Epigenetic-Targeted Treatments for H3K27M-Mutant Midline Gliomas

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Abstract

Diffuse intrinsic pontine glioma (DIPG) is a lethal midline brainstem tumor that most commonly occurs in children and is genetically defined by substitution of methionine for lysine at site 27 of histone 3 (H3K27M) in the majority of cases. This mutation has since been shown to exert an influence on the post-translational epigenetic landscape of this disease, with the loss of trimethylation at lysine 27 (H3K27me3) the most common alteration. Based on these findings, a number of drugs targeting these epigenetic changes have been proposed, specifically that alter histone trimethylation, acetylation, or phosphorylation. Various mechanisms have been explored, including inhibition of H327 demethylase and methyltransferase to target trimethylation, inhibition of histone deacetylase (HDAC) and bromodomain and extraterminal (BET) to target acetylation, and inhibition of phosphatase-related enzymes to target phosphorylation. This chapter reviews the current rationales and progress made to date in epigenetically targeting DIPG via these mechanisms.

Keywords

Diffuse intrinsic pontine glioma · DIPG · H3 K27M · DMG · Trimethylation · Phosphorylation · Acetylation · Demethylase · HDAC · BET

6.1 Introduction

Diffuse intrinsic pontine glioma (DIPG), now characterized by the World Health Organization (WHO) as diffuse midline glioma of the brainstem with H3K27M mutation, is a devastating disease that predominately affects young children [1]. Although rare, DIPG is one of the leading causes of cancer-related morbidity and mortality in children, and despite best practice of radiation therapy, the tumors inevitably progress with average survival of less than 12 months [2–4]. Therefore, there is a great need to investigate possible other therapeutic modalities that may have a greater impact on prognosis.

The establishment of stereotactic surgery in recent years as a safe approach in well-selected patients has allowed us to explore the molecular nature of DIPG, which historically was viewed as unnecessary by some due to the lethality of this diagnosis. Molecular analyses of the biological tissue has uncovered the vast majority of these tumors possess a histone H3 gene mutation that most commonly occurs at either H3.1

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(HIST1H3B/C) or H3.3 (H3F3A) and results in a H3K27M mutation (histone 3 lysine substitution for methionine at site 27 (H3K27M)) [5, 6]. This specific mutation has since been established to prognosticate a worse prognosis in pediatric brain tumors overall [7]. In the largest pretreatment biopsy study to date, mutations in these genes were detected by sequencing in 57 of 62 (92%) subjects diagnosed with presumed DIPG based on radio-clinical parameters [6]. These findings reaffirmed the previous clinical reports of the H3K27M mutation being prototypical of DIPG and confirmed its pathognomonic significance [5, 8]. It was this accumulating evidence that eventually gave way to WHO reclassification of DIPG tumors in 2016 [1]. Not only have these relatively recent advancements in our genetic understanding of DIPG uncovered the growing relevance of the H3K27M mutation in these tumors, they have also uncovered a number of novel epigenetic modifications to residues of the H3 histone tail that likely contribute to the tumorigenic burden of this disease [9–11].

6.2 The Histone and Epigenetic Landscape

Understanding the biology of the H3K27M mutation, and the subsequent posttranslational modifications (PTMs), is required to develop effective targeted therapies for DIPG. In brief, histones are the primary protein component of chromatin in eukaryotic cells, synthesized during DNA synthesis. DNA is packaged around a histone core made of eight histone bodies, and this octamer is able to help regulate DNA transcription activity based on selective and reversible PTMs found on the tails of the histone bodies [12, 13]. These PTMs effectively act as molecular switches for the genes and their transcription, which may be altered in cancers based on the presence of aberrant PTMs [14]. At the H3 N-terminal tails, PTMs include methylation at lysine and arginine sites, acetylation at lysine sites, and phosphorylation at serine sites [14–17]. There is a paradigm within the histone literature to refer to enzymes that facilitate/catalyze these PTMs as "writers," e.g., methyltransferases and histone acetyltransferases (HATs), and to enzymes that act to remove PTMs as "erasers," e.g., demethylases and histone deacetylases (HDACs) [18]. Finally, there is another set of enzymes termed "readers," which recognize and translate these PTMs into cellular programs [19]. The rationale in targeting these epigenetic enzymes, primarily that of writers and erasers, is to alter the epigenetic landscape of DIPG tumors in such a way their associated tumorigenic tendencies can be silenced or even reversed.

Biologically, the H3K27M mutation in DIPG tumors results in significant epigenetic changes in methylation and acetylation at multiple H3 sites [20, 21]. There is effectively a global loss of H3K27 trimethylation (H3K27me3) when the H3K27M mutation is present, which was the earliest detected, and most defined, epigenetic change in DIPG and observed in 100% of the H3K27M cases (Fig. 6.1) [6, 9, 15, 22]. This epigenetic change, along with other PTMs such as acetylation at H3K27 (H3K27ac) and alterations in phosphorylation at adjacent H3S28 (pH3S28), is theorized to accommodate and drive the tumorigenic burden of DIPG [15–17, 23]. Baker and colleagues [21] recently demonstrated that targeted H3K27M depletion restores H3K27me3, increases differentiation, and extends latency of diffuse intrinsic pontine glioma growth in vivo, highlighting the significance of this mutation and its consequent epigenetic alterations. Given the lack of alternative mutations characteristic for this type of tumor, targeting the altered epigenetic landscape presents a worthy therapeutic approach to ameliorate the dismal prognosis of DIPG (Table 6.1), with one avenue being the reversal of associated epigenetic changes such as H3K27me3 restoration.

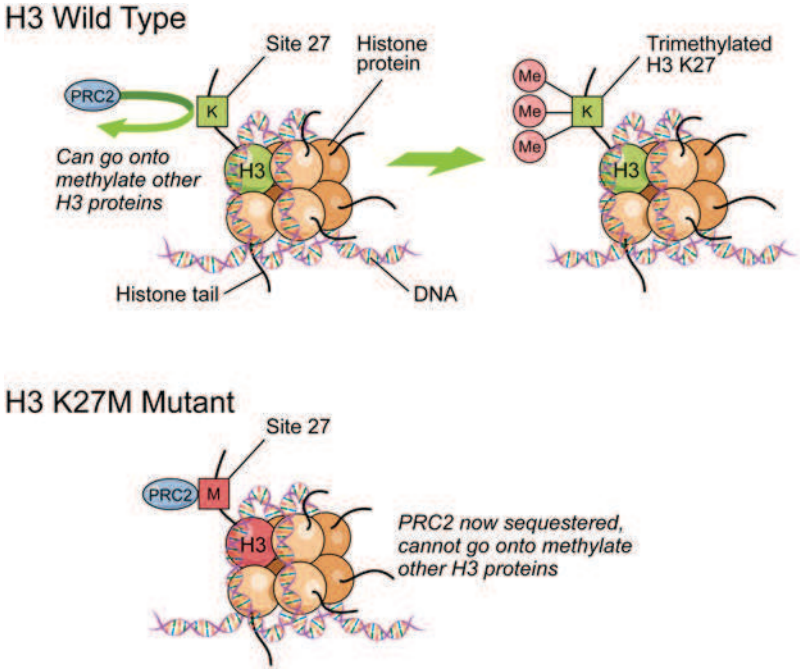

Fig. 6.1 Epigenetic consequence of the H3 K27M mutation. In the H3 wild type, the PRC2 complex is able to (tri-) methylate the lysine (K) at site 27 of the histone tail of histone 3 (H3 K27). When the H3 K27 is substituted for a methionine (M), i.e., the H3 K27M mutation, the PRC2 complex is sequestered, meaning that it is no longer available to methylate other H3 proteins at the K27 site, resulting in global loss in H3 K27 trimethylation

6.3 H3K27M and Lysine Methylation

6.3.1 Molecular Biology of Lysine Methylation

The specific residue at which lysine methylation occurs dictates the biological consequence of the PTM, as across the H3 body there are distinct transcriptional sites. Methylation at H3K4 and K36, for instance, are typically associated with transcriptionally active chromatin, whereas methylation at H3K9 and H3K27 are more associated with transcriptionally repressed chromatin and gene expression [20]. With respect to DIPG, H3K27 is primarily methylated by histone-lysine *N*-methyltransferase enhancer of zeste homolog 2 (EZH2), a component of the polycomb repressive complex 2 (PRC2), and is demethylated in its trimethylated form by the KDM6 subfamily K27 demethylases jumonji domain-containing 3 (JMJD3) and ubiquitously transcribed tetratricopeptide repeat, X chromosome (UTX) [24–27]. When trimethylated (H3K27me3), this H3 PTM is recognized by the PRC1 complex which represses transcription by multiple mechanisms, including ubiquitination of H2A at K119 and compaction of chromatin [18]. The loss of H3K27me3 due to the inhibition of PRC2 activity by the H3K27M mutation in DIPG cells therefore alleviates transcription repression of potential oncogenes to increase tumorigenic burden [9]. Therefore, reversal or restoration of the H3K27me3 loss, by either augmenting repressed PRC2 methyltransferase activity or inhibiting JMJD3/UTX demethylase activity, is a potentially targeted therapeutic approach for H3K27M tumors.

6.3.2 Targeting Methylation

6.3.2.1 Demethylase Inhibition

To date, the attempts to target the loss of H3K27me3 in DIPG have focused primarily on inhibiting demethylase activity. David James and colleagues [28] reported the first, and to date only, evidence of how directly targeting lysine methylation in DIPG can be of therapeutic benefit by

Table 6.1 Details of relevant publications targeting the H3K27 epigenome of the H3K27M-mutant midline gliomas

Study	Location	Epigenomic target	Intervention	Mechanism	Diagnosis (es)	Primary conclusion(s)
Hashizume et al. 2014 [28]	Chicago, USA	Lysine methylation	GSKJ4	Demethylase (JMJD4) inhibitor	DIPG	Favorable antitumor effects in vitro and in vivo
Mohammad et al. 2017 [31]	Copenhagen, Denmark	Lysine methylation	GSK343 and EPZ6438	Methyltransferase (EZH2) inhibitor	DIPG	Favorable antitumor effects in vitro and in vivo
Wiese et al. 2016 [35]	Goettingen, Germany	Lysine methylation	EPZ6438	Methyltransferase (EZH2) inhibitor	DIPG and H3K27M-wild-type glioma	Similar antitumor effects in vitro between DIPG and H3K27M-wild-type glioma
Grasso et al. 2015 [44]	Portland/ Stanford, USA	Lysine acetylation	Panobinostat and vorinostat	HDAC inhibitor	DIPG	Favorable antitumor effects in vitro with H3K27me3 restoration
Hennika et al. 2017 [49]	Durham, USA	Lysine acetylation	Panobinostat	HDAC inhibitor	DIPG and H3K27M-wild-type glioma	Similar antitumor effects in vivo between DIPG and H3K27M-wild-type glioma
Piunti et al. 2017 [23]	Chicago, USA	Lysine acetylation	JQ1	BET inhibitor	DIPG	Favorable antitumor effects in vitro and in vivo
Zhang et al. 2017 [56]	Guangzhou, China	Lysine acetylation and methylation	JQ1 and EPZ6438	BET inhibitor and methyltransferase (EZH2) inhibitor	DIPG	Favorable antitumor effects in vitro and in vivo

their work testing GSKJ4 in vitro and in vivo. GSKJ4 was developed by GlaxoSmithKline as the first small-molecule catalytic site inhibitor that was selective for the H3K27me3-specific JMJ subfamily and was first tested in T-cell acute lymphoblastic leukemia with encouraging results [27, 29]. Of great interest was in addition to reduced cell proliferation in DIPG, James and colleagues [28] demonstrated that the GSKJ4 downregulated genes experience restoration of H3K27me3 upon treatment, signaling its translational therapeutic potential.

James and colleagues [28] found that when exposed to GSKJ4, multiple H3K27M-mutant DIPG cell lines demonstrated evidence of decreased cell viability, increased S-phase arrest, increased apoptosis, and decreased clonal growth when compared to wild-type gliomas, H3G34V-mutant gliomas, and isogenic human astrocyte cell lines. Specificity for JMJD3 in DIPG was confirmed by siRNA depletion studies, with similar results found only when JMJD3 was targeted, but not UTX. In orthotopic H3K27M xenograft models, GSKJ4 resulted in not only decreased cell proliferation and increased apoptotic activity within the tumor but also prolonged survival compared to H3K27-wild-type models. Most relevant, postmortem examination of these tumors demonstrated significant H3K27me3 restoration confirming on target drug effects with GSKJ4 treatment. To date however GSKJ4 is not FDA-approved, and thus despite these promising results, there has been no clinical trial established to investigate how these effects could translate in DIPG patients, or any other pediatric cancer for that matter.

6.3.2.2 Methyltransferase Inhibition

An extension of targeting lysine methylation in DIPG has been inhibiting the EZH2 methyltransferase component of the PRC2 complex. Although this may initially present as counterintuitive to the paradigm of H3K27me3 restoration, it derives from the observation that there are still low levels of H3K27me3 in H3K27M mutant tumors which EZH2 is responsible for [15]. The significance of this is that the retained trimethylated regions are posited to contribute to the transcriptional program specific for K27M-mutant DIPG by means of increasing EXH2 affinity at oncogene promoter regions [30, 31]. It has been proposed that this locus-specific retention of H3K27me3 acts together with the more universal decline of H3K27me3 to promote tumorigenesis by a concerted silencing of tumor suppressor genes, such as with EZH2 and silencing of p16 [9, 23, 32]. The precise mechanism of this phenomenon remains to be fully elucidated.

Mohammad et al. [31] reported that small-molecule inhibition of EZH2 by GSK343 and EPZ6438 [33, 34] resulted in favorable antitumor effects in vitro and in vivo using patient-derived H3K27M-mutant DIPG cell lines, with the expected loss of H3K27me3 at sites that had originally retained methylation despite having the H3K27M mutation. However, the significance of this aspect in targeting methylation modifications in DIPG remains unclear, for these implications are not universal. Wiese et al. [35] reported that in their in vitro studies, using one of the same EZH2 inhibitors EPZ6438, this did not result in any significant cytotoxic difference in H3K27M-mutant DIPG and H3K27-wild-type pediatric glioma cell lines. These differences may be explained in part by varying EZH2 expression levels in DIPG.

A degree of caution is recommended when considering the targeting of EZH2 until the role of the retained H3K27me3 in DIPG is better distinguished from that of the lost H3K27me3. This is because although overexpression of EZH2 may restore H3K27me3 at lost H3 sites in DIPG, it also has been correlated with tumor cell proliferation and invasive growth in multiple adult cancers [36], including brain tumors [37]. Admittedly, even though evidence in pediatric brain tumors is more scarce, anecdotal reports of similar trends in medulloblastoma by Taylor and colleagues [38] would indicate alternative pathways that increase tumorigenesis independent of H3K27me3 restoration following EZH2 inhibition is not an impossible scenario in the setting of DIPG.

6.4 H3K27M and Acetylation

6.4.1 Molecular Biology of Lysine Acetylation

Acetylation of H3 lysine sites reduces the charged attractions between DNA and the histone core within the nucleosome, resulting in greater exposure of DNA in an open chromatin structure which becomes more susceptible to active transcription [39]. Acetylation is achieved by "writer" HATs, and this occurs in natural antagonism to "eraser" HDACs, which remove this PTM. Typically, HATs associate themselves with active genes of transcription, and HDACs associate themselves with inactive genes. The relevance of H3 acetylation is that it is able to recruit specific bromodomain and extraterminal (BET) proteins, which contain bromodomain-containing 4 (BRD4), that recognize acetylated H3 lysines including H3K27ac [40]. Subsequently, transcription initiator cofactors are recruited to respective promoters and ultimately lead to active transcription after phosphorylation by RNA Pol II [41]. Although not clear, it has been suggested that aberrant acetylation at H3K27 in the presence of H3K27M contributes to DIPG tumorigenesis by dysregulating the PRC2 balance between the oncogenes involved in maintaining an undifferentiated state versus oncogenes involved in the differentiation process [23, 42, 43]. There is evidence to suggest that increase in H3K27ac results in partial restoration of the H3K27me3 loss in DIPG by means of alleviating H3K27M-induced PRC2 inhibition, leading to investigations if H3K27ac can be augmented

Table 6.2 Details of relevant clinical trials targeting the H3K27 epigenome of the H3K27M-mutant midline gliomas

Clinical trial identifier	Epigenomic target	Intervention	Phase	Organizing site	Diagnosis	Start date	Finish date	Target size	Current status[a]
NCT02717455	Lysine acetylation (HDAC)	Panobinostat	I	St. Jude Children's Research Hospital, USA	DIPG	June 2016	October 2019	40	Recruiting
NCT03566199	Lysine acetylation (HDAC)	Panobinostat by CED	I/II	University of California, San Francisco, USA	DIPG	May 2018	September 2020	24	Recruiting
NCT03632317	Lysine acetylation (HDAC)	Panobinostat with everolimus	II	University of Michigan, USA	DIPG	June 2019	September 2025	32	Recruiting
NCT02420613	Lysine acetylation (HDAC)	Vorinostat with temsirolimus	I	M.D. Anderson Cancer Center, USA	DIPG	October 2015	October 2020	18	Recruiting
NCT01189266	Lysine acetylation (HDAC)	Vorinostat	I/II	National Cancer Institute, USA	Pediatric high-grade glioma	August 2010	November 2020	80	Active, not recruiting
NCT02296476	Lysine acetylation (BET)	MK-8628 (OTX015)	II	Private, USA	Glioblastoma	October 2014	October 2015	12	Terminated due to lack of activity

[a]As of June 2019

therapeutically to rescue the H3K27M-induced H3K27me3 loss phenotype and result in antitumor effect for DIPG [44, 45].

6.4.2 Targeting Acetylation

6.4.2.1 HDAC Inhibition

The use of HDAC inhibitors has emerged in the cancer field as a potent potential therapy in many cancer types [46, 47]. Correspondingly, there has been much hope that such promising responses could be translated for DIPG patients, in particular given its association with H3K27me3 restoration.

Panobinostat (LBH589) is the most investigated HDAC inhibitor in the DIPG world, with it being a Food and Drug Administration (FDA)-approved nonselective small molecule trialed originally in hematological malignancies with encouraging clinical outcomes [48]. Grasso et al. [44] screened 83 compounds across 14 patient-derived DIPG cell lines and observed sensitivity of multiple cell lines to HDAC inhibitors, with panobinostat the most effective in terms of reducing cell viability. These results showed for the first time since the characterization of the H3K27M mutation that this class of epigenetic therapy may also be of benefit in DIPG, and the epigenetic rescue of H3K27me3 by increasing H3K27ac was validated in their in vitro studies; however the mechanism by which this happens is currently not known [45]. Subsequent in vivo modelling by Grasso et al. [44] showed that in orthotopic H3K27M-wild-type DIPG xenografts, systemic panobinostat led to both tumor volume reduction and prolonged survival compared to vehicle-treated controls. Unfortunately, they did not report the outcomes of in vivo H3K27M-mutant xenografts. However, they did demonstrate synergy between panobinostat and demethylase inhibitor GSKJ4 in H3K27M-mutant DIPG, highlighting the potential for combinatorial therapy and epigenetic interplay to lead to greater therapeutic impact in DIPG.

Next, Becher and colleagues [49] sought to extend the findings of Grasso et al. [44] by utilizing a genetically engineered mouse model (GEMM) of DIPG driven by H3.3-K27M expression with known global loss of H3K27me3, which was thought to be another biological model in which therapeutic hypotheses could be validated. Although they did observe increased H3K27ac in response to the therapy, they also observed multiple results that would temper the implications of the prior study. First, panobinostat showed efficacy against all patient-derived and murine DIPG and brainstem glioma cell lines, irrespective of H3 mutation status. Second, in vivo, extended consecutive daily treatment of both genetic and orthotopic xenograft models with 10 or 20 mg/kg systemic panobinostat consistently led to significant toxicity. Third, when reduced to well-tolerated doses of panobinostat, there was no overall survival difference compared to vehicle-treated GEMM models. Taken collectively, the work by Becher and colleagues [49] suggested that although there may be promise in HDAC inhibition to treat DIPG, this may not be specific to the epigenetic landscape of DIPG and, furthermore, compounded by the universal importance of histone acetylation in normal physiology, that off-target effects following systematic administration cannot be discounted.

Nevertheless, due to the initial encouraging results of panobinostat found by Grasso et al. [44], as well in clinical trial of other cancers [50, 51], a number of clinical trials (clinicaltrials.gov) have been established to investigate whether or not the theorized epigenetic targeting benefits of HDAC inhibition in DIPG can be translated into the clinic (Table 6.2). In brief, NCT027171455 (previously identified as NCT02899715) is a Phase I trial and was the first trial of panobinostat in DIPG spurred by the results of Grasso et al. [44], investigating safety and feasibility of systemic delivery of panobinostat by means of dose escalation; NCT03566199 is a Phase I/II trial evaluating panobinostat in nanoparticle formulation delivered to the brainstem by means of convection-enhanced delivery for DIPG patients with history of radiation therapy; and NCT03632317 is a Phase II trial investigating systematic delivery of 30 mg/m^2 panobinostat in combination with

3 mg/m^2 kinase inhibitor everolimus in newly diagnosed DIPG. All three trials are US-based and are currently active and recruiting at the time of writing.

It is worth noting finally that another FDA-approved HDAC inhibitor vorinostat (suberanilohydroxamic acid) had also been identified as a therapy of interest in DIPG from a 51-drug-screen as 1-of-8 nonmolecularly charged, lipophilic, and relatively small-sized drugs likely to passively diffuse through the blood brain barrier (BBB) [52]. Although Grasso et al. [44] showed vorinostat possessed antitumor effects in DIPG models, these effects were not as pronounced as that of panobinostat, with concerns emerging for how effectively the drug did penetrate the BBB. Nevertheless, there are two active US-based clinical trials examining the therapeutic benefit of vorinostat in DIPG – NCT02420613 is a Phase I trial investigating vorinostat in combination with kinase inhibitor temsirolimus in newly diagnosed and progressive DIPG, and NCT01189266, a Phase I/II study investigating vorinostat in combination with local radiation therapy in newly diagnosed DIPG only.

6.4.2.2 BET Inhibition

The BET bromodomain of BRD4 recognizes H3K27ac and binds to the exposed chromatin structures caused by the acetylation to activate transcription [41, 53]. It has been hypothesized that competitive binding of BET inhibitors to the bromodomain pocket can displace the BRD4 from active chromatin of H3K27ac in DIPG cells, therefore resulting in the inactivation of pertinent oncogene transcription [41, 54].

Piunti et al. [23] investigated in their works the application of BET inhibitor JQ1 [55] to H3K27M-mutant DIPG, which similar to other tested epigenetic therapies was first evaluated in the setting of hematological malignancies with encouraging in vitro antitumor effects and downregulation of specific oncogenes. Their in vitro work in patient-derived DIPG cell lines demonstrated significant dose response in cell viability, which was accompanied by the expected decrease in H3K27ac. Corresponding to transcription downregulation, RNA sequencing revealed that after JQ1 treatment, the genes transcriptionally modulated by JQ1 showed H3K27M, active transcription marks, and BRD2/4 occupancy around their promoters indicating they are direct targets affected by JQ1 treatment. In terms of in vivo results, they observed favorable tumor shrinkage and prolonged survival in orthotopic DIPG xenograft models following administration of JQ1, with the corresponding epigenetic change of H3K27ac reduction, compared to vehicle-treated controls. These findings were successfully recapitulated using another BET inhibitor (I-BET151). Finally, compared to the demethylase inhibitor GSKJ4, the authors observed significantly longer overall survival when using JQ1.

To date, there is no clinical trial established to investigate BET inhibition in DIPG, although we note such trials do exist for malignant adult glioblastoma (NCT02296476). One consideration to bear in mind moving forward is the potential of combinatorial epigenetic therapy in DIPG. With respect to BET inhibition, Zhang et al. [56] demonstrated combinational therapy with JQ1 and EZH2 inhibitor EPZ6438 resulted in synergistic in vitro antitumor effects in patient-derived H3K27M-mutant DIPG cell lines, as well as prolonged overall survival in orthotopic DIPG xenograft models when combined versus individual therapy. Therefore, future clinical trial designs may benefit from incorporating epigenetic therapy from more than one target PTM.

6.5 H3K27M and Phosphorylation

The final epigenetic histone PTM in H3K27M tumors to consider is that of phosphorylation. Although H3K27 residues do not typically undergo phosphorylation, the adjacent serine H3S28 can be phosphorylated in the presence of H3K27me3 [57]. It is thought that pH3S28 causes the displacement of the PRC2 complex from the H3K27me3 peptide, resulting in H3K27me3 loss and increased transcriptional activity of otherwise repressed oncogenes. It should be noted that the activation of these polycomb group target genes

likely depends on a multiple number of factors in addition to the pH3S28, including recruitment of specific transcription factors and other H3 epigenetic changes [58, 59]. Therefore, the target potential of phosphatase/kinase and dephosphatase enzymes remains an area that has yet to be explored in DIPG relevant to the H3K27 site. However, a recent study by Schramm et al. [60] identified serine/threonine protein phosphatase 2A (PP2A) as a top depleted hit in patient-derived DIPG cell lines using next-generation sequencing and validated its lethal potential by genetic knockdown of the PP2A structural subunit PPP2R1A. Furthermore, therapeutic phosphatase inhibition by LB-100 treatment resulted in more favorable antitumor and apoptotic effects in H3K27M-mutant versus wild-type patient-derived cell lines. The epigenetic relevance of this finding is that PP2A is known to interact with the serine residues across histone H3 such as H3S28 [61]. Therefore, pH3S28 may prove another worthy epigenetic target to consider for H3K27M-mutant DIPG in the future.

Looking forward, it is known that phosphorylation occurs at other H3 serine sites which themselves may have possible significance in the case of managing these H3K27M tumors. A Phase I trial of a selective aurora kinase inhibitor AT9283 in a series of solid pediatric brain tumors, including DIPG, showed decrease in phosphorylation at H3S10 in 17 of 18 patients treated at high dose, indicating a rescue role possibly [62]. Furthermore, phosphorylation at H3S31 has been linked with both protection of euchromatin from the spreading of pericentric heterochromatin and a role in marking some H3.3 for replacement with canonical histone H3, which could prove relevant in the context of mutated H3.3K27M [63].

6.6 Future Considerations

Major validation of these findings to date is required to justify epigenetic targeting as a viable and feasible approach in DIPG treatment. Concerns about macroscopic and molecular specificity, as well as capacity to overcome the BBB, are the primary translational barriers that impede the interpretation of how effective these epigenetic-targeted therapies can be in practice based on the current literature. Furthermore, whether or not combinatorial therapy, targeting multiple epigenetic changes associated with increased tumorigenic burden in DIPG is an avenue worthy of exploration.

Finally, as we continue to work towards a more robust molecular understanding to DIPG, surgical intervention to gain biological sample for analysis is not without its risks. Therefore, preference for a less invasive modality that can provide biological information about the tumor remains optimal. There is emerging evidence to suggest that a liquid biopsy, targeting biofluids, such as cerebrospinal fluid (CSF) and blood plasma, could be a feasible alternative for brain tumors in general [64, 65]. The promise of liquid biopsy of circulating nucleosomes focusing on H3K27, and even other H3 sites of known phosphorylation, could afford us an insight into the changes in the epigenetic landscape of DIPG and be used to follow response to various targeted treatments [66]. There have been encouraging results to date about the feasibility of this concept in other cancers, and the epigenetic landscape of DIPG would suggest this tumor type too may be amenable to noninvasive monitoring [67, 68].

6.7 Conclusions

The complete epigenetic landscape of H3K27M-mutant DIPG is still being elucidated. Nonetheless, targeting distinct epigenetic changes such as H3K27me3, H3K27ac, and pH3S28 has shown therapeutic potential in vitro and in vivo. However, as there likely remains a complex interplay between epigenetic parameters in the tumorigenic burden of DIPG, it is difficult to ascertain whether or not targeting one specific change in isolation will be sufficient to translate into clinically meaningful benefits. We wait with much anticipation for the finalization of multiple epigenetic (H3K27ac)-based clinical trials in DIPG to begin to address these translational unknowns.

Greater understanding of these molecular interactions will better inform us of how to best

target the collective, characteristic epigenome of DIPG. Furthermore, barriers such as tumor specificity and access to the tumor site will need to be overcome in order to fully evaluate how effective epigenetic-targeted therapies can be in DIPG patients, with the predicted emergence of liquid biopsy, a tool that will assist in bridging this bench-to-bedside divide.

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