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REVIEW ARTICLE

Diffuse Intrinsic Pontine Glioma: New Pathophysiological Insights and Emerging Therapeutic Targets

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Abstract: ***Background***: Diffuse Intrinsic Pontine Glioma (DIPG) is the leading cause of brain tumor-related death in children, with median survival of less than one year. Despite decades of clinical trials, there has been no improvement in prognosis since the introduction of radiotherapy over thirty years ago.

Objective: To review the clinical features and current treatment challenges of DIPG, and discuss emerging insights into the unique genomic and epigenomic mechanisms driving DIPG pathogenesis that present new opportunities for the identification of therapeutic targets.

Conclusion: In recent years, an increased availability of biopsy and rapid autopsy tissue samples for preclinical investigation has combined with the advent of new genomic and epigenomic profiling tools to yield remarkable advancements in our understanding of DIPG disease mechanisms. As well, a deeper understanding of the developmental context of DIPG is shedding light on therapeutic targets in the microenvironment of the childhood brain.

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Keywords: Childhood cancer, Diffuse Intrinsic Pontine Glioma (DIPG), epigenetics, histone mutation, pediatric glioma, pediatric neurodevelopment.

EPIDEMIOLOGY

Diffuse Intrinsic Pontine Glioma (DIPG) is a devastating, aggressive brain tumor of childhood arising in the ventral pons. Though brainstem tumors are rare among adults, they comprise approximately 10-15% of pediatric brain tumors [1]. Half of all malignant pediatric gliomas occur in the brainstem, and indeed DIPG is the most common tumor subtype in this anatomical region, constituting 80% of brainstem gliomas overall [2]. With an estimated 200-400 children affected by DIPG annually in the United States, it is the second most common malignant brain tumor of childhood [3]. The prognosis is bleak: in the absence of effective therapies, DIPG is uniformly fatal and is the leading cause of childhood brain tumor death. Median age at diagnosis is 6-7 years, with median survival of 9 months; 90% of children will die from the disease within 2 years of initial diagnosis, with less than 1% surviving after 5 years [4, 5].

CLINICAL CHARACTERISTICS

Because DIPG progresses rapidly, children typically experience symptoms for a month or less before they are diagnosed [6]. The clinical signs and symptoms of DIPG result from compression or dysfunction of anatomic structures at and near the ventral pons where the tumor arises. Dysconjugate gaze and resulting diplopia associated with cranial nerve VI dysfunction (*abducens palsy*) nearly always occur as an initial sign and sensitive positive predictor of DIPG, and carry a poor prognostic significance [5]. Facial weakness or asymmetry may also result from damage to cranial nerve VII. Extremity weakness, hyperreflexia, and Babinski sign on neurologic examination may result from damage to the long motor tracts passing through the pons. Impairments of gait, coordination or speech (*ataxia, dysmetria, dysarthria*) indicate involvement of projections to the adjacent cerebellum. Together, this combination of multiple cranial neuropathies, long tract and cerebellar signs is known as the "classic triad" of DIPG presentation, though up to half of patients may not demonstrate such typical findings [5-8]. In fewer than 10% of cases, dorsal extension of the tumor blocks CSF flow leading to hydrocephalus, a condition of increased intracranial pressure that results in headache, nausea and fatigue and can progress to obtundation [4, 6, 8].

DIAGNOSIS

The diagnosis of DIPG is based on the clinical history and examination combined with radiographic findings. Because DIPG is diffusely infiltrative, intermixing with healthy tissue, the tumor margins appear poorly demarcated

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on neuroimaging. The tumor classically involves greater than 50% of the pontine axial diameter, engulfs the basilar artery, and does not enhance with gadolinium on MRI. These characteristic findings on diagnostic imaging distinguish DIPG from the less aggressive focal brainstem cancers [5, 6].

At present, surgical biopsy is not routinely obtained to confirm diagnosis in the United States. Undertaking the perceived risks of the procedure is not considered necessary, as neuroimaging is sufficient for diagnosis in typical cases of DIPG, and histopathological analysis has no current role in guiding therapeutic management at the present state of the field. The infrequency of DIPG biopsies has contributed to the scarcity of tumor tissue available for molecular study, but in the past decade this reluctant approach to the procedure has begun to shift. More recent evidence now suggests that that the risks of morbidity associated with stereotactic biopsy are minimal and may have been previously overestimated, and that biopsy can be both safe and diagnostically useful in cases where radiographic findings are unclear [9, 10]. In France, image-guided stereotactic biopsies are widely offered to patients and their families with the goal of obtaining treatment-naïve tissue for histopathological study, next-generation sequencing, and other research [10]; in fact, a recent 2011 French multidisciplinary consensus has formally recommended biopsy for research purposes [11]. Currently, international and multi-institutional efforts to share tumor tissue and resources resulting from biopsies, as well as from a growing pool of early post-mortem autopsy tissue donations by patients and their families, have resulted in expanded possibilities to study the disease. This has given way to a wide expansion of our understanding of its biology in recent years. As researchers begin to better understand the molecular markers of DIPG and how these associate with disease trajectory and prognosis, some now speculate that eventual development of disease stratification and targeted therapies may warrant revisiting the role of biopsy for DIPG patients in the future.

HISTOPATHOLOGICAL FEATURES AND PATTERN OF SPREAD: CLUES TO THE DIPG CELLULAR ORIGIN

On histopathological analysis, DIPG is classified within the fibrillary astrocytoma family, in contrast to the less aggressive pilocytic astrocytomas also arising in the brainstem [5]. By the World Health Organization (WHO) grading system, it is classically a high-grade lesion, most often representing glioblastoma multiforme (WHO grade IV) or high-grade anaplastic astrocystoma (WHO grade III), with less frequent lesions characterized as well-differentiated astrocytoma (WHO grade II) [5, 12]. Although variability in histologic grade occurs, this spectrum does not always correlate with clinical outcome; lower-grade DIPGs behave in an equally aggressive manner to their high-grade counterparts, and mutational profiles do not parse by WHO grade [13]. This highlights enduring inadequacies of the current cytological and histological grading system for disease stratification, and suggests a future role for the integration of molecular features in refining the present system.

Historically, DIPG was regarded as a disease largely restricted to the brainstem [14], but accumulating evidence now underscores its potential for dissemination along the neuraxis, prompting reexamination of the traditional view. Indeed, invasion of brainstem structures proximal to the pons (medulla and midbrain) and extension along white matter tracts into the cerebellum or thalamus occurs in more than half of patients, but additionally, leptomeningeal dissemination and supratentorial extension are well-described on autopsy and neuroimaging, and spinal cord involvement less frequently occurs [12, 13, 15, 16]. A recent case series recognized a subventricular pattern of spread, with radiographic and histologic evidence of involvement of the subventricular zone and frontal horns of the lateral ventricles in ~65% of cases [16].

Intriguingly, the subventricular zone represents a neural stem cell niche in the postnatal brain, and a stem-like population of cells found specifically in the human pons is proposed to represent the DIPG cell of origin. These pontine precursor cells, which appear by marker expression to be Olig2+ precursors likely in the oligodendroglial lineage [17, 18] are found specifically in the childhood brainstem within the very region of the ventral pons where DIPG arises. Moreover, these pontine precursor cells peak in number first during infancy and then again during middle childhood; this second peak corresponds to the age (6-7 years) of highest DIPG incidence [17]. Consistent with this finding, analysis of postmortem DIPG specimens reveal that most express the markers Olig2 and/or Sox2 providing further support that DIPG originates from an Olig2+ neural precursor cell [17, 19]. In a recent study incorporating MRI-based morphometric and histologic analyses, robust proliferation of Olig2+ progenitors and increased myelination of white matter tracts were found to accompany a striking postnatal volume expansion of the ventral pons observed from birth to middle childhood [18].

Further studies are needed to elucidate if DIPG demonstrates a particular tropism for the subventricular zone, but if confirmed, this could join other lines of evidence that provide insights into a developmental approach to understanding DIPG pathogenesis. Regardless, the now well-documented phenomenon of neuraxis spread demonstrates a need to revisit the traditional clinical strategy of treating DIPG solely as a localized disease of the brainstem.

CURRENT TREATMENT CHALLENGES

Because DIPG grows diffusely and infiltrates critical brainstem structures, surgical resection is not possible. Radiation therapy has remained the mainstay of treatment for the past three decades since its introduction. At most treatment centers, the standard recommendation is conventionally fractionated local field radiotherapy with dose range of 54-60 Gy for a period of 6 weeks [20]. Radiotherapy provides temporary improvement or stabilization of symptoms and extends overall survival by an average of 3 months; median survival is less than 5 months without radiation [21]. Though both clinical and radiographic responses are initially observed, local recurrence invariably occurs. Given the prevalence of neuraxis spread also

contributing to morbidity and mortality, some clinicians now advocate extending the field of radiotherapy, perhaps to include whole brain radiation [13, 16]. Multiple clinical trials attempting to optimize the fractionation schedule have not been fruitful. Hyperfractionated therapy (smaller, more frequent doses) did not improve overall survival in multiple clinical trials and furthermore was associated with increased morbidity [22-25]. Newer studies suggest that while hypofractionated therapy (higher doses over a shorter period of 3 weeks) does not improve prognosis, it may offer comparable overall survival with reduced burden on the patient and family, allowing for an average of less than 10% of remaining survival time to be spent in the hospital receiving treatment [4, 26, 27].

Many clinical trials of the past three decades have explored the use of various chemotherapeutic agents for DIPG, employing conventional and high-dose strategies as well as targeted agents. Chemotherapy has been attempted at time points before, during and after radiation therapy. Despite all efforts, no improvement in overall survival has been demonstrated [28-35]. Historically, these trials were performed largely without guidance by direct preclinical experimental data and were designed as analogues of therapeutic strategies used for adult high-grade glioma. As will be discussed, this approach is problematic: although adult and pediatric high-grade gliomas may lie on the same disease spectrum with certain shared histopathological features, emerging research suggests that DIPG exemplifies a distinct biology from its adult counterpart. Preclinical data for adult high-grade glioma thus cannot be assumed to generalize to DIPG.

The extent to which chemotherapy failure in previous clinical trials is due to inherent tumor cell resistance is unclear. Drug delivery to the pons, where DIPG is located, poses a major therapeutic challenge. Due to strict regulation by the blood-brain barrier, drugs intended to treat DIPG may not effectively reach their target. To address this challenge, Convection Enhanced Delivery (CED) has been proposed as a promising approach to bypass the blood-brain barrier and provide localized drug delivery in higher concentrations without systemic side effects. In this system, a catheter is placed into the region of the tumor by stereotactic surgery, and an attached pump locally delivers the drug under positive pressure [36]. In a recent clinical trial, CED was demonstrated to feasibly deliver topotecan to the brainstem; however, this trial highlighted the need for further technical optimization [37]. Small studies utilizing co-infusion of MRI surrogate tracers with CED have been useful in characterizing volume of distribution, pattern of anatomic distribution, and other important properties of convective drug delivery in this system, and have demonstrated how modifications of technical parameters can affect these properties [38, 39]. Co-infused surrogate tracers can be used to monitor drug infusion and demonstrate appropriate distribution of a given agent for faithful assessment its efficacy [38, 39]. A phase I safety trial of pontine CED, designed to optimize technical parameters, is ongoing (clinicaltrials.gov ID NCT01502917), and CED continues to gain attention as a promising area of development in the treatment of DIPG.

EMERGING PRECLINICAL TOOLS AND RESOURCES

In decades past, the lack of substantial progress in developing therapeutic strategies that improve DIPG survival underscores the fact that, until recently, DIPG was largely an understudied disease. This was due in part to a dearth of available tissue specimens resulting from the absent role of surgery or biopsy in the management of the disease. In recent years, rapid autopsy protocols have emerged as a feasible means of generating postmortem tissue samples for experimental study, and multiple institutions are now actively enrolling patients for postmortem donation [40-42]. Combined with available biopsy samples, tissue generated from rapid autopsy has yielded viable neurosphere [17, 43-45] and adherent [46] primary cell cultures, and the pool of available DIPG cell lines, while modest, continues to grow worldwide. Since 2010, faithful animal models of DIPG have been developed using both xenograft and genetic approaches. Monje and colleagues stereotactically implanted patient-derived DIPG tumor cells into the pons of immunodeficient mice to create the first patient-derived DIPG xenograft model [17]. Drawing from commonly observed genetic alterations in gliomas, Becher and colleagues used the RCAS/tv-a system to develop a genetically engineered mouse model *via Pdgf-B* overexpression in nestin-expressing cells when combined with loss of the tumor suppressor *Ink4a-ARF* [47], or combined with p53 loss and the H3.3K27M mutation [8]. Ongoing efforts by the *DIPG Preclinical Consortium*, formed in 2011 as an international collaboration of DIPG researchers, will attempt to harness the emerging availability of DIPG cell lines and animal models to ascertain efficacy of potential drugs at the preclinical level, directing future clinical trials [48].

NEW PATHOPHYSIOLOGICAL INSIGHTS

In recent years, a rapid expansion in our understanding of the biology of DIPG has occurred alongside the expansion of available autopsy/biopsy tissue and animal models for molecular analysis. The availability of this material, paired with the advent of next generation sequencing tools, has enabled groundbreaking new research revealing a complex genomic and epigenetic landscape that characterizes DIPG as a unique entity, distinct from high-grade gliomas that arise in adulthood or at other neuroanatomical locations.

Perhaps most revolutionary in our understanding of the molecular mechanisms of DIPG pathogenesis has been the discovery that most (78%) DIPG tumors contain a specific, recurrent mutation in one of two genes encoding histones, key chromatin components that play important roles in regulating the epigenome [49, 50]. About 60-75% of these identified mutations occur in *H3F3A* (H3 histone, family 3A), a gene encoding histone variant H3.3, which replaces histones as necessary in the event of nucleosome disruption [51, 52]. Another identified recurrent mutation, mutually exclusive of mutant *H3F3A*, is known to affect histone variant H3.1, usually *via* an alteration in *HIST1H3B* (histone cluster 1, H3b) and very rarely in *HIST1H3C* (histone cluster 1, H3c) [53, 54]. Histone H3.1 plays a role in packaging newly synthesized DNA during S-phase [52]. In both H3.3 and

H3.1, the alteration is a specific missense mutation resulting in the substitution of lysine with methionine at position 27 (K27M) [49, 50]. This position is located within the N-terminal tail of the histone; importantly, post-translational modification of histone tails by methylation, acetylation or ubiquitylation of lysine residues is known to mediate the epigenetic regulation of gene expression and alter nucleosome structure. The addition or deletion of such modifications is facilitated by "writers" and "erasers" and result in altered interactions with transcription modifiers, as mediated by "readers." While mutations in writers, erasers or readers have recently been implicated in other oncogenic pathways, it appears that in DIPG the epigenetic aberrancy directly results from mutation of the histone alone [45, 53-55]. Indeed, DIPG represents the first identified example of the implication of a histone mutation in oncogenesis and disease [56-59]. Remarkably, the H3K27M mutation is heterozygous in 100% of DIPG cells, and remains so in both treatment-naïve and treatment-exposed samples, and within low- and high-grade tumor regions [45, 49, 53-55, 60]. This strongly suggests clonal selection, emphasizing the robust selective advantage that the H3K27M mutation likely confers.

Understanding the changes in the epigenetic landscape and gene expression initiated by the H3K27M substitution is now the current focus of intense research. H3K27M is a gain-of-function mutation that exerts broad transcriptional effects by disrupting lysine trimethylation at histone H3 lysine 27. The H3K27 trimethylation mark (H3K27me3) is normally established by methyltransferase activity of Polycomb Repressive Complex 2 (PRC2) *via* nucleosome interaction with the EED subunit of PRC2 [61, 62]. PRC2 is known to silence gene transcription in order to regulate stem cell differentiation in development, and mutations in subunits of PRC2 itself have been previously implicated in oncogenesis [63]. The substitution of lysine with methionine at position 27 interferes with the enzymatic activity of PRC2 *via* interaction of the K27M mutant with the EZH2 subunit, leading to robust, aberrant derepression of gene transcription normally silenced by PRC2 [61]. In DIPG samples, this occurs in the absence of altered EZH2 expression [64, 65]. Strikingly, while the mutant H3 variants represent only a fraction (~3-17%) of the total histone H3 population in DIPG cells, the heterozygous *H3F3A* mutation nonetheless exerts a dominant-negative effect observed in human DIPG samples as well as in *in vivo* and *in vitro* models; it initiates a global pattern of reduced histone trimethylation or dimethylation of the entire population of H3K27 across all H3 variants including wildtypes, resulting in upregulated expression of genes associated with H3K27me3 loss [61, 64-66]. Furthermore, ectopic H3K27M expression in other cell types is sufficient to reduce overall levels of H3K27me3 *in vitro* [61, 65]. Gain of H3K27me3 at certain gene loci, including those of known tumor suppressors (*e.g.*, *p16Ink4a*), also occurs in association with the *H3F3A* mutation, leading to decreased expression of these genes; this suggests another potential mechanism by which altered transcriptional regulation could contribute to tumorigenesis [65, 66]. Additionally, there is an observed increase in H3K27me3 in regions simultaneously trimethylated at H3K4, a mark that usually promotes active gene expression [65]. This contradictory combination of "silent" and "active" marks signifies that the associated target genes are "bivalent"—*i.e.*, uniquely primed for expression upon H3K27 trimethylation loss—and indeed, these target genes were found to be involved in oncogenic as well as developmental pathways [65].

H3K27M-mutated primary DIPG tumor specimens also exhibit an altered DNA methylation profile with overall reduction in DNA methylation compared to H3K27-wildtype tumors, suggesting an additional mechanism of epigenetic dysregulation leading to altered gene expression and tumorigenesis [66, 67]. Discrete patterns of DNA methylation have been identified that reflect altered transcription of genes involved in Myc or Hh signaling, suggesting distinct molecular subgroups involving aberrant activation of either pathway [67]. Of note, DNA hypomethylation and changes in H3K27me3 may represent related processes, as DNA hypomethylation is known to shift the targeting of PRC2 complexes interacting with chromatin, leading to altered H3K27me3 distribution and downstream changes in transcriptional activity [68]. In DIPG, some but not all genes that exhibit DNA hypomethylation at their promoter regions are also associated with loss of H3K27me3, suggesting that if there is a link between these two processes in DIPG, it is not complete [66]. Regardless, both processes likely act as major epigenetic drivers of gene upregulation in DIPG, as the vast majority (74%) of upregulated genes exhibited reduced H3K27me3 either alone or together with DNA hypomethylation, while downregulated genes did not demonstrate corresponding changes in H3K27me3 or DNA methylation [66]. Thus, DNA hypomethylation and loss of H3K27me3 in DIPG alter transcriptional activity in a parallel and perhaps partially related manner, shifting the epigenetic landscape towards a less differentiated, more tumorigenic state. DIPG also exhibits increased mRNA expression of Ten Eleven Translocation (*TET*) 1 and 2; these enzymes play roles in the conversion of repressive methylation marks at the 5-position of cytosine to 5-hydroxymethylcytosine, which itself is a mark associated with active transcription [69]. This pattern of decreased cytosine methylation and increased 5-hydroxymethylcytosine marks in the setting of increased *TET1* and *TET2* expression likely contribute to the pathologic gene expression changes observed in DIPG.

While evidence of broad transcriptional alterations occurring in such a vast majority of DIPG tumors provides compelling support for epigenetic mechanisms as a key drivers of DIPG pathogenesis, the cellular and molecular context in which the H3K27M mutation results in tumorigenesis is not yet fully clear. New and ongoing efforts to elucidate such mechanisms will be key to the identification of novel therapeutic targets. Indeed, epigenetic modifying agents, such as demethylase or deacetylase inhibitors, are emerging as a promising class of agents. H3K27 is known to be demethylated by JMJD3 and UTX [70], and pharmacological inhibition of JMJD3 with GSKJ4 was recently found to selectively decrease cell viability in H3K27M-mutated tumor cells *in vitro* in a dose-dependent manner [71]. Additionally, treatment with GSKJ4 reduced tumor growth and increased survival in *in vivo* H3K27M-

mutant orthotopic xenograft brainstem tumor models, with treated tumors exhibiting significantly increased levels of H3K27me3 overall, as expected [71]. Additionally, histone deacetylase (HDAC) inhibitors have also emerged from a chemical screen as effective agents for reducing cell viability in a panel of 16 patient-derived DIPG cultures; the multi-HDAC inhibitor panobinostat showed particular promise, potently reducing both tumor cell viability *in vitro* and orthotopic xenograft tumor growth *in vivo*. These effects were associated with increased H3 acetylation and H3K27 trimethylation, and in fact panobinostat was found to act synergistically with the demethylase inhibitor GSKJ4 [72]. These data provide evidence that pharmacological agents targeting epigenetic mechanisms of DIPG pathogenesis represent a promising therapeutic avenue for further investigation.

Adding a further layer of complexity, the H3.3 and H3.1 K27M mutations also associate with distinct genetic alterations, which may act in concert with dysregulated epigenetic mechanisms to promote tumorigenesis. The H3.1 and H3.3 mutations also segregate by clinical features, highlighting that these represent specific molecular subgroups: H3.1K27M mutant tumors appear to occur at a younger age (median age at diagnosis is 4-5 years), are more frequent in females and are associated with a slightly longer median survival (15 months) relative to H3.3K27M mutant tumors [45, 53-55]. The H3.1K27M substitution is uniquely associated with a recurrent gain-of-function somatic mutation in *ACVR1* (activin A receptor, type 1). *ACVR1* encodes the serine threonine kinase ALK2, and constitutive activation of ALK2 in the setting of mutant *ACVR1* leads to aberrant activation of BMP signaling, with increased downstream phosphorylation of SMAD1, 5 and 8 leading to transcription of growth-promoting genes [45, 53-55]. BMP signaling plays important roles in the regulation of proliferation and differentiation in normal tissue development [73]. While the somatic *ACVR1* mutation is relatively unique to DIPG, analogous germline *ACVR1* mutations are known to cause fibrodysplasia ossificans progressiva (FOP), a heritable musculoskeletal disorder [74, 75]. Interestingly, FOP patients do not demonstrate any particular vulnerability to cancer, and the *ACVR1* mutation in isolation does not lead to tumorigenesis *in vivo* [45, 55]. However, quite rarely, the *ACVR1* mutation is observed in tumors in the absence of H3.1K27M [55]. Such evidence implies complex relationships between *ACVR1*, H3.1K27M, and oncogenesis; it may be that mutant *ACVR1* acts in concert with other genetic or epigenetic alterations to confer a selective advantage, but this interaction remains incompletely understood.

By contrast, the H3.3K27M mutation tends to be associated with somatic loss-of-function mutations in the tumor suppressor *TP53*; this combination is exhibited by 60-80% of *H3F3A*-mutated pediatric high-grade glioma samples [49, 52, 54, 76]. Mutually exclusive mutations in *PPM1D*, downstream of p53 in the apoptosis pathway, have also been observed in H3.3K27M-mutated DIPG in 9-23% of cases [45, 55, 77]. H3.3K27M is also uniquely associated with amplifications or gain-of-function mutations in *PDGFRA* (alpha-type platelet-derived growth factor receptor, a component of the Ras/PI3K/AKT pathway) not observed in adult disease [78-83]. Drawing subtype nomenclature from the adult glioblastoma literature, this combination has been described as an "oligodendroglial subtype" of DIPG that appears to be the most clinically aggressive and resistant to radiotherapy [82]. Unfortunately, there is a considerable degree of heterogeneity in *PDGFRA* amplification within tumors, presenting a serious obstacle to the therapeutic strategy of using receptor tyrosine kinase inhibitors for DIPG given the presence of subpopulations of resistant cells that would lack the target [81].

Other genetic aberrations observed in DIPG include a relatively higher frequency of chromosome gains in 1q, 2q, 8q, and 9q and losses in 16q, 17p and 20p, a pattern that distinguishes DIPG from adult high-grade gliomas and pediatric gliomas of other neuroanatomical compartments [81, 84-86]. This highlights the unique selective pressures of the microenvironment at the precise location and stage of postnatal neural development at which DIPG arises. A recent study observed overexpression of the gene encoding developmental transcription factor *PAX3* (paired box 3) in 40% of examined human DIPG samples, characterizing a distinct molecular subgroup [87]. Additionally, amplifications of the cell-cycle control genes *CDK4*, *CDK6*, and the D-type cyclins (*CCND1*, *2* and *3*) have been found in up to 30% of tissue samples [80, 81, 85]. This suggests a possible role for *CDK4/6* inhibitors in DIPG therapy, and indeed a preclinical study of the *CDK4/6* inhibitor PD-0332991 induced cell-cycle arrest and increased survival in the PDGF-B;*Ink4a-ARF*$^{-/-}$ genetic mouse model of DIPG, but not the PDGF-B;p53$^{fl/fl}$ model [88]. In addition to suggesting CDK4/6 inhibition as a viable therapeutic approach, this study also emphasizes the key role that molecular profiling will likely play in disease stratification, particularly as subtype-specific drug development emerges from new preclinical studies. While the genomic and epigenomic landscape of DIPG is complex and appears to comprise several molecular subgroups, there is a striking degree of homogeneity of mutational profiles and DNA methylation patterns within these subgroups, highlighting the great potential for development of future targeted therapies.

Finally, a recent discovery that cortical neuronal activity promotes the growth of many high-grade glioma types, including DIPG, may indicate a future approach to DIPG therapeutics. Venkatesh and colleagues found that neuronal activity-regulated secretion of the synaptic protein neuroligin-3 induced PI3K-mTOR signaling in patient-derived DIPG cells, leading to increased rates of proliferation and growth; furthermore, neuroligin-3 expression was found to be inversely correlated with survival in human high-grade glioma [89]. These data suggest that targeting the secretion or activity of neuroligin-3 may represent previously unexplored therapeutic avenues for DIPG, and underscore the importance of recognizing the interactions between DIPG cells and their microenvironment towards a better understanding of the mechanisms of the disease for future pharmacologic development.

SUMMARY

DIPG is an aggressive and incurable brain cancer, representing the leading cause of pediatric brain tumor death. Growing diffusely in the ventral pons, it causes severely disabling neurologic symptoms that progressively destroy control and coordination of the face, pharynx and body. Surgical resection is not possible, radiation therapy results in merely transient stabilization of symptoms, and multiple chemotherapy trials designed after therapeutic strategies for adult glioma have not proven successful to date. Novel, effective therapies are urgently needed. Increased availability of tissue samples for preclinical investigation, alongside the development of experimental model systems and the advent of next-generation sequencing tools, now provide important tools to guide future drug development. Emerging research characterizes DIPG as a biological entity distinct from adult glioma and pediatric non-brainstem glioma, and its unique pathophysiological mechanisms reflect the particular selective pressures of the DIPG microenvironment at a precise location and timepoint in postnatal neurodevelopment. Advancements in our understanding of the broad transcriptional changes underlying DIPG pathophysiology reveals epigenetic dysregulation as a fascinating core driver of the disease, with accompanying genetic mutations and alterations likely contributing to pathogenesis in ways yet to be fully understood. Ongoing and future efforts to further define the cellular and molecular mechanisms mediating DIPG oncogenesis hold great promise for identifying new therapeutic targets and effective pharmacological approaches for the treatment of this devastating disease.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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