Cancer Science WILEY

Epigenetic regulation in medulloblastoma pathogenesis revealed by genetically engineered mouse models

Ryo Shiraishi[1,2] 🔟 | Daisuke Kawauchi[1] 🔟

[1]Department of Biochemistry and Cellular Biology, National Center of Neurology and Psychiatry (NCNP), Tokyo, Japan

[2]Department of NCNP Brain Physiology and Pathology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan

Correspondence
Daisuke Kawauchi, Department of Biochemistry and Cellular Biology, National Center of Neurology and Psychiatry (NCNP), 4-1-1 Ogawa Higashi-cho, Kodaira, Tokyo 187-8502, Japan.
Email: d.kawauchi@ncnp.go.jp

Funding information
Mochida Memorial Foundation for Medical and Pharmaceutical Research; Japan Agency for Medical Research and Development, Grant/Award Number: JP20ck0106534h; Japan Society for the Promotion of Science, Grant/Award Number: 20J13210

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 MB_{WNT}, MB_{SHH}, MB_{Grp3}, and MB_{Grp4} from this point forwards) in accordance with (epi)genetic and transcriptomic features.[1] MB subgroups exhibit distinct clinical features.[2-4] MB_{WNT} 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. MB_{SHH} occupies 30% of all MB cases and is mainly
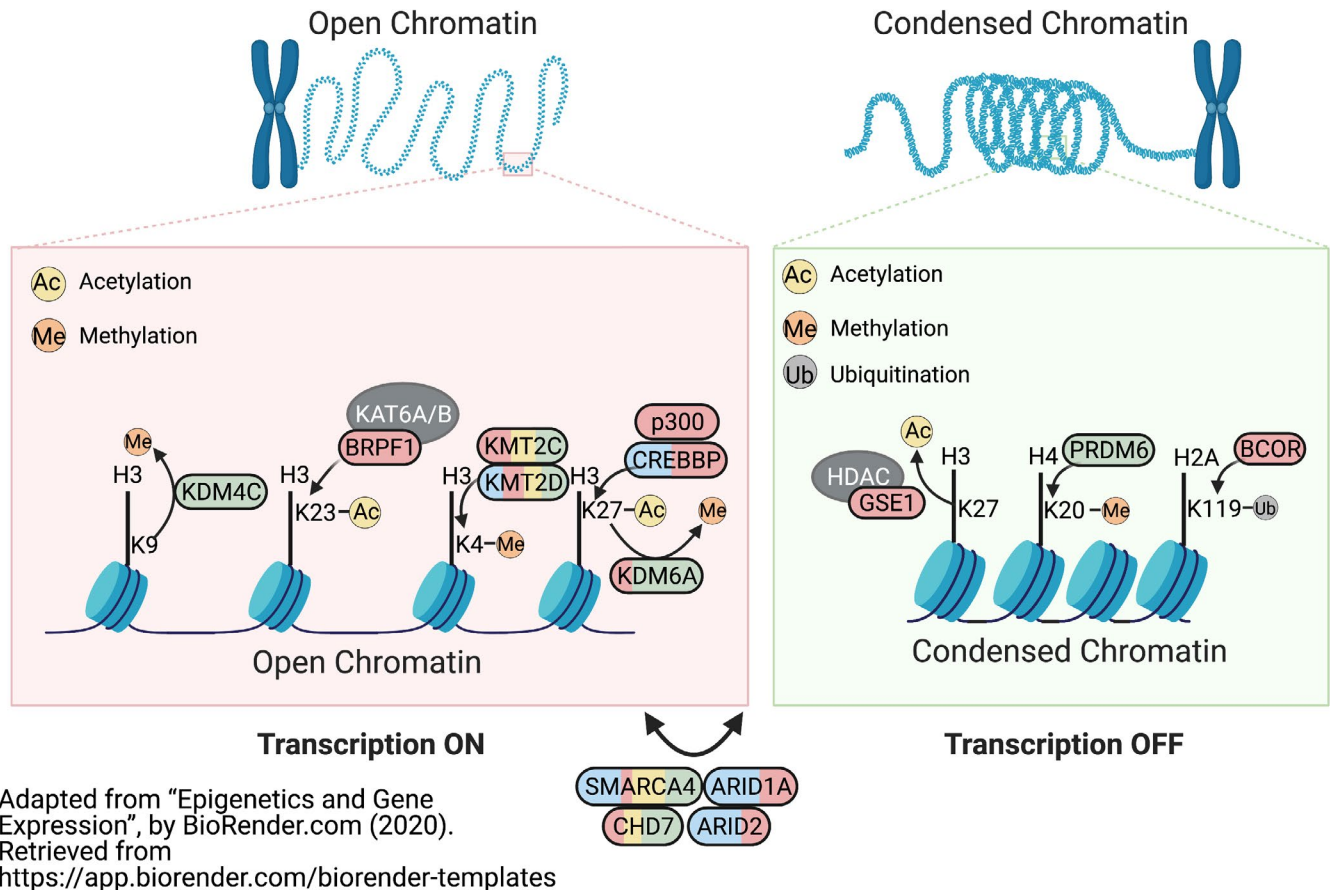

FIGURE 1 Schematic representation illustrating roles of chromatin regulators recurrently mutated in medulloblastoma. The blue, red, yellow, and green genes represent the ones mutated in MB$_{WNT}$, MB$_{SHH,}$ MB$_{Grp3}$, and MB$_{Grp4}$, respectively. Genes with more than 2 mutations that are reported in the PeCan portal (https://pecan.stjude.cloud/bubble/BT-MB) are shown in this figure

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.[5] 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.[6] 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.[7,8] In addition to these studies proposing a multilayered classification, many studies have identified subgroup-specific molecular features from various biological perspectives.[9-18] 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.[19-21] Although the generation of germline 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.[22] 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).[13,14] Moreover, pediatric tumors including MB have lower mutational burdens compared with adult tumors[23], 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
	Goup4	2.2
CREBBP	WNT	4.2
	SHH	7.2
GSE1	SHH	5.0
KDM4C	Group4	1.5
KDM6A	SHH	1.1
	Goup4	8.9
KMT2C	SHH	5.5
	Group3	3.3
	Goup4	7.4
KMT2D	WNT	7.0
	SHH	13.8
	Group3	6.1
	Group4	2.6
p300	SHH	1.1
PRDM6	Gourp4	10.4*
SMARCA4	WNT	21.1
	SHH	2.2
	Group3	9.4
	Group4	1.5

*Given that MB$_{Grp4}$ patients harboring *SNCAIP*-associated structural variants exhibit *PRDM6* upregulation,[11,14] the frequency of *PRDM6* abnormalities is determined as the frequency of the *SNCAIP* mutations.

2 | BIOLOGICAL FUNCTIONS OF CHROMATIN REGULATORS IN THE PATHOGENESIS OF MBS

2.1 | SHH subgroup

MB$_{SHH}$ 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 MB$_{SHH}$ cases, *Patched 1* (*PTCH1*), 49.6% in MB$_{SHH}$ cases, *Smoothened* (*SMO*), 17.3% in MB$_{SHH}$ cases), *Suppressor of fused homolog* (*SUFU*), 14.3% in MB$_{SHH}$ cases), and *Glioma-associated oncogene homolog 2* (*GLI2*), 7.5% in MB$_{SHH}$ cases) are frequently mutated or amplified,[13] 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,[24] previous studies have proposed the cells committed to GNP cell fate as a cellular origin of MB$_{SHH}$s. Several reports have revealed that GNP was a cell of origin of MB$_{SHH}$ 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).[25-27] These animal studies suggested that the mutations activating SHH signaling in GNPs are one cause of MB$_{SHH}$ 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 MB$_{SHH}$. 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).[28,29] 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.[28,29] 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).[30] The balance between mitotic and postmitotic states of GNPs is strictly regulated by multiple signaling pathways[31]; disruption of cell cycle exit also influences MB$_{SHH}$ 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 MB$_{SHH}$[32] and accelerates MB$_{SHH}$ formation via repression of the cell cycle exit of GNPs.[33] In addition, factors promoting differentiation of GNPs, such as WNT, bone morphogenetic proteins (BMP) and NeuroD1, antagonize tumor cell proliferation.[34-36] 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.[37] Indeed, HDACs have been shown to regulate GLI1 activity from multiple directions. HDACs directly bind to the *Gli1* 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 flx/flx; Ptch1+/-	Sonic hedgehog	Genetic deletion of Bcor by Cre-mediated DNA recombination in Atoh1-expressing cells including GNPs in the Ptch1 heterozygous medulloblastoma-prone background.	[45]
BRPF1	SmoM2-YFP flx/+	Sonic hedgehog	In vivo transfection of plasmids encoding Cre and Brpf1 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 flx/flx; SmoM2-YFP flx/+	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 flx/flx; SmoM2-YFP flx/+	Sonic hedgehog	Cre-mediated deletion of Smarca4 in Atoh1-positive cell lineages in the SmoM2-expressing medulloblastoma-prone background.	[38]
KMT2D	Nestin-Cre; Kmt2d flx/flx	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 flx/flx, 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_{SHH} and MB_{Grp3}, 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.[38] These studies implied that Rack-1 could regulate SHH signaling non-epigenetically and epigenetically via HDAC stabilization. Therefore, these data indicated that MB_{SHH} 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_{SHH} progression. An early milestone study using Ptch1 heterozygous mice (Ptch1+/- mice), a faithful MB_{SHH} 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_{SHH} 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_{SHH} pathogenesis, further revealing mutation-dependent characteristics of individual tumors (Figure 2C).

Recurrent mutations unveiled by next-generation sequencing on human MBs give us a clue to the secondary genetic hits for MB_{SHH} 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_{SHH} tumorigenesis and mutations of chromatin modifiers are potential second hits.

BCL6 interacting corepressor (BCOR) is one of the recurrently mutated chromatin regulators in MB_{SHH} and has been considered as a potential second hit. BCOR was initially identified as an interacting partner of BCL6 transcriptional repressor.[43] Subsequently
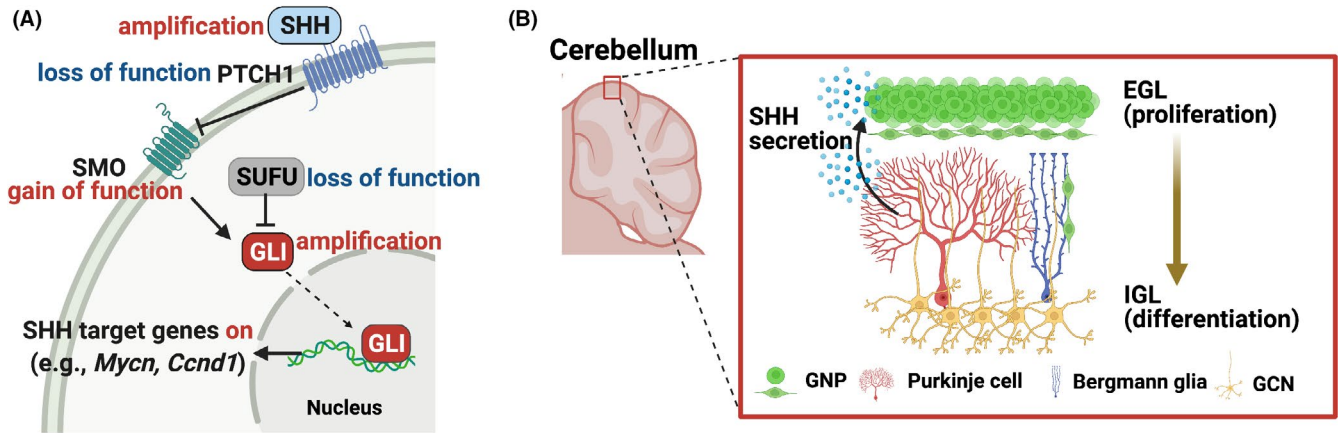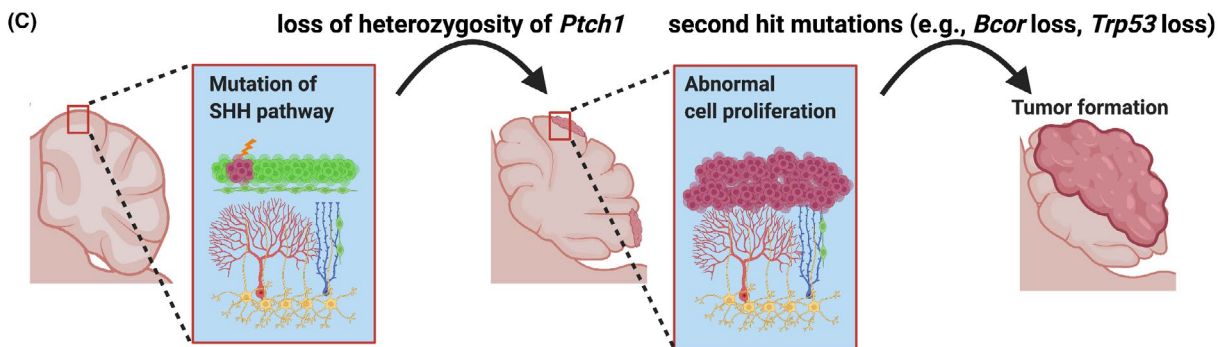
FIGURE 2 Normal differentiation of cerebellar granule neuron progenitors (GNPs) and the pathogenesis of MB_SHH. A, Illustration depicting SHH activation in GNPs. When SHH binds to PTCH1, inhibition of SMO by PTCH1 is released, resulting in the activation of the downstream transcription factor, GLI. SUFU inhibits the activation of GLI. The active form of GLI translocates to the nucleus and initiates the transcription of downstream target genes. MB_SHHs carry mutations that affect the components of this cascade to constitutively activate the pathway. B, GNP proliferation is stimulated by sonic hedgehog (SHH) secreted from Purkinje cells at the external granule layer (EGL). After exiting cell cycle, GNPs inwardly migrate along the fibers of Bergmann glia followed by maturation to granule cell neurons (GCNs) in the internal granule layer (IGL). C, Multistep tumorigenesis of MB_SHH. Not only the mutations of the SHH pathway but also the second hit mutations are required for malignant transformation

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.[44] 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 GNPs (Figure 3A,B).[45] 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),[46] the mutations of BCOR in human MB_SHHs mostly yielded truncated mutants with an intact BCL6 binding domain, but lacking the PUFD domain, which is essential for proper formation of PRC1.1.[47] 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,[13] 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_SHHs. A recent study revealed the cooperative contribution of BRPF1 mutations and SHH pathway mutations to the oncogenesis of adult MB_SHH.[48] In this report, the expression of SmoM2, a constitutively active form of Smo[49,50] in differentiated cerebellar GCNs was shown to induce de-differentiation of GCNs and generated a tumor resembling adult
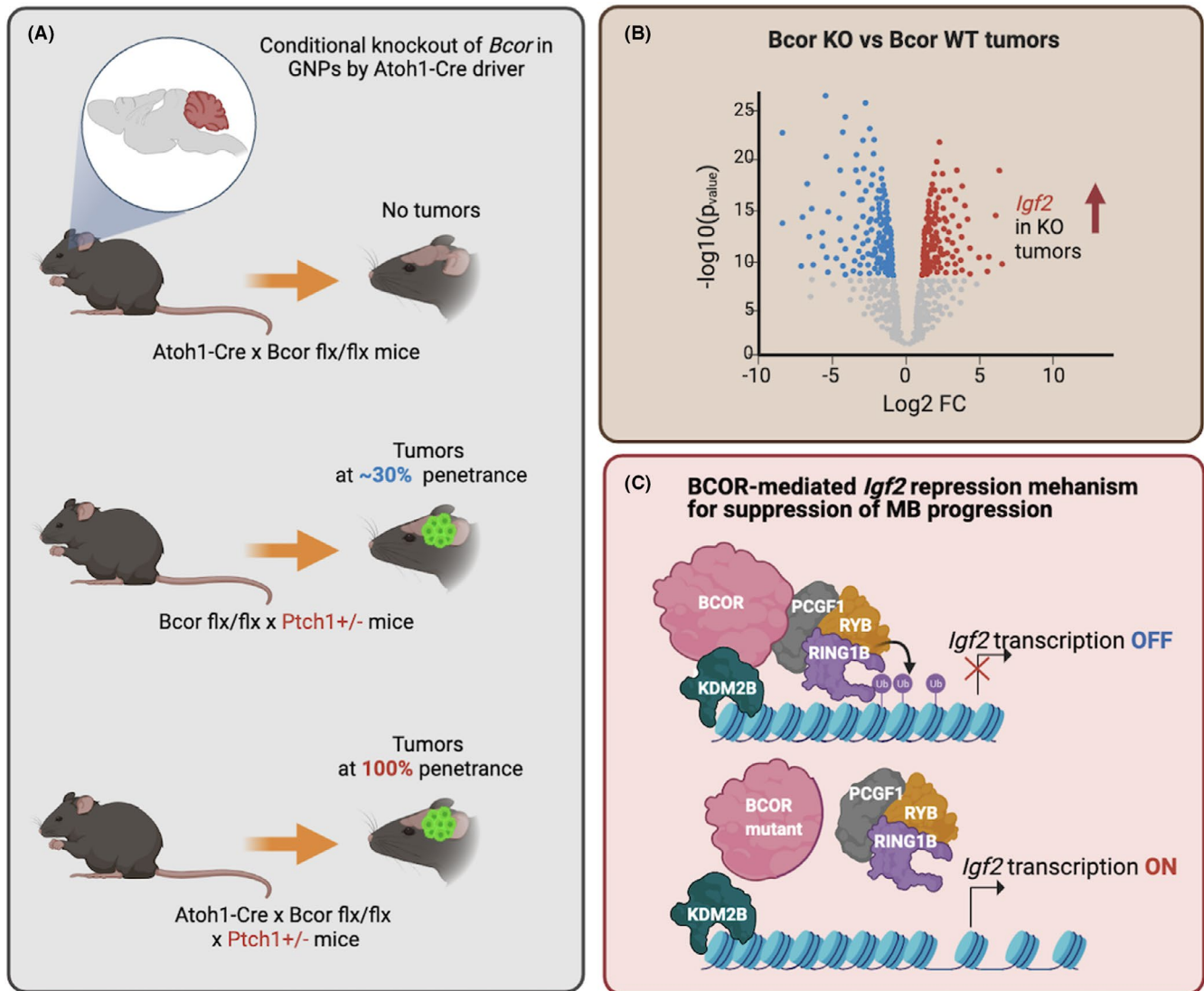
FIGURE 3 A, B, *Bcor* functional loss induces the upregulation of *Igf2* in *Ptch1*^{+/-} preneoplastic lesions and tumors, therefore causing the acceleration of tumorigenesis. C, *Igf2* activation can be explained by dysfunction of BCOR-mediated polycomb repressor complex 1.1 in the genomic locus of *Igf2*

MB$_{SHH}$s. 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$_{SHH}$s remains to be identified.[48] 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$_{SHH}$s. 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$_{SHH}$s.

Another example has underlined the importance of the timing of somatic deletion in MB$_{SHH}$ pathogenesis. *CREB binding protein* (*CREBBP*), a transcriptional co-activator harboring HAT activity, is

also predominantly mutated in adult MB$_{SHH}$s.[13] 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.[51] 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$_{SHH}$s has been shown to acetylate non-histone proteins, including TP53, to regulate their transcriptional activities.[52] The acetylation of TP53 is essential for its transcriptional activity,

therefore affecting cell cycle arrest, senescence, and apoptosis.[53] Considering that the most frequent mutations in *CREBBP* and *p300* are observed in their HAT domains,[13] MB$_{SHH}$s carrying mutations in *CREBBP* and *p300* may influence TP53 activity. A recent study showed that TP53 inactivation enhanced MB$_{SHH}$ tumorigenesis by escaping from oncogene-induced senescence.[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$_{Grp3}$ and MB$_{Grp4}$ were originally named non-WNT/SHH MBs. While our recent study has identified Rous sarcoma oncogene (SRC) signaling as an oncogenic driver of MB$_{Grp4}$,[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$_{Grp4}$s and unipolar brush cells, while the MB$_{Grp3}$ transcriptome does not resemble any specific types of cerebellar cells statistically.[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$_{Grp3}$. *MYC* amplification was observed in 17% of MB$_{Grp3}$ cases.[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.[1,56,57] *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 *growth factor independent 1 transcription repressor* (*GFI1*) through genomic rearrangement specific to MYC-amplified MB$_{Grp3}$ may drive their tumorigenesis.[12] Indeed, the upregulation of *GFI1B* was observed in 10.7% of MB$_{Grp3}$ cases.[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.[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.[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$_{Grp3}$ 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 *Lysine-specific histone demethylase 1a* (*Kdm1a*) for progression of MYC/GFI1-driven tumors.[59] Of note, while Gfi1 represses the TP53 pathway in the hematopoietic system,[60-62] the TP53 pathway for DNA damage responses and the Major histocompatibility complex class I (MHC-I) trafficking is still intact in this model,[63] therefore predicting the effectiveness of TP53 activation for therapeutic treatment on MYC/GFI1-driven MB$_{Grp3}$s, although preclinical studies using humanized models are still required. Second, although Enhancer of zeste homolog 2 (Ezh2) inhibitors are often proposed as MB therapies,[64-66] 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. *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.[67] These tumors exhibited upregulation of Ras-GTPase activators and Notch signaling due to dysregulation of DNA methyltransferase 3A (DNMT3A)-catalyzed DNA methylation and Sirtuin1/Bcl6-mediated histone modification.[67] Notably, while the tumors specifically expressed Natriuretic peptide receptor 3 (NPR3) and MYC, which are MB$_{Grp3}$-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$_{Grp3/4}$s.[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$_{SHH}$ formation.[38] In addition, a previous study with human *MYC*-amplified MB$_{Grp3}$ 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).[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,[69] whether *SMARCA4* missense mutations discovered in MB$_{WNT}$s and MB$_{SHH}$s share similar functions with those of MB$_{Grp3}$ 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[70] and regulates tumor cell proliferation in MB$_{Grp4}$

patient-derived cell lines.[71] The pro-proliferative effect by CHD7 silencing on human MB_Grp4 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.[71] Provided that loss-of-function mutations in _CHD7_ have been also detected in MB_SHH_s and MB_Grp3_s 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,[72] CUT&RUN[73] and CUT&Tag[74] 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).[75] 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.

DISCLOSURE
The authors have no conflict of interest.

ORCID
Ryo Shiraishi [ID] https://orcid.org/0000-0003-3971-605X
Daisuke Kawauchi [ID] https://orcid.org/0000-0002-1254-9725

REFERENCES
1. Taylor MD, Northcott PA, Korshunov A, et al. Molecular subgroups of medulloblastoma: the current consensus. _Acta Neuropathol._ 2012;123:465-472.
2. Maier H, Dalianis T, Kostopoulou ON. New approaches in targeted therapy for medulloblastoma in children. _Anticancer Res._ 2021;41:1715-1726.
3. Orr BA. Pathology, diagnostics, and classification of medulloblastoma. _Brain Pathol._ 2020;30:664-678.
4. Archer TC, Mahoney EL, Pomeroy SL. Medulloblastoma: molecular classification-based personal therapeutics. _Neurotherapeutics._ 2017;14:265-273.
5. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. _Acta Neuropathol._ 2016;131:803-820.
6. Zhukova N, Ramaswamy V, Remke M, et al. Subgroup-specific prognostic implications of TP53 mutation in medulloblastoma. _J Clin Oncol._ 2013;31:2927-2935.
7. Cavalli FMG, Remke M, Rampasek L, et al. Intertumoral heterogeneity within medulloblastoma subgroups. _Cancer Cell._ 2017;31:737-754 e736.
8. Schwalbe EC, Lindsey JC, Nakjang S, et al. Novel molecular subgroups for clinical classification and outcome prediction in childhood medulloblastoma: a cohort study. _Lancet Oncol._ 2017;18:958-971.
9. Northcott PA, Nakahara Y, Wu X, et al. Multiple recurrent genetic events converge on control of histone lysine methylation in medulloblastoma. _Nat Genet._ 2009;41:465-472.
10. Dubuc AM, Morrissy AS, Kloosterhof NK, et al. Subgroup-specific alternative splicing in medulloblastoma. _Acta Neuropathol._ 2012;123:485-499.
11. Northcott PA, Shih DJ, Peacock J, et al. Subgroup-specific structural variation across 1,000 medulloblastoma genomes. _Nature._ 2012;488:49-56.
12. Northcott PA, Lee C, Zichner T, et al. Enhancer hijacking activates GFI1 family oncogenes in medulloblastoma. _Nature._ 2014;511:428-434.
13. Kool M, Jones DT, Jager N, et al. Genome sequencing of SHH medulloblastoma predicts genotype-related response to smoothened inhibition. _Cancer Cell._ 2014;25:393-405.
14. Northcott PA, Buchhalter I, Morrissy AS, et al. The whole-genome landscape of medulloblastoma subtypes. _Nature._ 2017;547:311-317.
15. Forget A, Martignetti L, Puget S, et al. Aberrant ERBB4-SRC signaling as a Hallmark of Group 4 medulloblastoma revealed by integrative phosphoproteomic profiling. _Cancer Cell._ 2018;34:379-395 e377.
16. Suzuki H, Kumar SA, Shuai S, et al. Recurrent noncoding U1 snRNA mutations drive cryptic splicing in SHH medulloblastoma. _Nature._ 2019;574:707-711.
17. Waszak SM, Robinson GW, Gudenas BL, et al. Germline Elongator mutations in Sonic Hedgehog medulloblastoma. _Nature._ 2020;580:396-401.
18. Robinson G, Parker M, Kranenburg TA, et al. Novel mutations target distinct subgroups of medulloblastoma. _Nature._ 2012;488:43-48.
19. Bassett EA, Tokarew N, Allemano EA, et al. Norrin/Frizzled4 signalling in the preneoplastic niche blocks medulloblastoma initiation. _eLife._ 2016;5.
20. Maximov V, Chen Z, Wei Y, et al. Tumour-associated macrophages exhibit anti-tumoural properties in Sonic Hedgehog medulloblastoma. _Nat Commun._ 2019;10:2410.
21. Yao M, Ventura PB, Jiang Y, et al. Astrocytic trans-differentiation completes a multicellular paracrine feedback loop required for medulloblastoma tumor growth. _Cell._ 2020;180:502-520 e519.
22. Zuckermann M, Hovestadt V, Knobbe-Thomsen CB, et al. Somatic CRISPR/Cas9-mediated tumour suppressor disruption enables versatile brain tumour modelling. _Nat Commun._ 2015;6:7391.
23. Chalmers ZR, Connelly CF, Fabrizio D, et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. _Genome Med._ 2017;9:34.

24. Wechsler-Reya RJ, Scott MP. Control of neuronal precursor proliferation in the cerebellum by Sonic Hedgehog. *Neuron.* 1999;22:103-114.

25. Schuller U, Heine VM, Mao J, et al. Acquisition of granule neuron precursor identity is a critical determinant of progenitor cell competence to form Shh-induced medulloblastoma. *Cancer Cell.* 2008;14:123-134.

26. Yang ZJ, Ellis T, Markant SL, et al. Medulloblastoma can be initiated by deletion of Patched in lineage-restricted progenitors or stem cells. *Cancer Cell.* 2008;14:135-145.

27. Li P, Du F, Yuelling LW, et al. A population of Nestin-expressing progenitors in the cerebellum exhibits increased tumorigenicity. *Nat Neurosci.* 2013;16:1737-1744.

28. Machold R, Fishell G. Math1 is expressed in temporally discrete pools of cerebellar rhombic-lip neural progenitors. *Neuron.* 2005;48:17-24.

29. Wang VY, Rose MF, Zoghbi HY. Math1 expression redefines the rhombic lip derivatives and reveals novel lineages within the brainstem and cerebellum. *Neuron.* 2005;48:31-43.

30. Chedotal A. Should I stay or should I go? Becoming a granule cell. *Trends Neurosci.* 2010;33:163-172.

31. Behesti H, Marino S. Cerebellar granule cells: insights into proliferation, differentiation, and role in medulloblastoma pathogenesis. *Int J Biochem Cell Biol.* 2009;41:435-445.

32. Flora A, Klisch TJ, Schuster G, Zoghbi HY. Deletion of Atoh1 disrupts Sonic Hedgehog signaling in the developing cerebellum and prevents medulloblastoma. *Science.* 2009;326:1424-1427.

33. Ayrault O, Zhao H, Zindy F, Qu C, Sherr CJ, Roussel MF. Atoh1 inhibits neuronal differentiation and collaborates with Gli1 to generate medulloblastoma-initiating cells. *Cancer Res.* 2010;70:5618-5627.

34. Poschl J, Bartels M, Ohli J, et al. Wnt/beta-catenin signaling inhibits the Shh pathway and impairs tumor growth in Shh-dependent medulloblastoma. *Acta Neuropathol.* 2014;127:605-607.

35. Zhao H, Ayrault O, Zindy F, Kim JH, Roussel MF. Post-transcriptional down-regulation of Atoh1/Math1 by bone morphogenic proteins suppresses medulloblastoma development. *Genes Dev.* 2008;22:722-727.

36. Cheng Y, Liao S, Xu G, et al. NeuroD1 dictates tumor cell differentiation in medulloblastoma. *Cell Rep.* 2020;31:107782.

37. Yang H, Zhu Q, Cheng J, et al. Opposite regulation of Wnt/beta-catenin and SHH signaling pathways by Rack1 controls mammalian cerebellar development. *Proc Natl Acad Sci USA.* 2019;116:4661-4670.

38. Shi X, Wang Q, Gu J, Xuan Z, Wu JI. SMARCA4/Brg1 coordinates genetic and epigenetic networks underlying Shh-type medulloblastoma development. *Oncogene.* 2016;35:5746-5758.

39. Goodrich LV, Milenkovic L, Higgins KM, Scott MP. Altered neural cell fates and medulloblastoma in mouse patched mutants. *Science.* 1997;277:1109-1113.

40. Oliver TG, Read TA, Kessler JD, et al. Loss of patched and disruption of granule cell development in a pre-neoplastic stage of medulloblastoma. *Development.* 2005;132:2425-2439.

41. Kessler JD, Hasegawa H, Brun SN, et al. N-myc alters the fate of preneoplastic cells in a mouse model of medulloblastoma. *Genes Dev.* 2009;23:157-170.

42. Tamayo-Orrego L, Wu CL, Bouchard N, et al. Evasion of cell senescence leads to medulloblastoma progression. *Cell Rep.* 2016;14:2925-2937.

43. Huynh KD, Fischle W, Verdin E, Bardwell VJ. BCoR, a novel corepressor involved in BCL-6 repression. *Genes Dev.* 2000;14:1810-1823.

44. Gearhart MD, Corcoran CM, Wamstad JA, Bardwell VJ. Polycomb group and SCF ubiquitin ligases are found in a novel BCOR complex that is recruited to BCL6 targets. *Mol Cell Biol.* 2006;26:6880-6889.

45. Kutscher LM, Okonechnikov K, Batora NV, et al. Functional loss of a noncanonical BCOR-PRC1.1 complex accelerates SHH-driven medulloblastoma formation. *Genes Dev.* 2020;34:1161-1176.

46. Tiberi L, Bonnefont J, van den Ameele J, et al. A BCL6/BCOR/SIRT1 complex triggers neurogenesis and suppresses medulloblastoma by repressing Sonic Hedgehog signaling. *Cancer Cell.* 2014;26:797-812.

47. Junco SE, Wang R, Gaipa JC, et al. Structure of the polycomb group protein PCGF1 in complex with BCOR reveals basis for binding selectivity of PCGF homologs. *Structure.* 2013;21:665-671.

48. Aiello G, Ballabio C, Ruggeri R, et al. Truncated BRPF1 cooperates with smoothened to promote adult SHH medulloblastoma. *Cell Rep.* 2019;29:4036-4052 e4010.

49. Xie J, Murone M, Luoh SM, et al. Activating Smoothened mutations in sporadic basal-cell carcinoma. *Nature.* 1998;391:90-92.

50. Mao J, Ligon KL, Rakhlin EY, et al. A novel somatic mouse model to survey tumorigenic potential applied to the Hedgehog pathway. *Cancer Res.* 2006;66:10171-10178.

51. Merk DJ, Ohli J, Merk ND, et al. Opposing effects of CREBBP mutations govern the phenotype of Rubinstein-Taybi syndrome and adult SHH medulloblastoma. *Dev Cell.* 2018;44:709-724 e706.

52. Ito A, Lai CH, Zhao X, et al. p300/CBP-mediated p53 acetylation is commonly induced by p53-activating agents and inhibited by MDM2. *EMBO J.* 2001;20:1331-1340.

53. Reed SM, Quelle DE. p53 Acetylation: regulation and consequences. *Cancers (Basel).* 2014;7:30-69.

54. Hovestadt V, Smith KS, Bihannic L, et al. Resolving medulloblastoma cellular architecture by single-cell genomics. *Nature.* 2019;572:74-79.

55. Vladoiu MC, El-Hamamy I, Donovan LK, et al. Childhood cerebellar tumours mirror conserved fetal transcriptional programs. *Nature.* 2019;572:67-73.

56. Kawauchi D, Robinson G, Uziel T, et al. A mouse model of the most aggressive subgroup of human medulloblastoma. *Cancer Cell.* 2012;21:168-180.

57. Pei Y, Moore CE, Wang J, et al. An animal model of MYC-driven medulloblastoma. *Cancer Cell.* 2012;21:155-167.

58. Tao R, Murad N, Xu Z, et al. MYC drives group 3 medulloblastoma through transformation of Sox2(+) astrocyte progenitor cells. *Cancer Res.* 2019;79:1967-1980.

59. Lee C, Rudneva VA, Erkek S, et al. Lsd1 as a therapeutic target in Gfi1-activated medulloblastoma. *Nat Commun.* 2019;10:332.

60. Duan Z, Zarebski A, Montoya-Durango D, Grimes HL, Horwitz M. Gfi1 coordinates epigenetic repression of p21Cip/WAF1 by recruitment of histone lysine methyltransferase G9a and histone deacetylase 1. *Mol Cell Biol.* 2005;25:10338-10351.

61. Grimes HL, Gilks CB, Chan TO, Porter S, Tsichlis PN. The Gfi-1 protooncoprotein represses Bax expression and inhibits T-cell death. *Proc Natl Acad Sci USA.* 1996;93:14569-14573.

62. Khandanpour C, Phelan JD, Vassen L, et al. Growth factor independence 1 antagonizes a p53-induced DNA damage response pathway in lymphoblastic leukemia. *Cancer Cell.* 2013;23:200-214.

63. Garancher A, Suzuki H, Haricharan S, et al. Tumor necrosis factor overcomes immune evasion in p53-mutant medulloblastoma. *Nat Neurosci.* 2020;23:842-853.

64. Vo BT, Li C, Morgan MA, et al. Inactivation of Ezh2 upregulates Gfi1 and drives aggressive MYC-driven group 3 medulloblastoma. *Cell Rep.* 2017;18:2907-2917.

65. Ballabio C, Anderle M, Gianesello M, et al. Modeling medulloblastoma in vivo and with human cerebellar organoids. *Nat Commun.* 2020;11:583.

66. Natsumeda M, Liu Y, Nakata S, et al. Inhibition of enhancer of zest homologue 2 is a potential therapeutic target for high-MYC medulloblastoma. *Neuropathology.* 2019;39:71-77.

67. Dhar SS, Zhao D, Lin T, et al. MLL4 is required to maintain broad H3K4me3 peaks and super-enhancers at tumor suppressor genes. _Mol Cell_. 2018;70:825-841 e826.

68. Dykhuizen EC, Hargreaves DC, Miller EL, et al. BAF complexes facilitate decatenation of DNA by topoisomerase IIalpha. _Nature_. 2013;497:624-627.

69. Fernando TM, Piskol R, Bainer R, et al. Functional characterization of SMARCA4 variants identified by targeted exome-sequencing of 131,668 cancer patients. _Nat Commun_. 2020;11:5551.

70. Feng W, Kawauchi D, Korkel-Qu H, et al. Chd7 is indispensable for mammalian brain development through activation of a neuronal differentiation programme. _Nat Commun_. 2017;8:14758.

71. Badodi S, Dubuc A, Zhang X, et al. Convergence of BMI1 and CHD7 on ERK Signaling in Medulloblastoma. _Cell Rep_. 2017;21:2772-2784.

72. Buenrostro JD, Giresi PG, Zaba LC, Chang HY, Greenleaf WJ. Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. _Nat Methods_. 2013;10:1213-1218.

73. Skene PJ, Henikoff JG, Henikoff S. Targeted in situ genome-wide profiling with high efficiency for low cell numbers. _Nat Protoc_. 2018;13:1006-1019.

74. Kaya-Okur HS, Janssens DH, Henikoff JG, Ahmad K, Henikoff S. Efficient low-cost chromatin profiling with CUT&Tag. _Nat Protoc_. 2020;15:3264-3283.

75. Tang Y, Gholamin S, Schubert S, et al. Epigenetic targeting of Hedgehog pathway transcriptional output through BET bromodomain inhibition. _Nat Med_. 2014;20:732-740.