H3K27M-Mutant Diffuse Midline Glioma

Subjects: Oncology

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H3K27M-mutant diffuse midline glioma is a rare childhood cancer originating in midline brain structures. The H3K27M mutation substitutes an amino acid on histone H3 that promotes gene expression and tumor growth. This cancer has a dismal prognosis and requires new and better treatment approaches. Thus, innovative treatment approaches are greatly needed to improve clinical outcomes for these patients.

diffuse midline glioma | H3K27M-mutant | immunotherapy

1. Introduction

Diffuse intrinsic pontine glioma (DIPG) was recently reclassified as *diffuse midline glioma, H3K27M-mutant* in the 2016 World Health Organization (WHO) classification of central nervous system tumors [1][2]. H3K27M-mutant diffuse midline glioma (DMG) is the second most common childhood malignant brain tumor with an incidence of 200–300 cases annually in the United Stated [3]. Accounting for nearly two-thirds of childhood brainstem tumors, H3K27 diffuse midline gliomas typically present in 3–10 year old children, with a median overall survival of 9–12 months post diagnosis [4].

“H3K27M” is the abbreviated descriptor of a recurrent somatic gain-of-function mutation, resulting from a lysine 27 to methionine (p.Lys27Met: K27M) substitution in histone 3 (H3) variant (**Figure 1**) [5][6]. The H3K27M mutation leads to the global loss of H3K27 trimethylation and subsequent gain of H3K27 acetylation, which has been linked to oncogenesis through upregulation of proto-oncogenes and suppression of cellular differentiation [7]. Despite only a single gain-of-function mutation, diffuse midline gliomas with the H3K27M mutation carry a worse prognosis than wild-type midline gliomas [1][8][9].

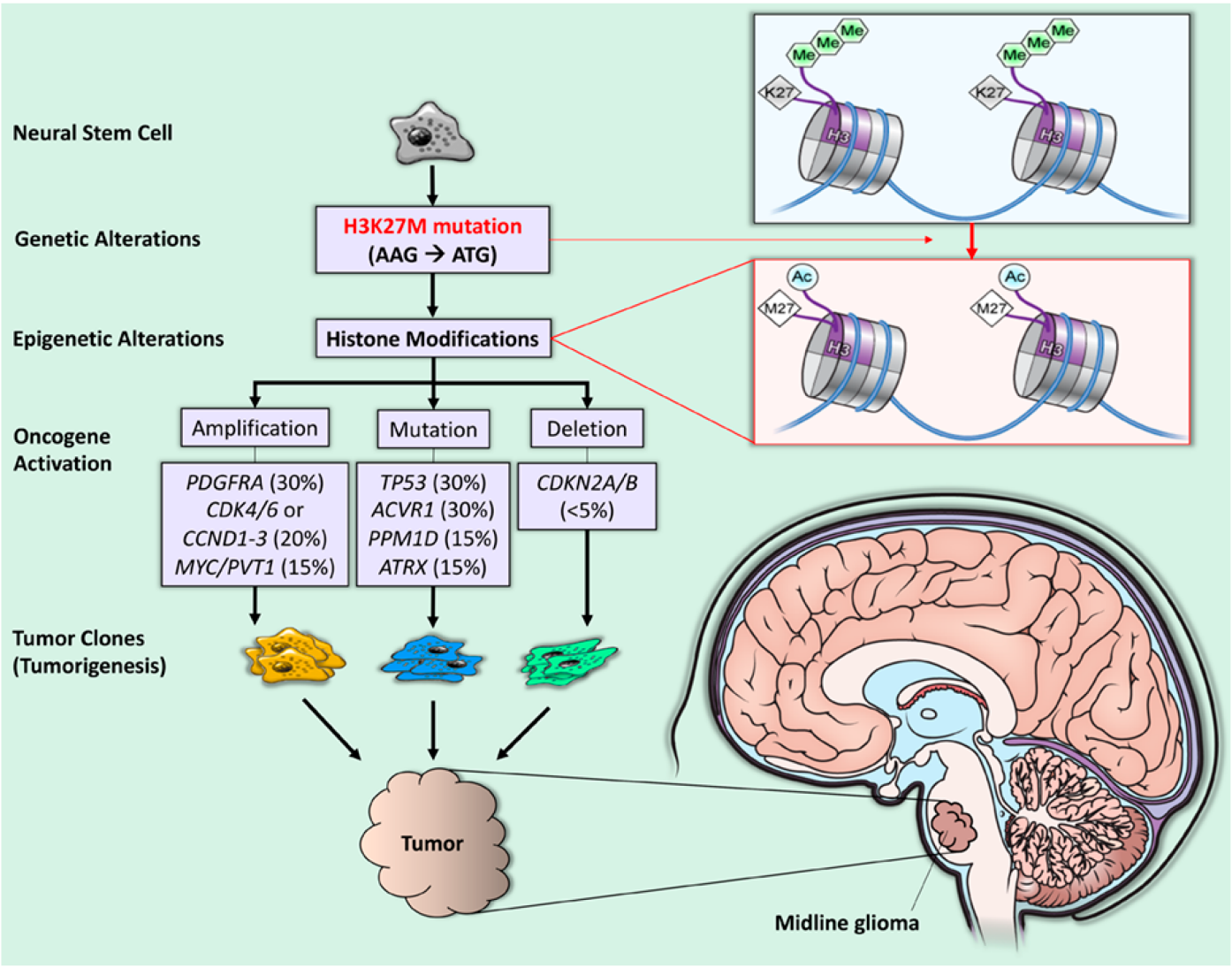

Figure 1. H3K27M mutation and tumorigenesis in diffuse midline glioma. The H3K27M mutation is a recurrent somatic gain-of-function missense mutation (AAG → ATG), resulting in a lysine 27 to methionine (p. Lys27Met: K27M) substitution in histone 3 (H3) variants (purple quadrant). The blue line represents double-stranded DNA wrapped around histones (short, segmented cylinders) regulating normal gene expression. The H3K27M mutation leads to the global loss of H3K27 trimethylation (green hexagons) and subsequent gain of H3K27 acetylation (blue circles), which is linked to oncogenesis (gene amplification, mutation, and deletion) and, subsequently, tumorigenesis [1].

2. Diagnosis

Characteristic clinical symptoms and pathognomonic radiographic findings lead to a presumptive H3K27M-mutant diffuse midline glioma diagnosis. Conventional magnetic resonance imaging (MRI) typically demonstrates a hyperintense signal on T2-weighted images and expansion of the pons (taking up at least 2/3 of the pons) and sometimes adjacent brainstem. Enhancement patterns for H3K27-mutant diffuse midline gliomas vary and may not always be present at disease presentation (**Figure 2**A,C) [10].

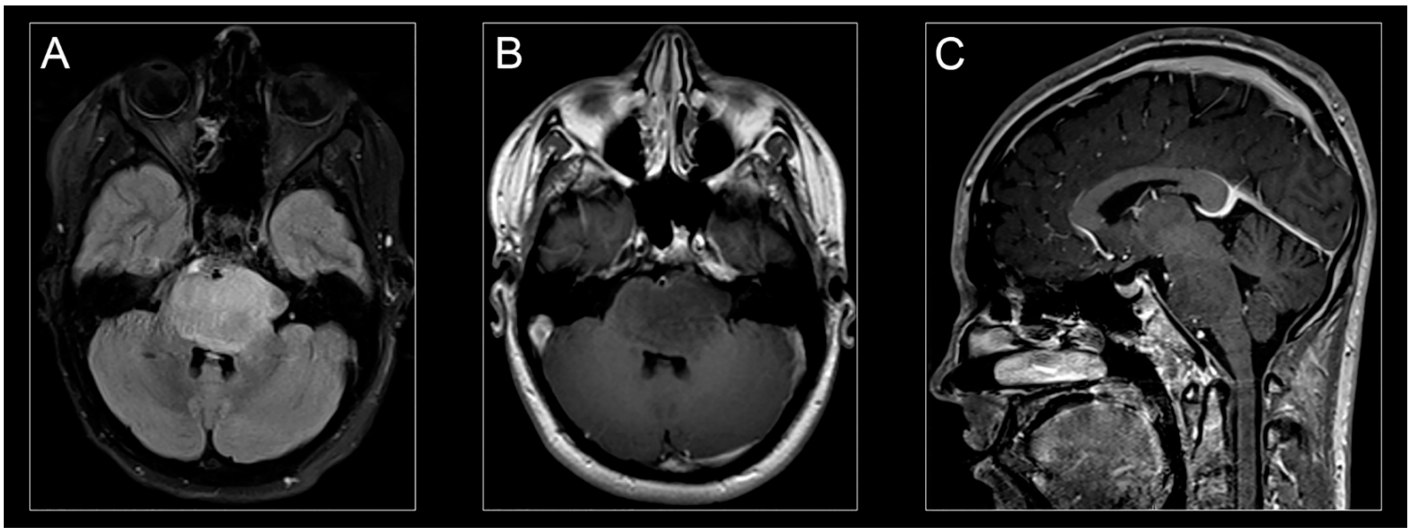

Figure 2. H3K27M-mutant diffuse midline glioma on MR imaging. Classic appearance of H3K27M-mutant diffuse midline glioma on MR-imaging: hyperintense signal on an axial T2-weighted fluid-attenuated inversion recovery (FLAIR) image (**A**), hypointense signal on an axial T1-weighted post-contrast image (**B**), and isointense signal on a sagittal T1-weighted post-contrast image (**C**).

Histological and molecular tumor characteristics traditionally have not guided treatment strategies in H3K27M-mutant DMG [11]. However, previous studies have shown that stereotactic biopsy of pontine tumors has relatively low morbidity and modest prognostic importance [12][13][14][15][16][17][18]. Thus, the development of molecularly guided treatments predicated on tumor biopsy tissue findings would enhance the diagnostic value of stereotactic biopsy [18].

Since repeat biopsy is rare for DMG patients, recent advances have suggested that liquid biopsies (through serum) could measure treatment response by detecting H3K27M mutations in circulating tumor DNA [19][20]. Specifically, levels of H3K27M plasma circulating tumor DNA may correlate with radiographic tumor responses and could be monitored before and after treatment.

3. Pathogenesis

The current understanding of the molecular biology of H3K27M-mutant DMG has resulted from the analysis of tumor tissue obtained through stereotactic surgical biopsy or postmortem tissue procurement [5]. In a recent study of tumor tissue from 215 pediatric patients with H3K27M-mutant diffuse midline glioma, Castel et al. showed that the tumor gene expression profile was more closely tied to molecular tumor characteristics than tumor location or survival [21]. They also found that embryological differentiation (midline vs. hemispheric) more accurately characterized these tumor cells' origin than the structure involved (e.g., brainstem vs. thalamus). To further characterize the H3K27M-mutant DMG cell of origin, Monje et al. identified cells of origin that resemble oligodendrocyte precursor cells. Their finding was supported by more recent RNA-seq experiments by Filbin et al. [22][23]. These findings support (1) the rationale in the updated 2016 WHO classification identifying DIPG more

precisely by its genotypic and molecular profile (H3K27M-mutant), and (2) treating these tumors with molecularly driven rather than location-driven therapies.

A recurrent somatic gain-of-function mutation, leading to a lysine 27 to methionine (p.Lys27Met: K27M) substitution in histone 3 (H3) variants, characterizes more than 85% of DMGs [5][6][24]. The K27M substitution affects histone variant H3.3 and H3.1, resulting from mutations to the *H3F3A* and *HIST1H3B/C* genes, respectively, with over 70% occurring in *H3F3A* [25]. The H3.3K27M gliomas are typically associated with loss-of-function mutations (p53) and gain of function of platelet-derived growth factor alpha (PDGFRA), while H3.1K27M gliomas have been associated with mutations in Activin A receptor type 1 (ACVR1). H3K27M mutation globally reduces H3K27 trimethylation (H3K27me3), leading to elevated expression of gliomagenesis genes [7][26][27]. Thus, research focused on reversing the H3K27 mutation effects through targeted upstream target inhibition or epigenetic therapy remains a viable treatment option [28][29][30].

4. Treatment

4.1. Therapeutic Targeting: Preclinical Development

The change in the 2016 WHO classification of DIPG to H3K27M-mutant diffuse midline glioma highlighted the need to identify potential therapeutic targets in the histologic and molecular tumor microenvironments. Furthermore, recognizing the roles of the histone mutation as the initiating feature and epigenetic modifiers as drivers of tumor pathogenesis has also influenced developing treatment strategies for treating H3K27M-mutant diffuse midline gliomas [1][31].

4.2. Targeting H3K27M Mutation

H3K27M-mutant DMG has a distinct epigenetic landscape characterized by dysregulated histone acetylation and methylation. One therapeutic option for these tumors would be histone-modifying drugs that reverse epigenetic silencing [32]. In a recent study, panobinostat, a histone deacetylase inhibitor, potently inhibited cell proliferation, viability, and clonogenicity of human and murine H3K27M cells in vitro [33]. In genetically engineered tumor-bearing mice, systemic panobinostat administration produced higher drug concentration in brainstem tumor tissue than normal brain tissue, reduced tumor cell proliferation, and increased H3 acetylation level [33].

4.3. Targeting ACVR1 Mutation

Although most preclinical studies of diffuse midline glioma target H3K27M-related mechanisms, *ACVR1* mutations remain an important driver of tumorigenesis in more than 30% of H3K27M-mutant diffuse midline gliomas [31][34]. Since ACVR1 encodes the serine/threonine kinase (ALK2), ALK2 inhibitors could play a role in H3K27M DMG treatment. Recently, ALK2 inhibition improved survival in orthotopic xenograft mice bearing H3.3K27M, ACVR1R206H tumors compared to control mice without the ACVR mutation [35].

4.4. EZH2 Inhibition

Overexpression of enhancer of zeste homolog 2 (EZH2), an enzyme involved in histone methylation, has been associated with a more dismal prognosis in DMG patients with H3K27M mutations [36]. Mohammad et al. found that small-molecule EZH2 inhibitors abolished tumor growth in an H3K27M-mutant diffuse midline glioma mouse model by inducing protein $p16^{INK4A}$, a tumor-suppressing molecule [37].

4.5. Metabolic Inhibitors

Several other approaches for targeting H3K27M DMG have also been proposed that target cellular metabolic pathways. Recently, Khan et al. demonstrated that polyamine synthesis is upregulated in DIPG and, therefore, could be targeted through a synthetic lethality-based approach using a polyamine synthesis inhibitor, difluoromethylornithine (DFMO). Since DIPG cells preferentially escape DFMO inhibition through upregulation of the polyamine transporter SLC3A2, adding polyamine transport inhibitors (AMXT 1501) potentiated tumor-selective cytotoxicity in vitro and in orthotopic animal models [38].

4.6. Immunotherapy

Several studies have investigated immunotherapy as a potential therapy for H3K27M DMG. Preclinical research for H3K27M DMG has incorporated recent chimeric antigen receptor (CAR) T-cell therapy advances. CAR T-cell therapy directed against GD2 (disialoganglioside), a tumor-associated cell surface antigen, is intriguing because patient-derived H3K27M DMG cell lines and neuroectodermal tissues almost uniformly express GD2 [39]. Additionally, in vitro exposure to anti-GD2-CAR T cells significantly depleted cultured H3K27M-mutant cells in a dose-dependent manner [39]. However, the brain penetrance of this CAR T-cell directed therapy is uncertain. Mount et al. did demonstrate brain penetrance in their murine xenograft model, although such penetrance was associated with neurotoxicities including peritumoral inflammation and resultant hydrocephalus [39]. Nevertheless, the tumoricidal effect of GD2-targeted CAR T cells in in vitro assays supports further development and testing of this approach in animal models. Interestingly, immunotherapeutic trials for neuroblastoma, osteosarcoma, and melanoma which target GD2 were successful [39].

5. Management

5.1. Radiation Therapy

Radiation therapy is the only treatment that improves life expectancy. A 54–60 Gy radiotherapy tumor dose over 6 weeks has been the standard treatment recommendation for H3K27M-mutant diffuse midline glioma over the past 20 years, delaying tumor progression for up to 3 months in 70–80% of patients [2][40][41]. Hyperfractionated therapy is less effective than conventional therapy and may increase radiation toxicity and other morbidities [42][43][44]. However, hypofractionated radiotherapy may reduce the burden of treatment for caretakers by significantly reducing hospitalizations and improving the quality of life [45][46].

Radiation may also have synergistic effects if combined with chemotherapy or immunotherapy for treating H3K27M-mutant DMG. Radiosensitizing agents such as gemcitabine selectively target rapidly dividing tumor cells and potentiate radiotherapy. In a phase I/II study, gemcitabine, a pyrimidine analog, and concurrent radiotherapy were well tolerated and without dose-limiting toxicity. However, overall survival was similar to that of historical controls (mOS of 8.7 months) [47].

5.2. Tumor-Localized Therapy

Technological and neurosurgical advancements in stereotactic MRI-guided infusion catheter placement, catheter design, and drug distribution monitoring have paved the way for clinical studies and trials of intratumoral drug delivery via convection-enhanced delivery (CED) [48]. CED is a regional drug delivery method utilizing small hydrostatic pressure gradients to drive the bulk flow of a drug through the extracellular spaces of the central nervous system. CED is a promising therapeutic delivery technique for treating patients with H3K27M-mutant diffuse midline glioma. Several earlier studies have established the technical parameters for safely using CED in patients with H3K27M-mutant DMG [49][50][51][52].

Two clinical trials using CED to treat H3K27M-mutant diffuse midline glioma have recently been reported. Heiss et al. investigated the safety, infusion distribution, and potential efficacy of CED of IL13–*Pseudomonas* exotoxin in five pediatric patients with H3K27M-mutant diffuse midline glioma [53]. They observed short-term radiographic antitumor effects in two of the five patients treated. However, the IL13–*Pseudomonas* exotoxin was not distributed widely enough to reach the entire MRI-defined tumor volume in any patient. Poor target drug distribution contributed to the lack of efficacy in this trial. Drug distribution could have been improved by infusing through multiple catheters or priming the tumor microenvironment. In another clinical trial, Souweidane et al. used CED to safely infuse a murine monoclonal antibody targeting glioma-associated B7-H3 antigen, conjugated to the radioisotope conjugate ([^{124}I]-8H9) into the brainstem of pediatric patients with H3K27M-mutant DMG [54]. The primary endpoint was the maximum tolerated dose of [^{124}I]-8H9 administered by convection-enhanced delivery. No dose-limiting toxicities occurred with any dose, and the maximum-tolerated dose was not reached. Overall survival in this group was slightly better than historical controls (15.3 months). Survival increased more in patients in higher-dose cohorts.

5.3. Immunotherapy

Immunotherapy of H3K27M-mutant diffuse midline glioma is being tested as an adjuvant to traditional chemoradiation. Immunotherapy was safe when administered intravenously and concomitantly with radiotherapy [54][55][56]. Benitez-Ribas et al. recently reported a phase Ib immunotherapy clinical trial using autologous dendritic cells pulsed with an allogeneic tumor cell-line lysate to reactivate tumor-specific T cells in patients with newly diagnosed H3K27M-mutant diffuse midline glioma after irradiation [57]. Autologous dendritic cell vaccines were feasible, safely prepared, and generated an H3K27M-mutant diffuse midline glioma-specific immune response detected in peripheral blood mononuclear cells (PBMC) and cerebrospinal fluid (CSF). In another study, Fried et al. demonstrated that the immune-modulating antibody MDV9300 (pidilizumab) is a potentially promising treatment for

H3K27M-mutant DMG following radiotherapy [58]. Of the nine pediatric patients enrolled in the study, two were still alive nearly 30 months from diagnosis at the trial's conclusion, with radiographically defined disease stability [4][58]. Additionally, Tejada et al. reported the novel intratumoral use of DNX-2401, a replication-competent, genetically modified virus that stimulates an antitumor immune response, in a pediatric patient with H3K27M-mutant diffuse midline glioma [59]. Preclinical studies suggested that DNX-2401 (known as Delta-24-RGD) effectively induced both an oncolytic and an antitumor immune response in pediatric high-grade gliomas [60]. DNX-2401 is under investigation in an ongoing phase I clinical trial (NCT03178032) for recently diagnosed H3K27M-mutant DMG patients. Their findings further suggest that immunotherapy is a promising adjunctive treatment for H3K27M-mutant diffuse midline glioma.

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