



Diffuse midline glioma: review of epigenetics

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Abstract

Purpose Our understanding of diffuse midline glioma (DMG) biology inclusive of diffuse intrinsic pontine glioma has been revolutionized by the discovery of novel mutations on the tails of histone 3, leading to the reclassification in 2016 of ‘diffuse midline glioma, H3 K27M-mutant.’ Given the abundance of basic, translational, and clinical information put forth in recent years, a review of the epigenetics of diffuse midline glioma is warranted.

Methods Literature for the epigenetics of diffuse midline glioma published from 1989 to 2019 was reviewed by searching PubMed using the terms “diffuse intrinsic pontine glioma”, “pontine glioma”, or “midline glioma”. The final references list was generated on the basis of originality and relevance to the broad scope of our review.

Results The effects of H3K27M-mutation, while better understood, suggest multiple consequences on the chromatin landscape and DNA modification states, contributed to the progression of DMG. A rapid pace of translational development is occurring for epigenetic modifiers, and several classes of inhibitors have already made their way into clinical trial testing. As more agents become clinically accessible, immense effort is underway to understand the target effects, tumor penetration, and immune microenvironmental changes of epigenetic modification.

Conclusion We continue to seek a comprehensive understanding of the mechanisms that govern chromatin dysregulation and DNA modification in DMG, and in parallel we forge ahead with clinical testing of epigenetic modifiers. The determined efforts from bench to bedside, along with collaborative mindset and unified mission, will ultimately result in improved outcomes for DMG.

Keywords Epigenetic(s) · Histone(s) · H3K27M · Diffuse · Midline · Glioma

Genetics/Epigenetics of diffuse midline glioma (DMG)

Discovery of histone 3 mutations and emergence of new disease classification

Diffuse intrinsic pontine glioma (DIPG) is an aggressive tumor of the brainstem, and accounts for 75% of brain stem tumors in children [1]. Median survival of DIPG remains only 11.2 months despite decades of clinical trials [2]. Fortunately, our understanding of DIPG biology has been revolutionized by the discovery of novel mutations on the tails of histone 3 (H3) [3]. Histones are nuclear proteins that play an essential role in condensing DNA into nucleosomes and regulating chromatin [4]. The H3 family of proteins includes canonical H3.1 and variant H3.3, each with a protruding amino-acid tail which is subject to a number of post-translational modifications (PTMs) [5]. These PTMs, such as methylation and acetylation, have a range of effects

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on the epigenetic regulation of transcription and chromatin structure [6]. One such PTM, the trimethylation of lysine 27 on histone 3 (H3K27me3), is usually associated with gene repression [7]. Trimethylation of H3K27 is catalyzed by the enhancer of zeste homolog (EZH1 or 2) subunit of the Polycomb Repressive Complex (PRC2), and has been shown to maintain transcriptional silencing, allowing the preservation of cell identity [8].

Mutations in H3, specifically the lysine to methionine substitution at position 27 (H3K27M), were initially reported almost a decade ago and represented the first description of histone mutations in cancer [3, 9, 10]. These highly recurrent, somatic, non-synonymous mutations occur not only in ~80% of DIPGs (65% in *H3F3A*, and 15% in *HIST1H3B*), but in gliomas arising from other midline structures such as the thalamus or spinal cord, and confer a worse prognosis than wildtype cases [11–13]. Thus, in 2016 the World Health Organization (WHO) recognized a new entity of diffuse midline glioma, H3 K27M-mutant [12]. Functional studies have since shown H3K27M mutation to drive DMG growth in vitro and in vivo, suggesting that epigenetic misregulation promotes DMG tumorigenesis [14–16]. DMG H3K27M mutant tumors, despite carrying the H3K27M mutation on merely 5–17% of H3 histones, have a global loss of H3K27me3 and DNA methylation, ultimately leading to a loss of gene repression [10, 17]. However, the mechanism by which H3K27M confers these effects is still debated.

The role of polycomb repressive complex 2

Following the discovery of H3K27M, it was shown that H3K27M is a gain of function mutation which enables the sequestration and inhibition of PRC2 [10]. The lysine-to-methionine mutation targets the active site of SET-domain containing methyltransferases, such as that in EZH2, and effectively competes with native lysine. By doing so, it prevents the hydrolysis of the methyl-donor *S*-adenosylmethionine (SAM) [18]. This, as well as methionine's increased binding affinity for the active site of EZH2 relative to that of lysine, results in EZH2 being sequestered at the H3K27M site, inhibiting the complex and preventing global methylation at cognate residues. In support of this, a recent report by Diehl et al. demonstrated that the presence of bivalent dinucleotides containing native H3K27me3 plays a key role in the inhibition of PRC2 [19]. Within the PRC2 complex, the binding of H3K27M to the catalytic subunit EZH2, and of H3K27me3 to the allosteric activator EED, work cooperatively to inhibit the PRC2 complex. The binding of H3K27me3 to EED increases the affinity of PRC2 to the adjacent nucleosome (H3K27M), and thus further enables the sequestration of PRC2 to the chromatin site [19].

Although this was the first proposed hypothesis of H3K27M mediated effects, recent in vivo studies such as

those by Piunti et al. have shown that, in contrast to the hypothesis that H3K27M sequesters PRC2, the localization of H3K27M is inversely correlated with PRC2 occupancy [20]. Specifically, H3K27M is often deposited at transcriptionally active regions of chromatin, and its expression is associated with increased levels of H3K27 acetylation at specific genomic regions [20, 21]. Additionally, the PRC2 subunits EZH2 and SUZ12 are often excluded from chromatin on sites containing H3K27M. Together, this suggests that the formation of locus specific H3K27M/H3K27ac heterotypic nucleosomes results in the exclusion of PRC2 from chromatin binding, and subsequently a loss of H3K27me3. This demonstrates that, in contrast to previous reports, H3K27M and PRC2 do not have strong interactions in all chromatin contexts. As well, the p16 protein (p16) is a well-known negative cell cycle regulator, tumour suppressor, and target of PRC2 mediated transcriptional repression [22]. However, upon PRC2 repression, p16 was not shown to be upregulated in H3K27M cells. This supports mechanisms proposed in other models, which suggest that the pro-proliferative activity of PRC2 inhibited cells occurs through a p16-independent mechanism [23].

In addition to the development of these hypotheses, a recent study by Stafford et al. proposed a third hypothesis to try and resolve the mechanism by which both PRC2 and the overall chromatin landscape of DIPG are affected by H3K27M [24]. Bridging the gap between the two-prior propositions, this study demonstrated that the allosterically activated form of PRC2 binds more strongly to H3K27M, as shown by Lewis et al. [10, 24]. This binding occurs aberrantly at loci that are not typical of PRC2 recruitment and occurs early after H3K27M expression. However, the H3K27M-PRC2 binding is transient; upon binding, EZH2 undergoes a conformational change which renders it catalytically non-functional, and the PRC2 complex inhibited. At this point, PRC2 is released from the chromatin site, and the inhibition of PRC2 persists, without lasting deposition at H3K27M (Fig. 1).

Histone Effects Beyond PRC2

As well as provide an additional mechanism of PRC2 inhibition, Stafford et al. also demonstrated that H3K27M also results in the global increase of other histone activating marks [24]. This includes additional histone H3 lysine methylation marks such as the PRC2 antagonist H3K26me2/3, as well as increased H3K36me2/3, both of which are typically associated with areas of active transcription. These complement the well-established effect of DNA hypomethylation, which causes increased expression as a result of loss of gene silencing [17]. Taken together, these alterations reveal the effects of H3K27M surpass that of the H3K27 site and suggest multiple consequences of H3K27M on the chromatin

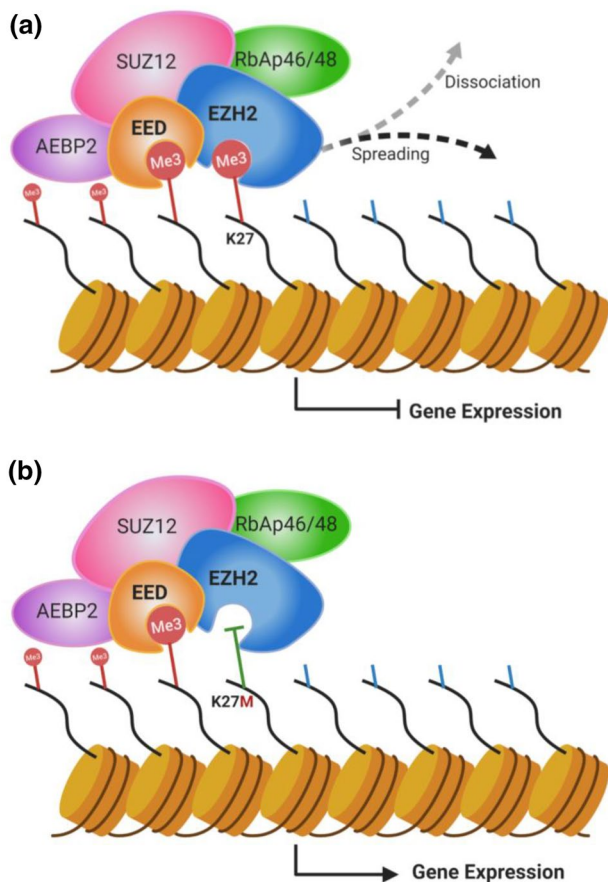


Fig. 1 **a** In wild-type cells, PRC2 complex is recruited to target genes and catalyzes the trimethylation of lysine 27 on the protruding histone H3 amino acid tail through the EZH2 catalytic subunit, maintaining gene repression. Following deposition, PRC2 spreads the trimethyl mark to additional target genes, or dissociates from the histone **b** Presence of K27M mutation on histone H3 inhibits the PRC2 complex, preventing the deposition of the trimethyl mark and subsequent spreading of PRC2

landscape and DNA modification states, suggested to contribute to the progression of DMG [24, 33].

Histone H3.1 and H3.3 Distinctions

The global effects of H3K27M in DMG can be assessed within the context of their specific H3K27M subgroups, H3.1K27M and H3.3K27M. Canonical H3.1 and variant H3.3 can be distinguished by their patterns of deposition throughout chromatin. The incorporation of H3.1 into chromatin is DNA replication dependent as H3.1 is only expressed during S-phase of the cell cycle, where it is incorporated throughout the genome with relative uniformity [25]. However, variant H3.3 is expressed throughout the cell cycle, and thus incorporation is DNA-independent. In contrast to H3.1, H3.3 is incorporated into actively transcribed regions of chromatin, as well as pericentric heterochromatin

and telomeres [25]. These differences suggest that the means by which these histones influence tumorigenesis may, in fact, not be identical.

The possibility that tumorigenesis may vary by type of H3 mutation is supported by the distinct secondary mutations associated with H3.1K27M and H3.3K27M tumours [26]. Within H3.1K27M tumours, there is a well-recognized co-occurrence with mutations in activin-receptor type 1 (*ACVRI*) and phosphoinositide 3-kinase (*PI3K*), whereas H3.3K27M tumours commonly co-occur with mutations or deletions in tumour suppressor 53 (*TP53*), and amplification of platelet-derived growth factor alpha (*PDGFRa*) [26]. Additionally, H3.3K27M tumours have shown enrichment for genes involved in the Ras homolog guanosine triphosphatase (Rho GTPase) activity over H3.1K27M tumours, promoting both tumour cell migration and invasion, and consistent with H3.3K27M DIPG primary tumours being more invasive, more aggressive, and less differentiated than H3.1K27M tumours [27–29].

Epigenetic modifier clinical experience for DMG

Given the mounting evidence that epigenetic alterations are associated with DMG development and progression, a rapid pace of translational development is occurring for epigenetic modifiers, and several classes of inhibitors have already made their way into clinical trial testing.

Histone deacetylase inhibition

Histone deacetylase (HDAC) inhibitors were the first class of epigenetic modifiers to be brought to DIPG clinical trial testing. Well before the discovery of histone mutations in DIPG, the Children's Oncology Group (COG) conducted a phase I/II clinical trial testing suberoylanilide hydroxamic acid (SAHA, Vorinostat), a histone deacetylase inhibitor, and local irradiation, followed by maintenance SAHA in children with newly diagnosed DIPG (NCT01189266). The study is no longer recruiting, and the results are yet to be published.

Following preclinical work described by Grass and colleagues [30], a multi-institutional phase 1 trial of the pan-HDAC inhibitor panobinostat for children with DIPG (NCT02717455) was launched through the Pediatric Brain Tumor Consortium (PBTC) in 2016. The maximum tolerated dose (MTD) of panobinostat administered to children with progressive DIPG on a schedule of 3 weeks on, 1 week off, Monday/Wednesday/Friday was 10 mg/m²/dose, much lower than anticipated [31]. The trial was amended to evaluate the MTD of panobinostat in children with non-progressive DIPG, treated on an alternate week dosing schedule

after completion of radiation therapy. The second stratum has been enrolling since October 2017 and is on-going.

Around similar time, a multi-institutional trial using molecularly targeted therapy for DIPG (NCT02274987) opened through the Pacific Pediatric Neuro-Oncology Consortium (PNOC). Patients enrolled from September 2014 through January 2016, and up to four FDA-approved drugs were used for every patient, dependent upon the tumor's molecular profile based on RNA sequencing as well as whole exome sequencing results [32]. Panobinostat was administered in doses ranging from 15–37 mg/m²/dose, at varying schedule and combination with other agents [33]. While the study achieved feasibility, the optimal dose and schedule of panobinostat remains in question. Furthermore, since its FDA approval in 2015, oral panobinostat has been used off label for pediatric DMG in a variety of doses and schedules. One published example has been its use concomitantly with reirradiation in children with progressive DIPG, at doses ranging from 22–25 mg/m²/dose, 3 times weekly for 2 weeks in 3-week cycles [34].

As the PBTC phase 1 trial nears completion, questions remain for oral panobinostat's ability to penetrate the blood tumor barrier effectively, even at the highest tolerated doses. Answers will come through a well powered efficacy trial, still several years away. In the mean-time, the clinical trial landscape is opening to alternative HDAC inhibitors as well as alternative routes of HDAC inhibitor delivery. Following promising small and large animal model data, PNOC's single institution phase I/II trial of MTX110 for children with DIPG opened in 2018 and delivers the water-soluble form of panobinostat intratumorally through convection enhanced delivery (CED) (NCT03566199) [35]. PNOC has also opened a multi-center target validation study of Fimepinostat, a novel oral, dual pan-HDAC and PI3K inhibitor for children with newly diagnosed DIPG and recurrent HGG in 2019 (NCT03893487). Further work is needed to define the optimal route, dose, schedule, and timing of HDAC inhibitors for children with DMG.

BMI-1 inhibition

The DMG trial landscape has already expanded beyond HDAC inhibitors to include targeted inhibition of BMI-1, a key regulatory component of the human PRC1 complex. Drawing from their preclinical work showing DIPG cell sensitization to radiomimetic drug-induced DNA damage by BMI-1 inhibition, the CONNECT consortium opened a multi-institutional phase 1b study of the BMI-1 inhibitor PTC596 (NCT03605550) in 2018 for children with newly diagnosed DIPG and HGG [36]. Enrollment is ongoing, providing PTC596 concomitantly with radiotherapy, followed by single-agent maintenance dosing.

Future epigenetic modifier strategies for DMG

Combination epigenetic therapy

We are years away from the results of a well powered efficacy study, but it is well presumed that agents such as HDACi will not succeed as monotherapy, and combinatorial approaches are needed to improve outcomes. To that extent, pre-clinical efforts are ongoing to test novel combinations. A recent comprehensive high-throughput drug screen followed by DMG xenograft testing identified potent synergy of panobinostat with the proteasome inhibitor marizomib. The models used were representative of H3WT, H3.3K27M, and H3.1K27M, and the authors propose the combination's efficacy is driven by a metabolic crisis ubiquitous within all DMG cells [37]. A multi-institutional phase 1 combination trial of panobinostat and marizomib for children with DMG is expected to open in early 2020.

Another recent study used a CRISPR screen to reveal the sensitization of DIPG cells to HDAC inhibition by LSD-1 knockout, and that Corin, a bifunctional inhibitor of HDACs and LSD1, potently inhibited DIPG growth in vitro and in xenografts. Mechanistically, Corin increased H3K27me3 levels suppressed by H3K27M histones, and simultaneously increased HDAC-targeted H3K27ac and LSD1-targeted H3K4me1 at differentiation-associated genes [38]. Although further identification of relevant clinical agents and the associated preclinical testing is needed, the combination of LSD1 inhibition with HDAC inhibition will likely be brought to clinical trial testing for DMG in coming years.

Effective penetrance

For epigenetic modification to reach clinical success, the chosen agents must achieve adequate exposure to the tumor and its sites of spread. Focal drug delivery methods such as CED will continue, as well as intra-arterial delivery methods such as the recently completed trial at Johns Hopkins (NCT01688401). Whether epigenetic modifiers may be delivered safely and effectively by focal delivery remains to be seen. In general, it is presumed that focal treatment will not be curative for DMG unless the therapeutic(s) reaches and eradicates all tumor cells prior to disease spread [39]. Most likely, systemic therapy will be a part of future success. Ongoing pre-clinical efforts to study systemically delivered epigenetic modifiers are placing importance on tumor penetrance. The HDAC inhibitor quisinostat has been identified as a possible superior

option due to its increased selectivity for HDAC1, and is currently undergoing further preclinical testing [40]. Regardless of which epigenetic agents are chosen for future clinical trials, we will likely see importance placed on trial design to determine intratumoral target validation.

Correlative studies for epigenetic modifier trials

As more agents become clinically accessible, a simultaneous effort is underway to understand the target effects of epigenetic modification.

Meaningful Biomarkers

An urgent need exists within DMG research to identify predictive biomarkers and validate them through clinical trials. Our understanding of the posttranslational modifications that occur with H3K27M mutation is growing—not only H3K27me3 and H3K27me2 reduction, but disruption of H3K4me3 and H3K36me2, histone acetylation, and DNA methylation [14, 17, 41]. How these aberrations lead to tumorigenesis, and how broadly reprogramming the epigenome through the aforementioned inhibitors leads to tumor reduction, is still not understood. Preclinical work such as that previously described using panobinostat single agent or in combination with marizomib and corin demonstrated efficacy not only for H3K27M mutant tumors, but H3 wild type as well [30, 37, 38]. More thorough mechanistic understanding will allow us to refine (or expand) our targeted populations. Currently, chromatin immunoprecipitation sequencing (ChIP-seq) and RNA sequencing are allowing for broader preclinical analysis of the transcriptome leading to a fuller contextual understanding. Alternatively, partial or total efficacy of epigenetic modifiers may lie in their effects beyond that of epigenome reprogramming.

Immune correlates

We are becoming increasingly aware of the immunologic effects of epigenetic modification. For example, DNA methylation inhibitors (DNMTi) and HDAC inhibitors (HDACi), in addition to broadly reprogramming the epigenome, alter the function of immune cells relevant to acquired immunity in various malignancies. HDACi have led to immunosuppressive effects such as upregulation of PDL-1 in adult melanoma, and DNMTi have upregulated PD1 in T cells of adult patients with MDS and AML [42, 43]. In other contexts, epigenetic therapy has enhanced immune activation. The combination of DNMTi and HDACi induced the secretion of T helper 1 (TH1) cell-type response cytokines, an immune signature associated with effective antitumour immunity in

both pretreated lung cancers and those treated with immune checkpoint blockade [44]. These findings highly the need to evaluate changes of the DMG immune microenvironment with epigenetic modifier treatment, and we will see immune correlates included more often within clinical trials.

Conclusion

We continue to seek a comprehensive understanding of the mechanisms that govern chromatin dysregulation and DNA modification in DMG, and their roles in tumorigenesis. In parallel, we forge ahead with clinical testing of epigenetic modifiers. Straddled between mechanistic discovery and clinical development lies preclinical testing, for which we are iteratively evaluating the appropriate models and thresholds of efficacy. The determined efforts from individuals working bench to bedside, along with collaborative mindset and unified mission, will ultimately result in improved outcomes for DMG.

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Compliance with ethical standards

Conflicts of interest All authors declare no competing interests or conflicts of interest.

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