1, MGMT, MPG)**

Radiotherapy is the standard of care for DIPG and adjuvant chemotherapeutics have been shown to be ineffective. Perturbations in DNA damage repair pathways in DIPG were first identified in 2010 by Zarghooni et al. Loss of heterozygosity (LOH) was identified in many genes involved in nucleotide excision repair, non-homologous end-joining (NHEJ), homologous recombination (HR), base excision repair (BER), and mismatch repair (MMR) by analysis of both SNP arrays and microsatellite markers (37). Poly (ADP-ribose) polymerase (PARP1), a protein essential for repair of single strand DNA breaks induced by alkylating agents, as well as repair of ionizing radiation induced double strand breaks by HR and NHEJ were found to be gained and/or overexpressed in 54% of DIPG, highlighting it as a potential therapeutic target (37). Furthermore, pathway analysis revealed a subset of patients with LOH or deletions in members of the BRCA DNA damage response pathway, including BRCA1 and BRCA2. Defects in either of these two genes have been implicated in promoting sensitivity to single strand DNA repair via PARP inhibition.

Temozolomide (TMZ) is a frontline DNA alkylating agent most often used in treatment of adult GBM. TMZ causes DNA damage by alkylating O6-guanine, N7-guanine, and N3-adenine residues. In adults, it was identified that a subgroup of patients with MGMT (O6-methylguanine DNA methyltransferase) promoter methylation had improved overall survival when treated with radiotherapy and concomitant TMZ compared to patients without MGMT promoter methylation (69). MGMT repairs TMZ alkylated O6-guanine nucleotides. However, the universal lack of response to TMZ in DIPG patients could not be attributed to this resistance mechanism, as DIPG have not been found to express MGMT (37). It has been recently described that TMZ resistance in the pediatric population can be attributed to an ATM-dependent regulation of 3-methylpurine-DNA glycosylase (MPG), an enzyme responsible for repair of alkylated N2 guanine and N3 adenosine residues (70). Further investigation into using radio-sensitizing and chemo-sensitizing agents in DIPG therapy is warranted.

**Isocitrate Dehydrogenase, ATRX, and Telomeres**

Mutations in the mitochondrial enzyme gene, isocitrate dehydrogenase 1 (IDH1), whose gene product catalyzes the oxidative decarboxylation of isocitrate to α-keto glutarate, are found in approximately 70–80% of adult low-grade astrocytomas (LGA) and anaplastic astrocytomas (AA), as well as secondary glioblastoma (GBM) (71). Furthermore, in adult gliomas, IDH1 mutations were found to be associated with ATRX mutations and alternative lengthening of telomeres (ALT) (72). Isocitrate dehydrogenase 2 (IDH2) mutations are also found in a subset of adult HGA but to date, no mutations in IDH1 or IDH2 have been detected in DIPG. Mutations of chromatin remodeling genes are less common in DIPG than in supratentorial HGA (3, 24). ATRX, which codes for the α-thalassemia/mental retardation syndrome X-linked gene, was found to be mutated in a subset of DIPG (~5%) but in contrast to pediatric supratentorial HGA, had no clear overlap with histone H3 mutations (3). Pediatric supratentorial HGA showed high overlap with ATRX or DAXX mutations (15–25%) and G34R/V-H3.3 alterations, as well as mutual exclusivity of IDH1/2 mutations (24, 73). Irrespective of tumor location, in the pediatric population ATRX mutations significantly overlap with TP53 mutations and are predominantly found in older children (3). ATRX and DAXX (death-domain associated protein) are genes encoding subunits of a chromatin remodeling complex required for histone H3.3 incorporation at telomeric regions. Telomeres are repetitive regions of DNA found on the ends of chromosomes and shorten during every cell division due to incomplete DNA replication (74). Over many cell divisions, telomeres may reach a critically short length, which results in cellular senescence (75). This fate can be avoided by expressing telomerase, an enzyme that can extend telomeres. Although telomerase is not expressed in most mature, terminally differentiated cells (76, 77), its expression and activity has been implicated in several brain cancers as a poor prognostic marker and potential therapeutic target (78–80). Extension of telomeres...
in certain cancers can be attained by a telomerase independent mechanism known as alternative lengthening of telomeres (ALT). Twenty percent of DIPG test positive for ALT by either TRF (telomere restriction fragment) assay or C-circles assay and ALT positive DIPG are exclusive carriers of the K27M-H3.3 mutation (40). ALT phenotype is also associated with an older age of diagnosis in DIPG and to date has not been detected in patients with low-grade astrocytoma histology (2, 40).

**DIPG Histopathology**

Diffuse intrinsic pontine glioma is a heterogeneous disease, and represents a varied histological spectrum. These tumors are very diffuse and often involve adjacent brain structures beyond the pons. Several studies report leptomeningeal dissemination and subventricular spread as a common occurrence seen in as many as one-third of DIPG, with tumor cells found as far rostrally as the frontal lobe (2, 81, 82). A review by Jansen et al. reported World Health Organization (WHO) central nervous system tumor classification on 108 biopsies from 13 studies, including 37 AA (WHO grade III), 27 GBM (WHO grade IV), 22 LGA (WHO grade II), 3 anaplastic oligoastrocytomas (WHO grade III), and 19 tumors with “not further specified” or undefined characterization (9). Data from this biopsy series would suggest that WHO grade III AA are the most common histological entity in brainstem glioma; however, autopsy based histopathological studies report WHO grade IV GBM to be the most common histology, potentially highlighting the caveat of limited tissue sampling during biopsy or anaplastic progress and/or treatment effect seen in late stage disease at autopsy. Autopsy based studies revealed that GBM histology was most common in DIPG. Of the 33 pediatric patients examined by Yoshimura et al., 29 were reported to be GBM and 4 with anaplastic astrocytoma histology (83). A larger autopsy based study of DIPG histology reported 42 GBM, 18 anaplastic astrocytoma, 8 low-grade astrocytoma, and 2 with features of primitive neuroectodermal tumor (PNET, WHO grade IV) (13).
Previous investigations into DIPG histology have also observed this rare PNET histology in the brainstem (84). Importantly, regional differences within one DIPG specimen can bias biopsy based diagnoses. Autopsy studies allow extensive tissue sampling and have highlighted intratumoral histopathologic heterogeneity (2). Areas within or around a grade IV astrocytoma may present with features of grade II or grade III histology that could be inadvertently targeted at biopsy (Figure 2).

Perhaps most importantly, DIPG histology is not a predictor of survival. Patients with low-grade histology do just as poorly as patients with high-grade histology. On multivariate Cox regression analysis, only histone H3 mutation is a predictor of worse

| Year | Subgroups | Molecular Features of Subgroups |
|------|-----------|--------------------------------|
| 2011 | Paugh et al. | Three molecular subgroups based on gene expression and copy number profiling. PDGFRA and RB1 copy number alterations common but not in subgroup specific manner. |
|      | HC1       | HC1: enriched for mesenchymal markers |
|      | HC2       | HC2: enriched for proliferative markers |
|      | HC3       | HC3: enriched for pediatric proneural markers |
| 2012 | Puget et al. | Two distinct subgroups based on gene expression and copy number profiling. |
|      | Mes       | Mesenchymal: enriched for mesenchymal and pro-angiogenic characteristics, with stem cell marker enrichment. |
|      | Pro       | Proliferative: displayed oligodendrogial features largely driven by PDGFRA alterations. |
| 2012 | Khuong-Quang et al. | Analyzed copy number between H3F3A mutant and wild-type DIPG. K27M-H3 mutation shown to be a marker of poor prognosis. |
|      | H3 wild-type | H3 wild-type: displayed focal gains/amplifications of 2p25.1 and 2p24.3 corresponding to ASAP2 and MYCN genes. |
|      | H3 mutant | H3 mutant: enriched for PDGFRA and PVT1/MYC gains and amplifications. Patients had poor overall survival. |
| 2013 | Sarasis et al. | Two subgroups based on mRNA, methylation and protein profiling. DIPG showed global DNA hypomethylation compared to normal brain and H3-mutant DIPG had unique methylation profile compared to wild-type DIPG. |
|      | N-Myc     | N-Myc: characterized by up-regulation of N-Myc. |
|      | HH        | Hedgehog: characterized by hedgehog signaling and up-regulation of HH pathway member PTC1 (Patch). |
| 2014 | Buczkowicz et al. | Three molecular subgroups based on methylation and gene expression profiling, copy number analysis, whole-genome and whole-exome sequencing. |
|      | MYCN      | MYCN: Chromothripsis of chr. 2p resulting in high level MYCN & ID2 amplification (all DIPG in this subgroup are H3 wild-type). |
|      | Silent    | Silent: Silent genomes with few copy number alterations and low mutation rate. Some DIPG with H3 and/or TP53 mutations. |
|      | H3-K27M   | H3-K27M: All K2M-H3 mutants, with enrichment for ACVR1 and TP53 mutations, PDGFRA, PVT1-MYC, TP53, Pten and RB1 copy number alterations and alternative lengthening of telomeres. |

FIGURE 3 | Summary of five publications between 2011 and 2014 outlining clinical and molecular features of diffuse intrinsic pontine gliomas based on various platforms and sample sizes; Paugh et al. (38), three subgroups – n = 27; Puget et al. (48), two subgroups – n = 23; Khuong-Quang et al. (3), two subgroups – n = 42; Sarasis et al. (65), two subgroups – n = 14; Buczkowicz et al. (40), three subgroups – n = 48.
overall survival, irrespective of histological grade, age of diagnosis, and sex (2, 3). Furthermore, DIPG patients, which at autopsy had grade II histology, but were mutated for histone H3, had clinical outcomes similar to what would be expected of high-grade tumors (2, 3). This important finding suggests brainstem glioma require their own WHO grading scheme, which needs to incorporate H3-mutation testing at biopsy.

**Molecular Subgroups of DIPG**

Several studies have embarked on molecular subgrouping of DIPG based on various molecular signatures, including gene expression profiling, copy number analysis, proteomics, mutational profiles, and methylation profiling (Figure 3). A study by Paugh et al. in 2011 profiled 27 DIPG by gene expression arrays and classified them into three subgroups based on hierarchical clustering and compared the subgroup specific enrichment scores to previously identified subgroups of adult GBM, highlighting significant enrichment in either mesenchymal, proliferative, or proneural markers (38). These data were also compared to genomic copy number abnormalities determined by single-nucleotide polymorphism arrays which highlighted frequent focal amplifications of PDGFRα and RB1. However, these CNAs were not restricted to any of the three identified subgroups. A subsequent study of gene expression profiles from 23 DIPG matched with copy number data from array CGH identified two subgroups using K-means clustering (48). These two subgroups were enriched for mesenchymal and proneural markers, respectively, with the proneural subgroup displaying oligodendrogial features and alterations of PDGFRα and PVT-1/MYC gains and amplifications to be present at high frequency in K27M-H3 mutant DIPG, whereas wild-type DIPG were enriched for MYCN amplification as determined by GISTIC2.0 analysis (3). A study from 2013 by Saratosis et al. utilized mRNA and methylation profiles with protein profiling of 14 DIPG specimens. This study also reported two subgroups, which were characterized by upregulation of N-Myc or Hedgehog signaling through mRNA expression and DNA hypomethylation (85).

The most comprehensive subgrouping of DIPG integrated CpG island methylation with WGS and WES, gene expression, and copy number profiling to find that DIPG are three molecularly distinct subgroups; MYCN, Silent, and H3-K27M (40). The MYCN subgroup did not contain any DIPG with histone mutations, but was characterized by high-level amplifications of MYCN and ID2 genes caused by chromothripsis on chromosome 2p. Furthermore, these MYCN amplified DIPG did not contain PVT-1/MYC, PDGFRα, or RB1 CNAs, which were previously described to be common in these brainstem neoplasms (40). The Silent subgroup did not contain many CNAs or mutations. In fact, this subgroup was characterized by stable genomes when compared to both MYCN and H3-K27M subgroup DIPG. Some DIPG in this subgroup contained histone H3, TP53, and ACVR1 mutations; however, they were found at a lower frequency. All patients within the largest subgroup, H3-K27M, had histone H3-mutations. This subgroup was further characterized by hypomethylated genomes and also enriched in ACVR1 mutations (which significantly co-occurred with K27M-H3.1 mutations), TP53/PPM1D mutations/homozygous deletions, alternative lengthening of telomeres, and CNAs of PVT-1/MYC, PSEN, PDGFRα, and RB1 (40).

**Conclusion**

In recent years, the combined increase in knowledge of DIPG histology, genetics, and epigenetics has been substantial. Mutations in previously undiscovered oncogenes, histone H3, and ACVR1, molecular subtypes of DIPG, and the rediscovered role for biopsy for this tumor entity are redefining what is clinically possible in treating these patients. The major challenges ahead are developing molecularly accurate pre-clinical models for testing of new therapeutics and integrating new genetic/epigenetic and histopathological knowledge for DIPG patient diagnosis, treatment, and future clinical trial design. In the near future, DIPG patients will benefit from mutational testing and molecular subgrouping at biopsy, which will provide more accurate prognosis and actionable tumor-specific genomic targets for these affected children.

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