Epigenetic regulation in medulloblastoma pathogenesis revealed by genetically engineered mouse models

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Abstract
Medulloblastoma is the most common malignant cerebellar tumor in children. Recent technological advances in multilayered ‘omics data analysis have revealed 4 molecular subgroups of medulloblastoma (Wingless/int, Sonic hedgehog, Group3, and Group4). (Epi)genomic and transcriptomic profiling on human primary medulloblastomas has shown distinct oncogenic drivers and cellular origin(s) across the subgroups. Despite tremendous efforts to identify the molecular signals driving tumorigenesis, few of the identified targets were druggable; therefore, a further understanding of the etiology of tumors is required to establish effective molecular-targeted therapies.

Chromatin regulators are frequently mutated in medulloblastoma, prompting us to investigate epigenetic changes and the accompanying activation of oncogenic signaling during tumorigenesis. For this purpose, we have used germline and non-germline genetically engineered mice to model human medulloblastoma and to conduct useful, molecularly targeted, preclinical studies. This review discusses the biological implications of chromatin regulator mutations during medulloblastoma pathogenesis, based on recent in vivo animal studies.

KEYWORDS
Cerebellar development, chromatin regulator, medulloblastoma, mouse model, pediatric brain tumor

1 | INTRODUCTION

The current consensus of MB classification recognizes 4 subgroups consisting of Wingless/int (WNT), sonic hedgehog (SHH), Group3, and Group4 (called as MBWNT, MBSHH, MBGrp3, and MBGrp4 from this point forwards) in accordance with (epi)genetic and transcriptomic features.¹ MB subgroups exhibit distinct clinical features.²-⁴ MBWNT accounts for approximately 10% of MB patients, mainly observed in ages 6-10 y or later and has an excellent prognosis with a 90% 5-y survival rate. MBSHH occupies 30% of all MB cases and is mainly
found in infants (<3 y) and adults (≥18 y). This subgroup is predominant in adult MBs, accounting for 60% of all adult patients. The overall survival rate is 70%, while specific molecular signatures such as Tumor protein p53 (TP53) status affect the outcome. MB Grp3 comprises 25% of all MB patients and mainly affects children of 2-5 y. This subgroup has the worst prognosis with approximately 50% overall survival. MB Grp4 accounts for 35% and has a good prognosis with approximately 90% overall survival. MB Grp3 and MB Grp4 have a male predominance compared with MB WNT and MB SHH, in which there is no particular predilection.

This molecular subgrouping, in combination with histological classification approved by the World Health Organization, should lead to greater accuracy of diagnosis and a decision of treatment regimen. Nevertheless, this basic classification still has limitations given the intertumoral molecular and histological heterogeneity within each subgroup. For example, MB SHH harboring the TP53 mutation is defined as a high-risk tumor compared with tumors without TP53 mutation. Therefore, more detailed analyses of the molecular signatures underpinning tumor heterogeneity may better predict prognosis and accurately determine molecular-targeted drugs. Recent analyses on large cohorts of MB patients have allowed for a deeper understanding of MB stratification: each subgroup can be subdivided into different subtypes that show distinct clinical outcomes and molecular signatures. In addition to these studies proposing a multilayered classification, many studies have identified subgroup-specific molecular features from various biological perspectives. Although these studies have predicted candidate genes responsible for MB pathogenesis, the comprehensive biological functions of these genes in primary MB formation and progression are unknown.

In vivo evaluation of cancer-specific mutation-driven oncogenicity using animal models provides insight into pro-tumorigenic signaling pathways, and may lead to the development of promising drugs. As the tumor cells grow in the brain microenvironment, these animal models have revealed both cell autonomous and non-cell autonomous oncogenic mechanisms. Although the generation of germ-line genetically engineered mouse models (GEMMs) is traditionally time consuming and costly, recent advances in clustered regularly interspaced short palindromic repeat (CRISPR)-based genome editing technologies have shortened the time to generate germline and non-germline GEMMs, therefore accelerating the investigation of tumor-relevant mutations. In addition, establishing and sharing PDX lines offer great opportunities to study how mutations of interest contribute to MB progression. Therefore, the era of post-genomics with animal models has already started toward rigid preclinical studies of MBs.
A marked hallmark of mutational patterns in MBs is recurrent mutations in chromatin modifiers that regulate various aspects of epigenetic processes (Figure 1 and Table 1). Moreover, pediatric tumors including MB have lower mutational burdens compared with adult tumors, implying that mechanisms other than genetic mutations are an important factor for MB formation, and prompting us to investigate epigenetic regulation in MB pathogenesis. In this review, we summarize the current knowledge on frequently mutated epigenetic regulators and their biological significance derived from animal models (Table 2). We focus on SHH and non-WNT/SHH subgroups, as no reports have been published on chromatin modifier functions in the WNT subgroup, to the best of our knowledge.

### TABLE 1 Summary of mutation frequencies of chromatin modifiers in MBs

| Gene     | Subgroup | Mutation frequency in each subgroup (%) |
|----------|----------|----------------------------------------|
| ARID1A   | WNT      | 8.5                                    |
|          | SHH      | 1.7                                    |
| ARID2    | WNT      | 4.2                                    |
|          | SHH      | 1.7                                    |
| BCOR     | SHH      | 3.9                                    |
| BRPF1    | SHH      | 2.2                                    |
| CHD7     | SHH      | 1.1                                    |
|          | Group3   | 1.7                                    |
|          | Group4   | 2.2                                    |
| CREBBP   | WNT      | 4.2                                    |
|          | SHH      | 7.2                                    |
| GSE1     | SHH      | 5.0                                    |
| KDM4C    | Group4   | 1.5                                    |
| KDM6A    | SHH      | 1.1                                    |
|          | Group4   | 8.9                                    |
| KMT2C    | SHH      | 5.5                                    |
|          | Group3   | 3.3                                    |
|          | Group4   | 7.4                                    |
| KMT2D    | WNT      | 7.0                                    |
|          | SHH      | 13.8                                   |
|          | Group3   | 6.1                                    |
|          | Group4   | 2.6                                    |
| p300     | SHH      | 1.1                                    |
| PRDM6    | Group4   | 10.4*                                  |
| SMARCA4  | WNT      | 21.1                                   |
|          | SHH      | 2.2                                    |
|          | Group3   | 9.4                                    |
|          | Group4   | 1.5                                    |

*Given that MB patients harboring SNCAIP-associated structural variants exhibit PRDM6 upregulation, the frequency of PRDM6 abnormalities is determined as the frequency of the SNCAIP mutations.

## 2 | BIOLOGICAL FUNCTIONS OF CHROMATIN REGULATORS IN THE PATHOGENESIS OF MB

### 2.1 | SHH subgroup

MBSSH is named after constitutively activated SHH signaling in this subgroup. Because of faithful animal models, we understand the biological underpinnings of this subgroup best. In this subgroup, SHH pathway genes (eg, SHH, 3% in MBSSH cases, Patched 1 (PTCH1), 49.6% in MBSSH cases, Smoothened (SMO), 17.3% in MBSSH cases), Suppressor of fused homolog (SUFU), 14.3% in MBSSH cases), and Glioma-associated oncogene homolog 2 (GLI2), 7.5% in MBSSH cases) are frequently mutated or amplified, resulting in driving abnormal activation of the pathway (Figure 2A). Therefore, SHH signal activation is a prerequisite to MB pathogenesis. Indeed, in line with the fact that SHH secreted from Purkinje cells is a potent mitogen for cerebellar GNPs during normal cerebellar development, previous studies have proposed the cells committed to GNP cell fate as a cellular origin of MBSHH. Several reports have revealed that GNP was a cell of origin of MBSHH through conditional hyper-activation of SHH signaling pathways using GEMMs (eg, conditional deletion of Ptch1, a negative regulator of SHH signaling and forced expression of a constitutively active form of Smo, a key activator of SHH signaling in GNPs). These animal studies suggested that the mutations activating SHH signaling in GNPs are one cause of MBSSH formation.

In addition to SHH signaling, other factors required for normal development of GNPs have been shown to play important roles in the pathogenesis of MBSSH. Genetic fate-mapping in mice has shown that GNPs originate from the most dorsal part of rhombomere 1, the so-called upper rhombic lip (uRL), GNPs start to leave the uRL from embryonic day 12.5 in mice and migrate along the surface of the cerebellar primordium to form the EGL. GNPs massively proliferate in the EGL and subsequently exit the cell cycle to migrate inwardly along Bergmann glial fibers, followed by maturation in the internal granule layer (Figure 2B). The balance between mitotic and postmitotic states of GNPs is strictly regulated by multiple signaling pathways; disruption of cell cycle exit also influences MBSSH pathogenesis. In addition to SHH-related proteins, atonal bHLH transcription factor 1 (ATOH1), a transcription factor required for GNP fate commitment, is indispensable for the oncogenesis of MBSSH and accelerates MBSSH formation via repression of the cell cycle exit of GNPs. In addition, factors promoting differentiation of GNPs, such as WNT, bone morphogenetic proteins (BMP) and NeuroD1, antagonize tumor cell proliferation. A recent study has shown that the receptor for activated C kinase (Rack-1), a multifaceted signaling adaptor protein, oppositely regulates SHH signaling and WNT signaling via the physical interaction with histone deacetylase (HDAC)1/2, leading to stability of HDACs. Indeed, HDACs have been shown to regulate GLI1 activity from multiple directions. HDACs directly bind to the GLI locus via interaction with SWI/SNF related, matrix associated, actin-dependent regulator of chromatin (SMARCA4), a main component of the SWI/SNF complex, while...
TABLE 2 Mice models used to investigate functions of chromatin regulators

| Gene     | Mice models                                                                 | Tumor subgroup | Description of mice models | Refs |
|----------|-----------------------------------------------------------------------------|----------------|---------------------------|------|
| BCOR     | Atoh1-Cre; Bcor<sup>flx/flx</sup>; Ptch1<sup>+/−</sup>                      | Sonic hedgehog | Genetic deletion of Bcor by Cre-mediated DNA recombination in Atoh1-expressing cells including GNP in the Ptch1 heterozygous medulloblastoma-prone background. | 45   |
| BRPF1    | SmoM2-YFP<sup>flx/+</sup>                                                   | Sonic hedgehog | In vivo transfection of plasmids encoding Cre and Brp1 truncated form(s) in the mice in which a constitutive active form of Smo (SmoM2) fused with YFP is activated in Cre-expressing cell. | 48   |
| CREBBP   | Atoh1-Cre (or CreERT2); Crebbp<sup>flx/flx</sup>; SmoM2-YFP<sup>flx/+</sup> | Sonic hedgehog | Deletion of Crebbp in Atoh1-expressing cells in the SmoM2-expressing medulloblastoma-prone background at embryonic (for Atoh1-Cre) and postnatal (for Atoh1-CreERT2) stages. | 51   |
| SMARCA4  | Atoh1-Cre; Smarca4<sup>flx/flx</sup>; SmoM2-YFP<sup>flx/+</sup>            | Sonic hedgehog | Cre-mediated deletion of Smarca4 in Atoh1-positive cell lineages in the SmoM2-expressing medulloblastoma-prone background. | 38   |
| KMT2D    | Nestin-Cre; Kmt2d<sup>flx/flx</sup>                                        | Group3         | Genetic ablation of Kmt2d in Nestin-positive neural stem cells using a Nestin-Cre driver mouse, resulting in the formation of spontaneous cerebellar tumors that express NPR3, a marker for Group3. | 67   |
| KDM1A    | CAG-CreERT2; Kdm1a<sup>flx/flx</sup>, orthotopic transplantation          | Group3         | Myc- and Gfi1-infected neural progenitors from the mice transplanted into the cerebellum of NOD/SCID gamma mice for Group3 formation. Subsequently, Kdm1a deletion was performed by tamoxifen administration into the recipient mice. | 59   |
| CHD7     | Orthotopic transplantation                                                  | Group4         | A Group4 patient-derived xenograft cell line expressing shRNA targeting CHD7 in the cerebellum of NOD/SCID gamma mice. | 71   |

*Note that cerebellar granule neuron progenitors and neural stem cells were genetically modified in the animal models of MB<sub>SHH</sub> and MB<sub>Grp3</sub>, respectively, provided that the respective cells have been thought to be their putative cell of origin. 25,26,56,57

Directly deacetylating and activating GLI1 protein. 28 These studies implied that Rack-1 could regulate SHH signaling non-epigenetically and epigenetically via HDAC stabilization. Therefore, these data indicated that MB<sub>SHH</sub> pathogenesis is driven by abnormal regulation of normal GNP developmental pathways.

Animal studies have provided further insights into the requirement of multiple mutations in MB<sub>SHH</sub> progression. An early milestone study using Ptch1 heterozygous mice (Ptch1<sup>+/−</sup> mice), a faithful MB<sub>SHH</sub> model, 39 showed that only 16% of mice developed tumors at 10-25 wk of age, despite high frequency (>50%) of preneoplastic lesions at 3-6 wk of age. 40 Of note, loss of heterozygosity of Ptch1, which activated SHH signaling, was observed in preneoplastic cells (PNCs), suggesting that SHH signaling activation alone is insufficient for MB<sub>SHH</sub> tumorigenesis. 40 A further study revealed that most PNCs differentiate and only a small population of PNCs becomes tumor cells. 41 They demonstrated that overexpression of v-myc avian myelocytomatosis viral related oncogene, neuroblastoma derived (Mycn) in PNCs led to enhanced penetrance of tumor incidence, suggesting that secondary genetic hits, in addition to aberrant SHH signaling activation, are required for fully malignant transformation. Two other animal studies have shown that additional secondary hits are required for transformation, including transformation-related protein 53 (Trp53, mouse homolog of TP53) mutation for evading cellular senescence and Frizzled 4 deletion for angiogenic remodeling. 19,42 Therefore, identification of secondary oncogenic hits is required for a complete understanding of MB<sub>SHH</sub> pathogenesis, further revealing mutation-dependent characteristics of individual tumors (Figure 2C).

Recurrent mutations unveiled by next-generation sequencing on human MBs give us clues to the secondary genetic hits for MB<sub>SHH</sub> formation. In particular, mutations in chromatin modifiers were identified as a striking hallmark of MBs, 11,13,14 strongly suggesting that epigenetic regulation could be of great importance in MB<sub>SHH</sub> tumorigenesis and mutations of chromatin modifiers are potential second hits.

**BCL6 interacting corepressor (BCOR)** is one of the recurrently mutated chromatin regulators in MB<sub>SHH</sub> and has been considered as a potential second hit. BCOR was initially identified as an interacting partner of BCL6 transcriptional repressor. 43 Subsequently
it was recognized as a component of non-canonical polycomb repressive complex 1.1 (PRC1.1), which ubiquitinates histone H2A at Lys119 (H2AK119ub1) to repress transcription of target genes. Recently, we elucidated the biological mechanisms underpinning BCOR mutated MB SHH and revealed that functional loss of Bcor resulted in the acceleration of tumorigenesis in Ptch1+/- mice by strong activation of insulin-like growth factor 2 (Igf2), a potent mitogen of GNP (Figure 3A,B). Of note, retroviral overexpression of Igf2 in Ptch1+/- GNPs was sufficient to drive tumorigenesis at complete penetrance. Although BCOR has been reported to transcriptionally repress Gli1/2 via functional interplay with B cell leukemia/lymphoma 6 (BCL6), the mutations of BCOR in human MB SHH mostly yielded truncated mutants with an intact BCL6 binding domain, but lacking the PUF6 domain, which is essential for proper formation of PRC1.1. Truncated mutants of BCOR failed to interact with RING1B, the catalytic subunit of PRC1.1. Consistent with these observations, H2AK119ub levels at the Igf2 locus decreased when Bcor was deleted. This finding indicated that Bcor loss-of-function mutations led to a reduction in H2AK119ub levels at the Igf2 locus probably because of an inability to recruit PRC1.1, subsequently allowing Igf2 upregulation (Figure 3C). Together with the observation that Bcor deletion itself does not induce tumor formation, mutations in BCOR will function as the secondary hit in MB SHH formation.

While BCOR mutations are observed in both pediatric and adult MB SHH, there are mutations of chromatin modifiers that occur in an age-specific manner, indicating that age-specific mutations are important for pathogenesis in some cases. Bromodomain and PHD finger containing 1 (BRPF1) is one of the recurrently mutated chromatin regulators and is mainly mutated in adult MB SHH. A recent study revealed the cooperative contribution of BRPF1 mutations and SHH pathway mutations to the oncogenesis of adult MB SHH. In this report, the expression of SmoM2, a constitutively active form of Smo in differentiated cerebellar GCNs was shown to induce de-differentiation of GCNs and generated a tumor resembling adult
This study also showed that the expression of truncated mutant forms of BRPF1, which is often associated with SMO mutations, enhanced de-differentiation and tumor formation together with SmoM2 expression. Of interest, the co-expression of mutant BRPF1 and SmoM2 altered chromatin accessibility at stem/progenitor-related genes, although which genes promoted the tumorigenesis in BRPF1-mutated MB SHHs remains to be identified. Whether de-differentiation is the fundamental event for adult MB SHH pathogenesis remains unknown, but this study implied that chromatin remodeling of mature GCNs to restore the precursor cell properties is involved in the tumorigenesis of adult MB SHHs. Therefore, mutations in chromatin modifiers may often affect transformation of GCNs in a stage-dependent manner, and reflects discoveries of these mutations in specific subtypes of MB SHHs.

Another example has underlined the importance of the timing of somatic deletion in MB SHH pathogenesis. CREBBP and E1A binding protein p300, another HAT protein mutated in MB SHHs, has been shown to acetylate non-histone proteins, including TP53, to regulate their transcriptional activities. The acetylation of TP53 is essential for its transcriptional activity, also predominantly mutated in adult MB SHHs. Most mutations are likely to affect its HAT domain, suggesting that loss of its HAT activity is a predisposition to tumorigenesis. A recent study has revealed that conditional deletion of a CREBBP C-terminal fragment including the HAT domain in GNPs at embryonic stages induced cerebellar hypoplasia and attenuated oncogenicity in a SmoM2-expressing tumor-prone background. By contrast, loss of Crebbp at an early postnatal stage increased the incidence of SmoM2-driven MBs, partly due to the failure of HAT-mediated transcriptional activation of brain-derived neurotrophic factor (Bdnf). This study implied that the secondary genetic mutation of CREBBP during MB SHH tumorigenesis occurs later in life.

In addition to chromatin regulation via histone acetylation, CREBBP and E1A binding protein p300 (p300), another HAT protein mutated in MB SHHs, has been shown to acetylate non-histone proteins, including TP53, to regulate their transcriptional activities. The acetylation of TP53 is essential for its transcriptional activity, required...

**FIGURE 3** A, B, Bcor functional loss induces the upregulation of Igf2 in Ptch1+/− preneoplastic lesions and tumors, therefore causing the acceleration of tumorigenesis. C, Igf2 activation can be explained by dysfunction of BCOR-mediated polycomb repressor complex 1.1 in the genomic locus of Igf2.
Therefore affecting cell cycle arrest, senescence, and apoptosis.\textsuperscript{53} Considering that the most frequent mutations in CREBBP and p300 are observed in their HAT domains,\textsuperscript{12} MB\textsubscript{SHH} carrying mutations in CREBBP and p300 may influence TP53 activity. A recent study showed that TP53 inactivation enhanced MB\textsubscript{SHH} tumorigenesis by escaping from oncogene-induced senescence.\textsuperscript{42} Future studies should investigate the relationship between HAT mutations and TP53 activity, which might provide important insights into the non-epigenetic functions of HATS.

### 2.2 | Group3 and Group4 (non-WNT/SHH subgroups)

MB\textsubscript{Grp3} and MB\textsubscript{Grp4} were originally named non-WNT/SHH MBs. While our recent study has identified Rous sarcoma oncogene (SRC) signaling as an oncogenic driver of MB\textsubscript{Grp4},\textsuperscript{15} driver mutations for non-WNT/SHH MBs remain to be further explored due to the diversity of (epi)genetic profiles across these subgroups.

As described above, oncogenic signaling exerts cell proliferation activity in a cell type-dependent manner. Recent advances in single-cell transcriptomics on murine developing cerebella obtained from multiple embryonic and postnatal developmental stages have revealed molecular similarities between MB\textsubscript{Grp5} and unipolar brush cells, while the MB\textsubscript{Grp3} transcriptome does not resemble any specific types of cerebellar cells statistically.\textsuperscript{54,55} Nevertheless, the possibility of cell fate/transcriptome changes by oncogenic activation cannot be excluded. Therefore, animal studies on which cell type(s) exhibits cell proliferation with non-WNT/SHH subgroup-specific mutations would provide insights into their cellular origin(s) and potential oncogenic mutations.

Myelocytomatosis oncogene (MYC) is a prominent hallmark of MB\textsubscript{Grp3}; MYC amplification was observed in 17% of MB\textsubscript{Grp3} cases.\textsuperscript{14} We and other groups have reported faithful animal models of MYC-driven MBs by transducing Myc in GNPs and Prom1-positive cerebellar neural stem cells (NSCs) in combination with inactivation of Trp53 function.\textsuperscript{\textsuperscript{54,57}} TP53 mutations occurred only in recurrent MYC-driven MBs, but not the corresponding primary tumors, further prompting us to identify new oncogenic mutations relevant to primary MYC-driven MBs. For example, high activation of \textit{growth factor independent 1 transcription repressor (Gfi1)} through genomic rearrangement specific to MYC-amplified MB\textsubscript{Grp4} may drive their tumorigenesis.\textsuperscript{12} Indeed, the upregulation of Gfi1B was observed in 10.7% of MB\textsubscript{Grp3} cases.\textsuperscript{12} Ectopic coactivation of MYC and Gfi1 in GNPs and cerebellar NSCs and subsequent cranial transplantation in mice induced tumor development in vivo, indicating the transformation capacities with these combinatorial mutations.\textsuperscript{12} Furthermore, a recent study has successfully transduced Myc alone using lentiviral infection in SRY (sex-determining region Y)-box 2 (Sox2)-positive cerebellar cells that developed MBs following transplantation.\textsuperscript{58} Therefore, while MYC-driven MBs could be derived from multiple cell populations, these animal models offer an opportunity to clarify the contribution of chromatin modifiers to MB\textsubscript{Grp3} tumors.

Several studies have linked Gfi1 activation and epigenetic regulation in MYC-driven MBs. First, the Myc/Gfi1 animal model revealed that Gfi1 requires a \textit{Lysine-specific histone demethylase 1a (Kdm1a)} for progression of MYC/Gfi1-driven tumors.\textsuperscript{59} Of note, while Gfi1 represses the TP53 pathway in the hematopoietic system,\textsuperscript{60-62} it was reported to be activated in neuroectodermal tumors.\textsuperscript{63} Therefore, the effectiveness of TP53 activation for therapeutic treatment on MYC/Gfi1-driven MB\textsubscript{Grp3,5} although preclinical studies using humanized models are still required. Second, although Enhancer of zeste homolog 2 (Ezh2) inhibitors are often proposed as MB therapies,\textsuperscript{54-66} Ezh2/PRC2-mediated Gfi1 repression machinery influences the aggressiveness of MYC-driven MBs, requiring careful consideration on using epigenetic drugs, such as Ezh2 inhibitors.

In addition to Gfi1-mediated chromatin modification, mutations in chromatin modifiers themselves have been also reported in non-WNT/SHH MBs. \textit{Lysine methyltransferase 2D (KMT2D)} insertions/deletions have been discovered in the Group3/4 subgroups and the SHH subgroup. In mice, conditional genetic ablation of both alleles of Kmt2d in Nestin-positive NSCs and progenitors in the central nervous system induced spontaneous MBs.\textsuperscript{67} These tumors exhibited upregulation of Ras-GTPase activators and Notch signaling due to dysregulation of DNA methyltransferase 3A (DNMT3A)-catalyzed DNA methylation and Sir2A1/Bcl6-mediated histone modification.\textsuperscript{67} Notably, while the tumors specifically expressed Natriuretic peptide receptor 3 (NPR3) and MYC, which are MB\textsubscript{Grp3} specific molecular markers, their molecular similarity to human counterparts remains to be further investigated, as KMT2D indels have been reported to be expressed in only specific subtypes (subtypes II, III, VII and VIII) in MB\textsubscript{Grp3,4}.\textsuperscript{14}

Contributions of non-WNT/SHH MB-associated mutations to tumorigenesis have been further analyzed in humanized models including cell lines and PDX models, in addition to GEMMs. Missense mutations in SMARCA4 have been reported and is one of the most frequently mutated genes in MYC-driven MBs. Genetic ablation of SMARCA4 in GNPs has been reported to cause cerebellar hypoplasia by preventing proliferation, resulting in attenuation of MB\textsubscript{SHH} formation.\textsuperscript{38} In addition, a previous study with human MYC-amplified MB\textsubscript{Grp3} tumor cells revealed that single amino acid changes in SMARCA4 caused a reduction in ATPase activity, subsequently leading to the attenuation of DNA decatenation capacity of DNA Topoisomerase 2A (TOP2A).\textsuperscript{68} Therefore, the lack of function of SMARCA4 could be involved in genomic rearrangement and aneuploidy. Although mutations discovered in MBs are mainly located in mutational hotspots identified by a previous pan-cancer analysis,\textsuperscript{69} whether SMARCA4 missense mutations discovered in MB\textsubscript{WNT} and MB\textsubscript{SHH} share similar functions with those of MB\textsubscript{Grp3} tumors remains to be investigated.

In addition to SMARCA4, another chromatin remodeler, Chromodomain helicase DNA binding protein 7 (CHD7), also regulates DNA decatenation in cerebellar granule cells via interaction with TOP2B\textsuperscript{70} and regulates tumor cell proliferation in MB\textsubscript{Grp4}. \textsuperscript{71}
patient-derived cell lines. The pro-proliferative effect by CHD7 silencing on human MBGrp4 cells is mediated by ERK pathway activation, resulting from the dysregulation of chromatin compaction at the Dual specificity phosphatase 4 (DUSP4) locus, a negative regulator of ERK signaling. Provided that loss-of-function mutations in CHD7 have been also detected in MBShh and MBGrp3 and even other types of solid tumors, whether negative correlation of CHD7 expression to activation of ERK signaling is conserved beyond subgroups remains to be studied.

3 | CONCLUSION

So far, mutations in chromatin modifiers have been thought to be a marked genetic event involved in MB formation. Nevertheless, understanding their roles in tumorigenesis is difficult as individual chromatin modifiers regulate gene expression differently in cell type-specific and stage-dependent manners, requiring brain biomaterials that are appropriate for the application of chromatin biology to brain tumor research. Despite the limited availability of biopsies and tumor materials from individual brains, recent advances in new chromatin technologies, including ATAC-seq, CUT&RUN and CUT&Tag would help to determine chromatin modifier functions, even with a limited number of primary cells. In addition, a consolidated worldwide PDX platform would also provide human tumor cells related to entities of interest, which could then be studied using these new technologies. Indeed, comprehensive chromatin analyses on PDX tumors have elegantly explained the mode of action of epigenetic drugs (eg, JQ1). Collectively, when compared with the last decade, the understanding of chromatin biology in cancer is advancing quickly, accelerating the establishment of new therapeutic avenues for MB patients.

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DISCLOSURE

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