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Epigenetic rewiring underlies *SMARCA4*-dependent maintenance of progenitor state in pediatric H3K27M diffuse midline glioma

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Summary:

Epigenetic reprogramming drives tumorigenesis in pediatric H3K27M diffuse midline glioma (DMG) by altering the canonical functions of chromatin remodeling complexes. These studies 1). Identified BRG1 (encoded by *SMARCA4*), the catalytic subunit of the mammalian SWI/SNF (BAF) chromatin remodeling complex, as a novel dependency in pediatric H3K27M glioma, 2). Investigated the molecular mechanisms underlying maintenance of the progenitor state, and 3). Demonstrated efficacy for BRG1 inhibitors.

Pediatric diffuse midline gliomas driven by lysine 27 to methionine mutations in histone H3.1 and histone H3.3 (collectively noted here as *H3K27M* DMG) arise most commonly in the pons, precluding surgical resection. Chemotherapy is ineffective. All patients ultimately succumb to their disease after tumors become refractory to standard-of-care radiotherapy. Epigenetic dysregulation in *H3K27M* DMG leads to a stalled developmental state resembling that of highly proliferative, stem-like oligodendrocyte precursor cells (OPC) (1).

The *H3K27M* mutation rewires the epigenome by inhibiting Polycomb Repressive Complex 2 activity, globally reducing repressive H3K27 trimethylation (H3K27me3) and increasing activating H3K27 acetylation (2,3). However, H3K27me3 at promoters of key PRC2 target genes is required for tumor proliferation and maintenance (2,3). This argues for a model in which the *H3K27M* mutation alters the epigenetic landscape and creates novel dependencies that may be targeted therapeutically.

In this issue of *Cancer Discovery*, Mo, Duan, Zhang and colleagues (4) and Panditharatna, Marques and colleagues (5) independently identified BRG1, the catalytic subunit of the mammalian SWI/SNF (BAF) complex, as a dependency in *H3K27M* DMG through focused CRISPR/Cas9 screening of epigenetic regulators. To elucidate how BRG1 loss sensitizes *H3K27M* DMG, they performed cell viability and colony formation assays in *H3K27M* DMG cell lines, observing that ablation of BRG1 reduced cell viability and the ability to form colonies. BRG1 loss also led to reduced proliferation and increased apoptosis.

Exogenous expression of BRG1 rescued the proliferation defects, providing evidence that BRG1 is essential for proliferation of *H3K27M* DMG cells *in vitro*. *In vivo*, sgRNA-mediated depletion of BRG1 in orthotopically implanted DMG cells reduced tumor growth and improved survival.

To delineate molecular mechanisms underlying BRG1-mediated tumorigenesis and maintenance of the OPC state, the authors analyzed *SMARCA4*-driven gene regulatory networks using single cell regulatory network inference and clustering (SCENIC) analysis. SCENIC combines correlative expression analysis of transcriptional regulators and putative target genes with regulatory sequence analysis to define gene regulons or modules (6). SCENIC identified *SMARCA4* regulons in the OPC, oligodendrocyte cell (OC), and cycling cell states.

Hypothesizing that BRG1 remodels chromatin to maintain the OPC state, Pandittharatna, Marques et al. performed chromatin immunoprecipitation followed by DNA sequencing (ChIPseq) for BRG1, H3K27ac, and H3K27me3 in patient-derived DMG neurospheres. BRG1 binding sites partially overlapped with H3K27ac peaks and did not overlap with H3K27me3 peaks, suggesting that BRG1 localizes to active enhancer regions. Assessing chromatin binding of BRG1 in DMG cell lines by Cleavage Under Targets and Release Using Nuclease (CUT&RUN) (7) followed by next generation sequencing, Mo, Duan, Zhang et al. confirmed that BRG1 peaks localized to enhancer (high H3K27ac, low H3K4me3) as well as promoter regions (high H3K4me3). Because BRG1 is part of a chromatin remodeling complex, the authors investigated the effect of BRG1 loss on chromatin accessibility. After genetic depletion of *SMARCA4*, transposase-accessible chromatin using sequencing (ATACseq) demonstrated decreased accessibility at BRG1 binding sites, specifically at OPC-like H3K27M DMG markers. These data indicated that BRG1 regulates chromatin accessibility and binds regulatory elements to maintain an OPC-like program.

Mo, Duan, Zhang et al. then sought to identify the BRG1-dependent genetic pathways that could drive tumorigenesis. Combining BRG1 CUT&RUN data, RNA sequencing of genes downregulated upon BRG1 depletion, and gene ontology analysis, the authors found that downregulated genes with BRG1 peaks in their promoters and enhancers enriched for extracellular matrix (ECM) and cell proliferation programs. These data argued for a model in which BRG1 binds to gene regulatory elements to direct expression of genes involved in cell proliferation and ECM pathways.

To gain insight into how BRG1 is recruited to chromatin to drive changes in gene expression in *H3K27M* DMG, the authors analyzed DNA sequence motifs of BRG1 binding sites in DMG cell lines. They found that BRG1 peaks are most highly enriched for a set of transcription factors including SOX10, which is expressed in neural crest cells during early development and is required for oligodendrocyte specification and differentiation (8). By co-immunoprecipitation, they found that BRG1 interacted with SOX10 in DMG cells and CUT&RUN data showed that BRG1 and SOX10 peaks co-localized. Depletion of SOX10 from DMG cells downregulated expression of ECM genes and reduced the ability of these cells to proliferate, migrate, and invade. Mo, Duan, Zhang et al additionally

depleted H3.3K27M in DMG cells. These experiments demonstrated that the dependence of DMG cells on BRG1 was due the *H3.3K27M* mutation. They do not perform depletion experiments in H3.1K27M DMG cells. Future work will be required to determine a mechanistic link between *H3.1K27M* mutation and BRG1 in DMG cells. Collectively, these data suggest that in *H3K27M* mutant DMG, SOX10 recruits BRG1 to chromatin to direct expression of ECM and cell proliferation genes, paving the way for therapeutic targeting of BRG1 as a rational treatment strategy.

To test the efficacy of pharmacologic inhibition of BRG1, the authors treated DMG cells with tool ATPase inhibitors of BRG1 (encoded by *SMARCA4*) and BRM (a second SWI/SNF ATPase encoded by *SMARCA2*). Treatment of human *H3K27M* DMG cells reduced cell viability, ability to form colonies, and cell proliferation. Importantly, combined treatment with radiation and an ATPase inhibitor significantly reduced the viability of DMG cells more than either treatment alone. As an additional therapeutic strategy, Panditharatna, Marques et al. utilized heterobifunctional proteolysis-targeting chimera (PROTAC) molecules to target BRG1 for proteosomal degradation. PROTAC treatment resulted in reduced proliferation and increased apoptosis *in vitro*.

Building upon their *in vitro* studies, they assessed the utility of pharmacologic inhibition or degradation of BRG1 *in vivo*. ATPase inhibitor treatment of a subcutaneous xenograft mouse model significantly inhibited tumor growth and improved survival. However, PROTAC treatment only marginally reduced tumor volume and did not improve survival, likely due to poor absorption and tissue penetrance. Although subcutaneous models fail to accurately recapitulate some aspects of the human disease, limiting our ability to generalize the results of these drug studies, targeting BRG1 is a rational therapeutic approach, especially in a disease with such a dismal prognosis.

In summary, the authors identified BRG1 as a dependency in pediatric *H3K27M* DMG. They outlined a molecular mechanism by which, in *H3K27M* mutant cells, SOX10 recruits BRG1 to gene regulatory elements to drive expression of ECM and cell proliferation pathway genes (Fig). Pharmacologically targeting BRG1 with either ATPase inhibitors or PROTACs resulted in antiproliferative effects and induction of apoptosis *in vitro* and reduced tumor volume and increased survival *in vivo*. Inhibiting BRG1 ATPase activity represents a potential therapeutic strategy for pediatric *H3K27M* DMG.

However, it is important to take a step back and ask why preclinical studies of inhibitors targeting epigenetic regulators in pediatric *H3K27M* DMG have thus far failed to provide meaningful benefits to patients. One technical reason may be the difficulty in generating blood brain barrier-penetrant versions of epigenetic inhibitors, a significant hurdle in advancing compounds clinically in patients with brain tumors. Although a degrader of BRG1 showed efficacy preclinically in prostate cancer model (9), PROTAC degraders of BRG1 generated by Panditharatna, Marques and colleagues failed to penetrate the blood brain barrier. Degraders that pass efficiently into the brain are required for treatment of brain tumors.

Another reason for therapeutic failure to date may be the plasticity of the epigenome and the importance of context dependence in defining the function of epigenetic regulators. *SMARCA4* was first described as a tumor suppressor gene (10); and how the *H3K27M* mutation rewires the epigenome to become dependent on BRG1 is not clear. Theoretically, targeting BRG1 may lead to secondary malignancies, especially with long-term treatment, and future studies should assess this risk. However, in pediatric cancers like *H3K27M* DMG where epigenetic dysregulation causes differentiation block, long-term treatment likely may not be necessary. Short-term treatment with BRG1 inhibitors (perhaps in conjunction with radiation) may relieve the differentiation block, shunting cells from a proliferative, less differentiated, OPC-like state to a differentiated non-proliferative or apoptotic state. BRG1 inhibition potentially offers much needed differentiation therapy for pediatric *H3K27M* DMG, with promise to improve survival for patients.

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Statement of Significance:

The authors identified the BRG1 ATPase as a dependency in pediatric H3K27M mutant diffuse midline glioma. SOX10 recruits BRG1 to regulatory elements to drive progression. Pharmacologically targeting BRG1 reduced tumor volume and improved survival in vivo. Inhibiting BRG1 ATPase represents a potential therapeutic strategy for pediatric H3K27M DMG.

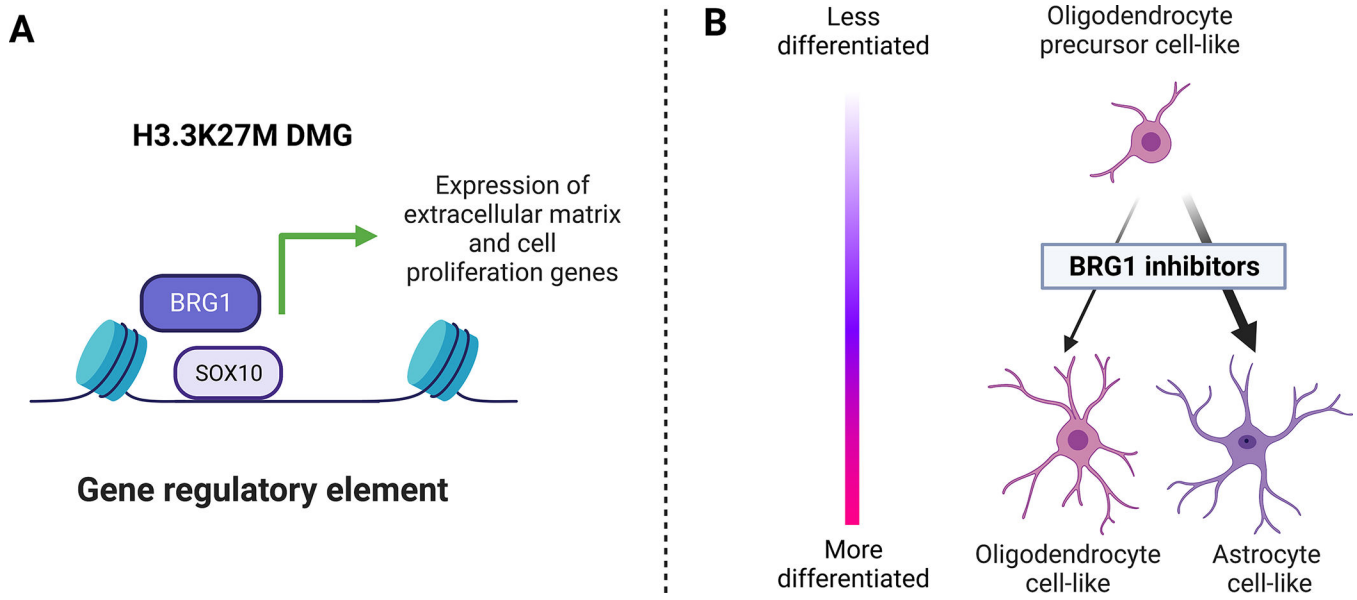

Figure 1. Molecular mechanism and treatment strategy for pediatric H3K27M diffuse midline glioma.

A. H3.3K27M rewires the epigenome to create novel dependencies on epigenetic regulators. The transcription factor SOX10 recruits BRG1 to gene regulatory elements that drive expression of extracellular matrix and cell proliferation genes. **B.** Targeting BRG1 ATPase activity with either ATPase inhibitors or PROTACs shifts cells from a highly proliferative oligodendrocyte precursor-like state to a more differentiated astrocyte-like state, resulting in decreased proliferation and increased apoptosis. Figure created with BioRender.com.