SPECIAL SECTION: REVIEW

Arrested development: the dysfunctional life history of medulloblastoma

Ran Tao,[1,2,5] Katie Han,[1,2,3,5] Stephanie C. Wu,[1,2,5] Jake D. Friske,[3,4] Martine F. Roussel,[4] and Paul A. Northcott[1,2]

[1]Center of Excellence in Neuro-Oncology Sciences, St. Jude Children's Research Hospital, Memphis, Tennessee 38105, USA; [2]Department of Developmental Neurobiology, St. Jude Children's Research Hospital, Memphis, Tennessee 38105, USA; [3]St. Jude Graduate School of Biomedical Sciences, St. Jude Children's Research Hospital, Memphis, Tennessee 38105, USA; [4]Department of Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, Tennessee 38105, USA

Medulloblastoma is a heterogeneous embryonal tumor of the cerebellum comprised of four distinct molecular subgroups that differ in their developmental origins, genomic landscapes, clinical presentation, and survival. Recent characterization of the human fetal cerebellum at single-cell resolution has propelled unprecedented insights into the cellular origins of medulloblastoma subgroups, including those underlying previously elusive groups 3 and 4. In this review, the molecular pathogenesis of medulloblastoma is examined through the lens of cerebellar development. In addition, we discuss how enhanced understanding of medulloblastoma origins has the potential to refine disease modeling for the advancement of treatment and outcomes.

Medulloblastoma (MB) is a World Health Organization (WHO) grade IV, embryonal tumor of the cerebellum predominantly diagnosed during childhood (Louis et al. 2016). Decades of elegant mouse modeling studies have demonstrated that MB initiates in the developing hindbrain when normal differentiation hierarchies are perturbed by growth-promoting genetic alterations (Wu et al. 2011; Northcott et al. 2019; Roussel and Stripay 2020). Disruptions in distinct cellular lineages underly the intertumoral heterogeneity associated with MB subgroups (WNT, SHH, group 3, and group 4), culminating in distinct diseases with varying ages of onset, metastatic potential, and survival (Taylor et al. 2012; Northcott et al. 2012a; Northcott et al. 2019; Hovestadt et al. 2020). Recent studies have further divided core subgroups into unique molecular subtypes, providing a framework for more precise clinical diagnoses that enhance risk stratification for specific patients (Cavalli et al. 2017; Northcott et al. 2017; Schwalbe et al. 2017).

Distinct clinical features characterize each MB subgroup as a sequela to their defining molecular traits (Fig. 1). Constituting ~10%–15% of all cases, WNT-MB occurs in children >4 years of age and often presents with localized disease at the time of diagnosis. The prognosis for WNT-MB is highly favorable, with a 5 year survival rate of >95% in children and 80%–95% when diagnosed in adulthood (Ellison et al. 2005; Clifford et al. 2006, 2015; Remke et al. 2011). Histologically, WNT-MB is predominantly of the classic variant and demonstrates hemorrhagic vasculature due to disruptions in blood–brain barrier integrity (Phoenix et al. 2016).

SHH-MB comprises ~30% of all MB diagnoses and presents with a bimodal age distribution (i.e., enriched in infants and adults). Outcomes for SHH-MB vary widely depending on the age of diagnosis, *TP53* mutation status, methylation subtype, and other prognostic biomarkers (Gajjar et al. 2021). For example, children harboring *TP53* mutations, either germline or somatic, exhibit poor outcomes and generally fail to respond to therapy (Zhukova et al. 2013). Conversely, SHH-2 infantile tumors and childhood SHH-MBs devoid of high-risk clinical and molecular features (i.e., *TP53* wild type, balanced chromosome 17p, no *GLI2* or *MYCN* amplification, M0 disease, and non-large cell/anaplastic [LCA] histology) have excellent outcomes that exceed 90% (Cavalli et al. 2017; Robinson et al. 2018; Gajjar et al. 2021). SHH-MB includes all histological variants delineated within the disease. Notably, desmoplastic/nodular (DN) and MB with extensive nodularity (MBEN) variants are restricted to the SHH subgroup, with high enrichment of MBEN seen in infants. In contrast, *TP53* mutant SHH-MBs are typically of the LCA variant and carry a poor prognosis (Orr 2020).

Group 3-MB and group 4-MB, sometimes referred to as non-WNT/non-SHH-MB, exist along a clinical and biological continuum and contribute to ~25% and ~35%–40%

[*Keywords*: cerebellum; developmental origins; medulloblastoma; mouse models; organoids; single-cell genomics; stem cell models]
[5]**These authors contributed equally to this work.**
Corresponding authors: paul.northcott@stjude.org, martine.roussel@stjude.org
Article published online ahead of print. Article and publication date are online at http://www.genesdev.org/cgi/doi/10.1101/gad.351936.124. Freely available online through the *Genes & Development* Open Access option.

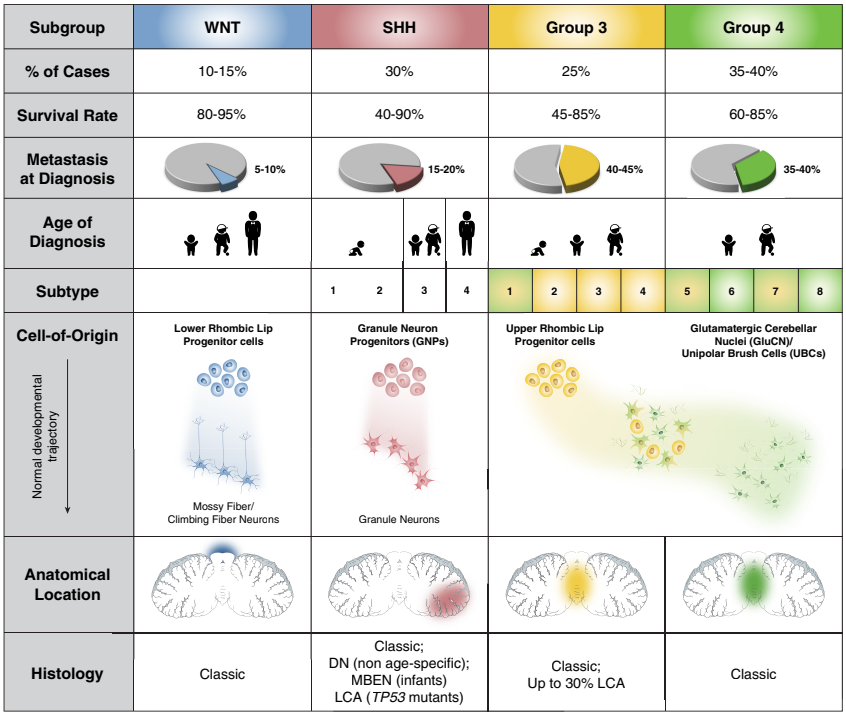

Figure 1. The molecular subgroups and subtypes of MB. Demographic, clinical, and anatomical features of the four molecular subgroups of MB, supplemented with cellular hierarchies of their respective lineages informed by developmental origins. M^+ and M0 are depicted by colored and gray portions of the pie chart, respectively. (DN) Desmoplastic/nodular, (MBEN) medulloblastoma with extensive nodularity, (LCA) large cell anaplastic.

of cases, respectively. Group 3-MB more commonly presents in early childhood, whereas group 4-MB is typically diagnosed in adolescence. Largely regarded as the most aggressive MB subgroup, group 3-MB has a heterogeneous survival rate (i.e., 45%–85%) and is frequently accompanied by metastatic disease. Although a subset of group 4-MB is also metastatic at diagnosis, 5 year survival rates are considered intermediate at ~75%. The average survival estimates for group 3/4-MB can be further subdivided by methylation subtype, where subtype 3 is associated with the worst outcome (5 year survival rate of ~33%), and subtype 7 is associated with the best outcome (5 year survival rate of ~90%) (Northcott et al. 2017, 2019; Sharma et al. 2019; Orr 2020; Gajjar et al. 2021). Histologically, most group 3/4-MBs are of the classic variant. However, up to 30% of group 3-MBs exhibit LCA histology, representing a very clinically aggressive subset of patients (Kool et al. 2012).

Cellular origins of MB are mechanistically linked to the recurrent genetic events suspected to drive each subgroup. Understanding the origins of MB subgroups is critical to characterizing dysregulated developmental pathways, generating faithful models, and designing effective treatment strategies. This review describes the chronological milestones and technologies used to reveal the cellular origins of MB subgroups, with later discussion of how knowledge of MB origins will enhance disease-modeling studies.

Origins of MB informed by mouse models

Foundational knowledge of MB origins has largely come from studying mouse tumor models in the context of development (Fig. 2A; Roussel and Hatten 2011; Roussel and Stripay 2020). The cerebellum derives from rhombomere 1 of the anterior dorsal hindbrain, where neurogenesis is initiated in two discrete germinal zones: the $PTF1A^+$ ventricular zone (VZ) and $ATOH1^+$ upper rhombic lip (RL) (Yamada et al. 2014; Leto et al. 2016; Haldipur et al. 2022). The VZ and upper RL give rise to GABAergic (GABAergic cerebellar nucleus neurons, Purkinje cells, interneurons, and astrocytes) and glutamatergic (granule neuron progenitors [GNPs], glutamatergic cerebellar nuclei [GluCN], and unipolar brush cells [UBCs]) cerebellar lineages, respectively. The lower RL, in contrast, originates from rhombomeres 2–8 and serves as the germinal zone for mossy fiber and climbing fiber neurons in the brainstem nuclei (Landsberg et al. 2005). When growth-promoting genetic alterations occur in specific contexts, lower and upper RL progenitors deviate from normal developmental trajectories, becoming susceptible to MB tumorigenesis.

WNT-MB

WNT-MB is enriched with mutations activating the namesake WNT signaling pathway. Among them, >85% of cases harbor mutations in *CTNNB1* that stabilize the protein (Eberhart et al. 2000; Thompson et al. 2006; Northcott et al. 2017). In a seminal study by Gibson et al. (2010), the gene expression signature of WNT-MB was effectively mapped to the lower RL and dorsal brainstem, rather than the upper RL and cerebellum. To assess the effect of constitutively activated WNT signaling in hindbrain progenitors, a genetically engineered mouse model (GEMM) carrying *Blbp-Cre* and a Cre-dependent dominant stable mutant allele of *Ctnnb1* was generated.

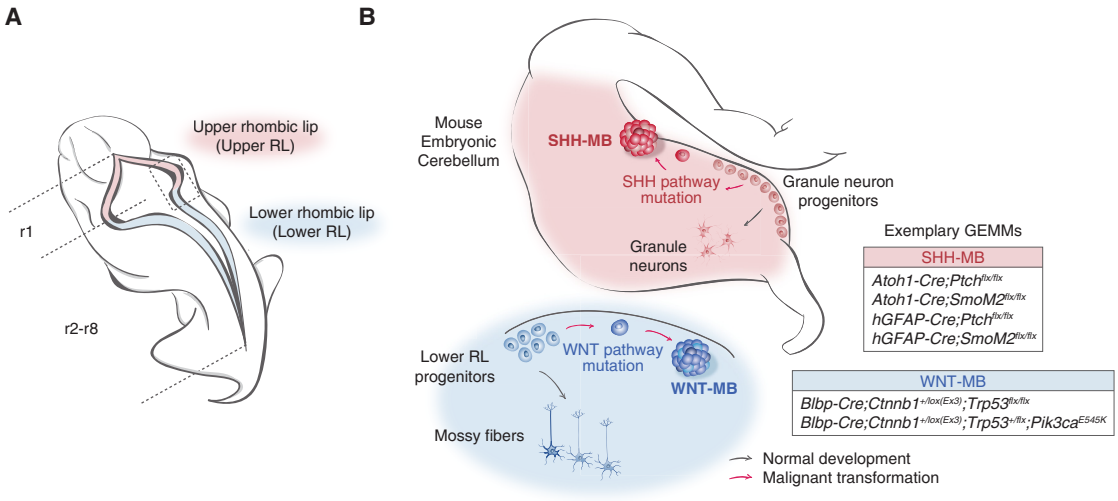

Figure 2. Mouse cerebellar development and inference of WNT-MB and SHH-MB origins from genetically engineered mouse models (GEMMs). (*A*) Schematic of E11.5 mouse embryo showing derivation of upper RL (red) and lower RL (blue) from rhombomere 1 (r1) and rhombomeres 2–8 (r2–r8), respectively. (*B*) Schematic of GEMM-informed cellular origin of WNT-MB and SHH-MB. Context-dependent oncogenic alternations hijack normal differentiation trajectories during development, restricting cells in their progenitor states with subsequent induction of MB formation. (*Top*) Embryonic mouse cerebellum (red). (*Bottom*) Dorsal brainstem (blue). (Black arrows) Normal differentiation trajectories of GNPs to GNs, and lower RL progenitors to mossy fiber neurons. (Red arrows) Malignant transformation of GNPs and lower RL progenitors upon their respective oncogenic events.

Although *Blbp-Cre* efficiently induced recombination in multiple hindbrain progenitors including VZ progenitors, GNPs, and OLIG3$^+$ progenitors in the lower RL at E14.5, proliferation of these progenitors was not significantly affected. Instead, these mice developed premalignancy at E16.5 in the dorsal brainstem that persisted into adulthood. When further crossed with *Trp53*$^{flx/flx}$ mice to inactivate *Trp53* in a Cre-dependent manner, ~15% of mice developed tumors that were histologically, anatomically, and molecularly similar to human WNT-MB (Gibson et al. 2010). Later, next-generation sequencing identified several genes somatically mutated in WNT-MB, including *SMARCA4*, *DDX3X*, *CDH1*, and *PIK3CA* (Jones et al. 2012; Pugh et al. 2012; Robinson et al. 2012). Knockdown of *Cdh1* reduced migration of lower RL progenitors to an extent similar to that of mutant *Ctnnb1*, whereas *Ddx3x* mutations increased proliferation of those progenitors. Moreover, combining *Pik3ca*^{E545K} mutant with *Blbp-Cre;Ctnnb1*$^{+/lox(Ex3)}$*;Trp53*$^{+/flx}$ mice led to a WNT-MB mouse model with 100% penetrance (Robinson et al. 2012). These findings in mice were supported by diagnostic imaging of human tumors, which showed that WNT-MB is predominantly extraparenchymal and frequently infiltrates the dorsal brainstem (Gibson et al. 2010; Wefers et al. 2014; Patay et al. 2015). Collectively, these findings from mouse models strongly suggest that WNT-MB arises from progenitors in the lower RL and dorsal brainstem (Fig. 2B).

SHH-MB

SHH-MBs exhibit pathognomonic genetic alterations in the SHH signaling pathway that promote constitutive mitogenic signaling, including *PTCH1*, *SMO*, *SUFU*, and *GLI2* (Kool et al. 2014; Northcott et al. 2017; Garcia-Lopez et al. 2021). During cerebellar development, proliferating GNPs migrate out of the upper RL to form the external granule layer (EGL), where they continue to expand and increase cerebellar volume in response to Purkinje cell-derived mitogens; namely, SHH. Upon exiting the cell cycle, postmitotic granule neurons (GNs) comprise the most abundant cerebellar cell type (~60% of all neurons in the mouse brain; ~80% of all neurons in the human brain) and ultimately traverse inward along Bergmann glial fibers to forge the internal granule layer (Roussel and Hatten 2011; Leto et al. 2016; Haldipur et al. 2022). Consistent with the pivotal role of SHH signaling in promoting GNP proliferation, SHH-MB is characterized by aberrant SHH signaling (Taylor et al. 2012; Leto et al. 2016; Northcott et al. 2017), indicative of GNPs as their probable cell of origin. Two parallel studies published by Schüller et al. (2008) and Yang et al. (2008) showed that activation of the SHH pathway in multipotent stem cells through *Ptch1* deletion induced an expansion of the stem cell population at E14.5. However, they remained capable of differentiating into astrocytes, oligodendrocytes, and neurons. Only after the stem cells committed to the GN lineage was there a massive expansion of RL and EGL at E16.5 with eventual SHH-MB development (Schüller et al. 2008; Yang et al. 2008). These observations suggest that the acquisition of GNP identity is required for SHH-MB initiation (Fig. 2B). However, GNPs are comprised of heterogeneous populations and cellular states, which potentially exhibit varying susceptibility to transformation. Postnatal NESTIN$^+$ cells located within the inner EGL contribute to GNP genesis and have been reported to exhibit increased tumorigenic potential compared with the bulk of ATOH1$^+$ GNPs (Li et al. 2013).

Likewise, rare and transient SOX2$^+$ GNPs in the developing EGL can also serve as the cell of origin for SHH-MB (Selvadurai et al. 2020). A recent study by Zhang et al. (2019) identified distinct progenitor populations during early tumorigenesis in a *Ptch1$^{fl/fl}$;hGFAP-Cre* MB model. A stem-like OLIG2$^+$ progenitor population expressing SOX2 and NESTIN represented tumor-initiating cells during both primary tumorigenesis and relapse. Depletion of mitotic OLIG2$^+$ progenitors by expressing a suicide gene (or *Olig2* gene knockout) inhibited tumor growth (Zhang et al. 2019). Whether these GNP subpopulations identified from independent studies refer to the same progenitors or represent distinct cell states or identities remains unclear.

Group 3/4-MB

Unlike WNT-MBs and SHH-MBs, groups 3 and 4 are not immediately associated with specific signaling pathways. *MYC* amplification contributes to ~17% of group 3-MB cases, with the remainder typically exhibiting elevated *MYC* expression (Robinson et al. 2012; Northcott et al. 2012b, 2017). In group 4-MB, the most recurrent genetic event is *PRDM6* activation by enhancer hijacking (Northcott et al. 2017). Genetic alterations targeting chromatin-modifying complexes (i.e., SWI/SNF, COMPASS, and PRC2) and developmental transcription factors (i.e., *OTX2*, *GFI1/GFI1B*, and *TBR1*) that are presumed to disrupt normal neurodevelopmental programs are recurrently seen in both subgroups (Bai et al. 2012; Bunt et al. 2012; Jones et al. 2013; Northcott et al. 2014, 2017, 2019; Boulay et al. 2017).

From mouse tumor models alone, developmental origins of group 3/4-MB have remained elusive, as numerous group 3-MB models have been generated from diverse cell populations, and accurate group 4-MB models are lacking. Original studies showed that group 3-like MB tumors can be generated by orthotopic implantation of CD133$^+$ (Prominin-1$^+$) neural stem cells or GNPs isolated from P6–P7 cerebellum and retrovirally transduced to overexpress *Myc* in combination with *Trp53* inactivation, either through overexpression of dominant-negative p53 (DNp53) or using *Trp53*-null GNPs (Kawauchi et al. 2012; Pei et al. 2012). By introducing the same gene perturbations using in utero electroporation (IUE) at E13.5, group 3-like MB models were generated in situ from different embryonic cerebellar progenitors (Kawauchi et al. 2017). In these mouse models, the inactivation of *Trp53*, a strong tumor suppressor, is required for tumorigenesis. Although *TP53* mutations are exceptionally rare in human group 3-MB patient tumors at diagnosis (Ramaswamy et al. 2016; Northcott et al. 2017), some reports have indicated their emergence at relapse (Hill et al. 2015). Group 3-MBs also exhibit frequent chromosome 17p deletion, most often in the context of isochromosome 17q, which may confer *TP53* haploinsufficiency (Northcott et al. 2012b). Using an oncogene combination observed in patient tumors, multiple studies showed that group 3-like MB tumors could be generated by overexpressing *MYC* and *GFI1/GFI1B* in postnatal neural stem cells or GNPs (Northcott et al. 2014; Vo et al. 2017; Lee et al. 2019). By using lentivirus-based approaches to target a broad range of cerebellar cell types including the slowly proliferating stem cells or progenitors, Tao et al. (2019) found that overexpressing *MYC* alone is sufficient to transform P5 Sox2$^+$ progenitors into group 3-like MB tumors without the need for perturbation of a second driver, indicating that these cells may be linked to group 3-MB origins. Besides orthotopic implantation and IUE-based group 3-MB mouse models, GEMMs have also been generated by overexpressing *MYCN* (GTML model) or *MYC* (GMYC) in Glt1$^+$ cells (Swartling et al. 2010, 2012; Mainwaring et al. 2023), although the specific identity of Glt1$^+$ cells has not been definitively characterized.

A bioinformatics study by Pöschl et al. (2014) systematically compared the gene expression profiles of 20 different MB mouse models versus 423 human MBs representing each subgroup. Using multiple bioinformatic methods, WNT-MB and SHH-MB mouse models matched their human counterparts. However, group 3-like MB mouse models generated by *Myc* overexpression and *Trp53* inactivation exhibited transcriptomes that matched to either group 3-MB or SHH-MB human counterparts depending on methodology. It is unclear whether inactivation of *Trp53*, which frequently occurs in SHH-MB but not in group 3-MB, shifted the transcriptome toward that of SHH-MB. In contrast, the GTML model driven by *MYCN* overexpression faithfully aligned to human group 3-MB (Pöschl et al. 2014).

Due to a lack of faithful mouse tumor models, the putative cellular origins of group 4-MB remained unknown until the recent emergence of single-cell transcriptome sequencing technologies and their application to cerebellar development, which we discuss below.

Insights into group 3/4-MB origins from cross-species transcriptomics

Advances in single-cell transcriptome profiling technologies have profoundly enhanced our understanding of cerebellar development in mice and humans. Cataloging the expression state of many thousands of single cells and nuclei has enabled an unbiased characterization of cerebellar cell types and states, inferring their differentiation trajectories and informing the molecular mechanisms governing lineage-specific differentiation hierarchies (Carter et al. 2018; Hovestadt et al. 2019; Vladoiu et al. 2019; Aldinger et al. 2021; Kozareva et al. 2021; Sarropoulos et al. 2021; Sepp et al. 2024; Yang et al. 2024). This information also grants a direct comparison of MB subgroups versus distinct cell lineages of the cerebellum to infer tumor cell of origin by searching for the "best match" between malignant MB cells and normal cerebellar counterparts.

In parallel studies using independent atlases of mouse cerebellar development, Hovestadt et al. (2019) and Vladoiu et al. (2019) were the first to apply these platforms to infer MB origins. In the study performed by Hovestadt et al. (2019), a mouse cerebellar atlas consisting of 13 developmental stages from E10 to P10 was used as a reference for

comparison with human MB expression data. This study found that SHH-MB was highly correlated with GNP populations, consistent with knowledge gleaned from GEMMs (Roussel and Stripay 2020). Interestingly, group 4-MB was highly similar to UBC and GluCN lineages, both of which originate from the upper RL. UBCs are glutamatergic interneurons that relay excitatory synapses to GNs and other UBCs (Mugnaini et al. 2011), whereas GluCN neurons project to different regions of the brain and modulate various cortical and premotor circuits (Bagnall et al. 2009). These findings suggested UBC and GluCN lineages as the putative origins of group 4-MB (Hovestadt et al. 2019), consistent with a previous study that implicated GluCN or upper RL progenitors based on overlapping activity of master transcription factors (Lin et al. 2016). In contrast, no high-confidence correlations were detected between any cerebellar populations and WNT-MB or group 3-MB (Hovestadt et al. 2019). Because WNT-MB is suspected to arise from lower RL progenitors, which were lacking in this purely cerebellar atlas, this negative result was not unexpected (Gibson et al. 2010). However, the lack of a confident cerebellar match for group 3-MB hinted that species-specific differences between humans and mice may have prevented the retrieval of a putative cell type match for this subgroup.

Through deconvolution of bulk MB transcriptomes using a mouse cerebellar atlas spanning nine developmental time points from E10 to P14, Vladoiu et al. (2019) showed that SHH-MB was predominantly comprised of GNPs and group 4-MB was predominantly comprised of UBCs, consistent with the findings above. The same group also observed varying similarities of group 3-MB to multiple cerebellar cell types, including neural stem cells, GNP and UBC lineages, and GABAergic interneurons (Vladoiu et al. 2019), in accordance with previous IUE-based mouse modeling studies (Kawauchi et al. 2017). A study performed by Jessa et al. (2019) characterized the developing pons and forebrain in mouse brains by single-cell transcriptome analysis and predicted the lower RL-derived mossy fiber neuron lineage as the origin of WNT-MB, corroborating previous insights derived from GEMMs (Gibson et al. 2010). Lower RL-derived mossy fiber neuron progenitors express high levels of *Wnt1* (Landsberg et al. 2005), providing an explanation as to why this lineage might be susceptible to constitutively active WNT signaling. A similar study in the context of human development has yet to be performed, as published human hindbrain reference atlases exclude brainstem populations.

Collectively, these studies reinforced suspected cellular origins of WNT-MB and SHH-MB while revealing UBC and GluCN lineages as the putative origin for group 4-MB, emphasizing the power of single-cell technologies and their utility in retracing the developmental history of malignancy.

Human cerebellar atlases inform group 3/4-MB origins

Although mouse modeling and cross-species genomics enabled genuine insights into MB origins, recent advances pertaining to the complexity of human cerebellar development have revealed key nuances that lack conservation in lower species (Fig. 3). First, the duration of cerebellar development is significantly longer in humans compared with mice; the human cerebellum requires 2–3 years to fully mature, whereas the mouse cerebellum only necessitates 30–35 days (van Essen et al. 2020; Haldipur et al. 2022). Second, both the human VZ and upper RL subdivide into anatomically distinct VZs and subventricular zones (SVZs), resulting in structural compartmentalization lacking in mouse cerebellar germinal zones. Notably, the human upper RL is characterized by a vascular bed that structurally separates SOX2$^+$ progenitors of the RL^{VZ} from ATOH1$^+$ GNPs and EOMES$^+$ UBC progenitors of the RL^{SVZ}. Distinct transcriptional signatures also distinguish the RL^{VZ} and RL^{SVZ}, with recapitulation of upper RL lineage trajectories (i.e., GNP/GN and GluCN/UBC) (Aldinger et al. 2021). This is in stark contrast to the mouse upper RL, which compartmentalizes into four molecular territories demarcated by differing expression patterns of *Wls*, *Atoh1*, *Pax6*, *Lmx1a*, and *Eomes* (Yeung et al. 2014). Third, the human RL uniquely undergoes protracted spatiotemporal expansion prior to internalization into the posterior vermis (Haldipur et al. 2019). RL progenitors exhibit high expression levels of *ARHGAP11B*, a human-specific gene known to induce neocortical expansion and foliation (Zhong et al. 2023). Similarly, Sepp et al. (2024) observed a significantly larger abundance of Purkinje cells in the early human fetal cerebellum (Sepp et al. 2024), which likely contributes to the vast increase in cerebellar volume via SHH-induced GNP proliferation. This extended duration of cellular proliferation and tissue expansion increases susceptibility to genetic events that predispose to cerebellar disease, thereby warranting further characterization of MB cellular origins in the developing human cerebellum.

Comparing the MB transcriptome with a human fetal cerebellar transcriptional atlas consisting of 52,419 single cells or nuclei helped clarify the origins of distinct MB subgroups. SHH-MB was confirmed to exhibit significant similarity to GNPs, consistent with previous cross-species transcriptomic studies and mouse modeling. Interestingly, both group 3-MB and group 4-MB aligned to a common RL^{SVZ}–GluCN/UBC differentiation trajectory, the latter of which could not be confidently separated due to the relatively shallow gene detection in the cerebellar reference atlas (Fig. 3; Smith et al. 2022). Using bulk RNA-seq data sets derived from microdissected human fetal cerebellar subcompartments including the RL^{VZ}, RL^{SVZ}, EGL, and Purkinje layer, the RL^{SVZ} signature, but not the RL^{VZ} signature, was enriched in both group 3-MB and group 4-MB (Smith et al. 2022). The RL^{SVZ} expression signature consisted of both photoreceptor and UBC expression programs that are highly expressed in group 3-MB and group 4-MB, respectively. Expression of these signatures placed group 3/4-MB into a transcriptional continuum. Recurrent genetic targets in group 3-MB and group 4-MB, such as *OTX2* amplification and *GFI1*/*GFI1B* enhancer hijacking, are also highly expressed in the RL^{SVZ}. These molecular characterizations were further complemented by MRI mapping of small group

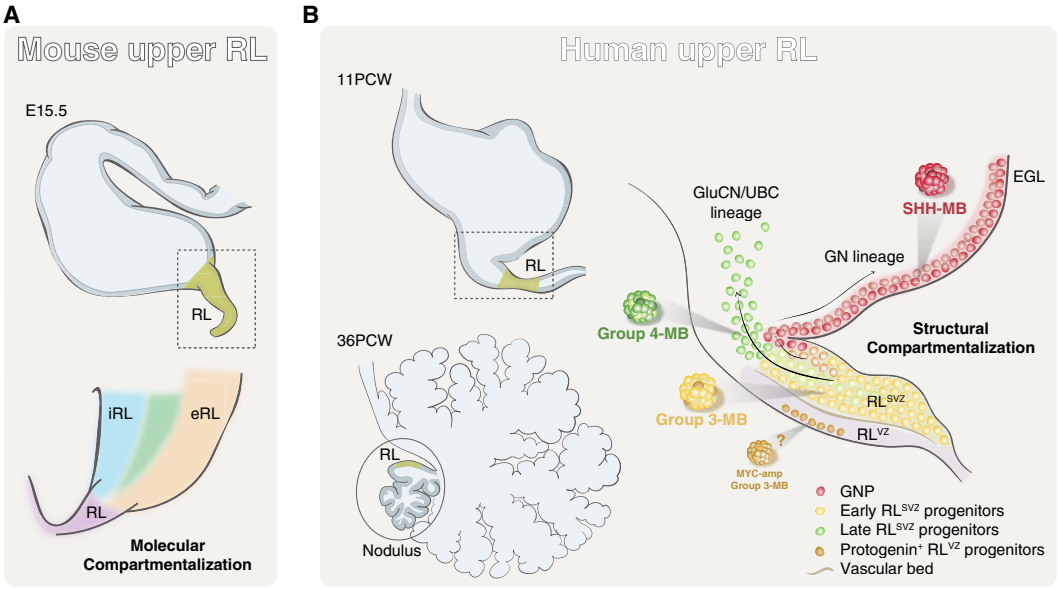

Figure 3. Novel insights into MB origins through comparative single-cell studies. (*A*) Developing mouse upper RL at E15.5. Varying expression patterns of *Wls*, *Atoh1*, *Pax6*, *Lmx1a*, and *Eomes* molecularly compartmentalize the mouse cerebellar RL into four distinct regions. (iRL) Interior face of the RL, (eRL) exterior face of the RL. (*B*) Developing human upper RL at 11 postconception weeks (PCW). A vascular bed structurally compartmentalizes the human cerebellar RL into transcriptionally distinct ventricular and subventricular zones (RL^{VZ} and RL^{SVZ}). The RL^{SVZ} contains progenitors to both GluCN/UBC and GN lineages. From single-cell comparative analyses, the cell of origin for group 3/4-MB is suspected to arise from the GluCN/UBC lineage, with group 3-MB developing from early, less differentiated RL^{SVZ} progenitors and group 4-MB developing from late, more differentiated RL^{SVZ} progenitors. By 36 PCW, the human upper RL involutes into the posterior vermis, specifically at the nodulus. (RL) Rhombic lip, (EGL) external granule layer, (GNP) granule neuron progenitor, (GN) granule neuron, (GluCN) glutamatergic cerebellar nuclei, (UBC) unipolar brush cell.

3/4-MBs caught early in their manifestation into the RL-derived nodulus, which together pinpointed the RL^{SVZ} as the probable origin of both group 3-MB and group 4-MB (Smith et al. 2022). Results from this study were largely substantiated in a parallel study by Hendrikse et al. (2022), where highly recurrent yet previously unreported somatic mutations in *CBFA2T2* and *CBFA2T3* were described in group 3-MB and group 4-MB and linked to their restricted expression in the RL^{SVZ} during early human cerebellar development. Likewise, deconvolution of bulk MB RNA-seq profiles confirmed the resemblance of group 3-MB and group 4-MB to the RL^{SVZ}–GluCN/UBC differentiation trajectory (Hendrikse et al. 2022). Using analogous human–human transcriptomic comparisons, two additional studies reached similar conclusions, both pinpointing the RL^{SVZ}–GluCN/UBC differentiation trajectory as the probable developmental origin of groups 3-MB and 4-MB (Williamson et al. 2022; Okonechnikov et al. 2023).

The studies above converged on similar findings and drew comparable conclusions using different bioinformatics methods, multiple cerebellar reference atlases, and diverse tumor data sets. However, some key discrepancies warrant further discussion, particularly those related to the origins of group 3-MB. For example, Okonechnikov et al. (2023) did not match group 3-MB to any specific lineage, although some similarities between certain group 3-MB subtypes and GN/UBC bipotent progenitors or early differentiating UBCs were implicated. In the study by Hendrikse et al. (2022), stem-like cells in the RL^{VZ} were suggested as the putative origin of group 3-MB subtype γ (i.e., group 3/4-MB subtype 2) using deconvolution. However, the results supporting this conclusion were based on a small proportion of cells, with the highest proportion of group 3-MB cells matching derivatives of the RL^{SVZ} and early UBCs (Hendrikse et al. 2022). In a recent follow-up study from the same group, Visvanathan et al. (2024) proposed Protogenin-positive, *MYC*-expressing stem cells in the RL^{VZ} as the origin of group 3-MB. These findings are in contrast to the Smith et al. (2022) study that aligned group 3-MB and group 4-MB to a shared lineage trajectory within the RL^{SVZ} and found no compelling evidence aligning group 3-MB to stem or progenitor cells of the RL^{VZ}. It is important to note that, as a Yamanaka factor, MYC can drive dedifferentiation and reprogramming (Poli et al. 2018; Sullivan et al. 2022). Thus, it remains plausible that the suggested similarity of some group 3-MB cells with high *MYC* expression to stem cells of the RL^{VZ} may be a byproduct of dedifferentiation driven by aberrant MYC activity, rather than being indicative of developmental origin. Future studies will be required to resolve this point of contention.

Mitogenic vs. differentiation defects dictate developmental susceptibilities

An interesting question in the field pertains to why different developmental lineages are uniquely susceptible to different categories of molecular alterations. Research

over the past two decades provided a plausible explanation for this question and informed distinct mitogenic versus differentiation vulnerabilities as mechanisms underlying MB subgroup-specific tumorigenesis. For instance, both WNT-MB and SHH-MB are driven by mutations that promote constitutive activation of mitogenic signaling pathways. In contrast, groups 3-MB and 4-MB are enriched with genetic alterations targeting chromatin-modifying complexes and developmental transcription factors that control cell identity and differentiation of specific cerebellar lineages (Bai et al. 2012; Bunt et al. 2012; Jones et al. 2012, 2013; Boulay et al. 2017; Northcott et al. 2017, 2019; Hovestadt et al. 2020). These findings suggest that disruption of normal developmental programs and differentiation trajectories underlies group 3/4-MB pathogenesis. Although this divergent mitogenic versus differentiation susceptibility hypothesis requires further experimental validation especially for groups 3-MB and 4-MB, resolving the distinct pathogenic processes underlying different MB subgroups will undoubtedly overcome current knowledge gaps and promote the development of more effective therapies.

MB intratumoral heterogeneity mirrors normal cerebellar differentiation programs

Because MB is a developmental disease, it has long been hypothesized that these tumors consist of heterogeneous populations mimicking normal differentiation trajectories (Gilbertson and Ellison 2008), with malignant stem/progenitor-like cells driving tumor growth and more differentiated tumor cells comprising the tumor bulk. Such hierarchies have been identified in both human patient samples and in various mouse models (Singh et al. 2003, 2004; Read et al. 2009; Ward et al. 2009; Vanner et al. 2014; Tao et al. 2019; Zhang et al. 2019; Selvadurai et al. 2020; Yao et al. 2020; Luo et al. 2021). However, the heterogeneous cellular states of human MB were largely unexplored until recently, when single-cell transcriptomics was first applied to limited patient cohorts. In a series consisting of eight patient tumor samples representing SHH, group 3, or group 4, Vladoiu et al. (2019) identified tumor cell clusters that mapped to cerebellar cell types along differentiation trajectories. By performing single-cell RNA-seq on 25 surgical samples representing all molecular subgroups, a parallel study by Hovestadt et al. (2019) found that MB exhibits subgroup-specific cellular hierarchies consisting of undifferentiated progenitor-like and differentiated neuronal-like populations. Correlation analysis of SHH-MB single cells revealed that undifferentiated progenitor-like tumor cells were more similar to UBC/GN progenitors and that more differentiated tumor cells resembled UBC/GN intermediate and differentiated GNs, consistent with the GN lineage as the origin for SHH-MB (Hovestadt et al. 2019). At the bulk sample level, group 3/4-MBs exhibit substantial molecular similarities, with ~20% described as "intermediate" tumors exhibiting overlapping signatures of both subgroups (Kool et al. 2008; Cho et al. 2011; Northcott et al. 2011, 2017). When analyzed at single-cell resolution, malignant cells consisted of distinct proportions of primitive progenitor-like cells and more differentiated cells, with group 3-MB cells being predominantly progenitor-like and group 4-MB cells being more differentiated. Group 3/4-MB intermediate tumors contained cells with both undifferentiated and differentiated cellular states, providing a cellular explanation for their intermediate classification based on bulk DNA methylation array (Hovestadt et al. 2019). Similar intratumoral heterogeneity was detected in independent MB single-cell transcriptome studies that showed that SHH-MB consists of variable proportions of GNP and GN, and that group 3/4-MB predominantly consists of cells of the UBC lineage (Riemondy et al. 2022; Okonechnikov et al. 2023).

Besides malignant cells of various progenitor and differentiation states, MB also contains nonmalignant cells in the tumor microenvironment. By single-cell transcriptomics, Riemondy et al. (2022) identified two main clusters of immune cells: myeloid cells and lymphocytes. Reclustering of the immune cells revealed diverse subpopulations of myeloid cells predicted to exert distinct neurodevelopmental or immune functions. Some subpopulations differed in proportion among MB subgroups, which may be linked to their unique biology. Analysis of lymphocytes revealed the presence of T cells, natural killer (NK) cells, and B cells in the MB microenvironment, although the rarity of these cells prevented an in-depth analysis (Riemondy et al. 2022).

MB may exhibit intratumoral heterogeneity not only at the level of cellular states but also spatially within the tumor microenvironment. Immunohistochemistry (IHC) of STMN2, a marker of the differentiated SHH-MB cell cluster, revealed that the STMN2 signal was predominantly localized to the nodules surrounded by $Ki67^{+}$, progenitor-like tumor cells (Riemondy et al. 2022). The spatial heterogeneity of SHH-MB was comprehensively characterized by two parallel studies, both of which focused on the MBEN histological subtype (Ghasemi et al. 2024; Gold et al. 2024). Single nuclear transcriptomic analysis of two distinct MBEN cohorts confirmed that their intratumoral heterogeneity recapitulates the GN lineage trajectory, with cell clusters from the beginning of the trajectory resembling early GNPs, whereas the intermediate clusters and the neuronal-like cluster at the end of the trajectory mapped to migrating GNPs and differentiated GNs, respectively. Spatial transcriptomic analysis via single-molecule RNA in situ hybridization or multiplexed IHC suggested that the early progenitor-like cells and differentiated cells localized into the internodular compartment and nodules, respectively, with intermediate cells found in both compartments (Ghasemi et al. 2024; Gold et al. 2024). Collectively, these findings indicate that intratumoral heterogeneity in MB mirrors normal cerebellar differentiation programs, aligning with lineage of origin.

Future opportunities for MB modeling illuminated through developmental origins

The divergence between human and mouse cerebellar development underlies the primary challenge of MB disease

modeling. Current GEMMs have only partially replicated the diversity of MB subgroups, likely owing to the lack of conservation in the developing RL that is fundamental to MB etiology (Wetmore et al. 2001; Yang et al. 2008; Ayrault et al. 2009; Gibson et al. 2010; Swartling et al. 2010, 2012; Pei et al. 2012; Kawauchi et al. 2017; Vo et al. 2018). Although much has been learned from established human MB cell lines (Bigner et al. 1990; Milde et al. 2012; Ivanov et al. 2016a,b) and patient-derived orthotopic xenografts (PDOXs) (Zhao et al. 2012; Brabetz et al. 2018; Rusert et al. 2020; Smith et al. 2020), these in vitro and in vivo models do not enable studies focused on tumor initiation, early progression, or the microenvironment, essential features of tumorigenesis that remain understudied, particularly in the context of groups 3-MB and 4-MB. Technological advances in human stem cells have gained strides in overcoming species-specific limitations, generating models with increasing biological fidelity to accommodate the heterogeneity observed in MB (Fig. 4).

Cellular reprogramming

Reprogramming of human somatic cells that either readily carry MB-predisposing germline mutations or have been genetically edited to mimic such events have provided a platform for studying malignant transformation. For example, induced pluripotent stem cells (iPSCs) derived from patients with Gorlin syndrome that exhibit germline mutation of the *PTCH1* gene (i.e., *PTCH1*$^{+/-}$) gave rise to MB-like tumors when subcutaneously injected into the flank of immunodeficient mice, as determined by histopathological attributes. Loss of heterozygosity in *PTCH1*$^{+/-}$ iPSCs through gene editing (i.e., *PTCH*$^{-/-}$) exacerbated the growth of such MB-like tumors (Ikemoto et al. 2020; Nagao et al. 2022).

Cellular reprogramming that favors the neuroectodermal lineage presents a refined system for MB modeling. Long-term self-renewing neuroepithelial-like stem (NES) cells are derived from embryonic stem cells (ESCs) or iPSCs through neural induction (Zhang et al. 2001; Gerrard et al. 2005) followed by continuous isolation and enrichment of neural rosettes, resulting in a self-renewing population that retains stable neurogenic properties (Koch et al. 2009; Falk et al. 2012). NES cells generated from Gorlin patients (*PTCH1*$^{+/-}$) gave rise to tumors that histologically and transcriptionally resemble SHH-MB upon orthotopic implantation (Susanto et al. 2020). MBs arising from aberrant overexpression of *MYCN* in NES cells exhibited transcriptomes comparable with SHH-MB and more accurately reflected DNA methylation profiles that were not observed in comparable mouse models (Diede et al. 2013; Huang et al. 2019). NES cells are distinguishable from hindbrain neuroepithelial stem (hbNES) cells, the latter of which are derived and expanded from early human embryos of Carnegie stage 15–17

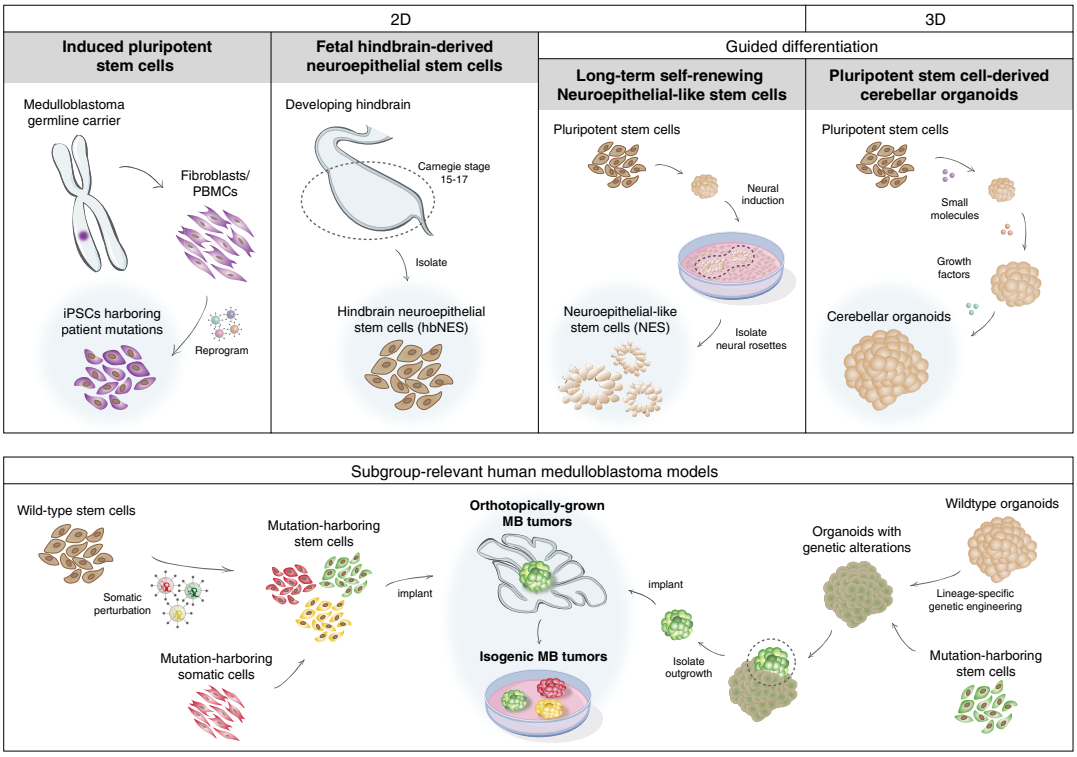

Figure 4. Advanced 2D and 3D systems for modeling MB in human cells. (*Top* panels) Summary of current 2D and 3D approaches for generating varying types of human pluripotent stem cells and cerebellar organoids as materials for MB modeling. (*Bottom* panel) Utilization of stem cell-based materials to engineer bona fide human MB models compatible with ex vivo and in vivo propagation. (iPSCs) Induced pluripotent stem cells, (PBMCs) peripheral blood mononuclear cells.

(Tailor et al. 2013). Upon *MYCN* overexpression in either cellular system, resulting tumors recapitulated transcriptional and epigenetic profiles of SHH-MB; however, hbNES cell-derived tumors were less aggressive than NES cell-derived counterparts (Čančer et al. 2019). The investigators attributed differences in aggressiveness to differential mTOR activity, potentially reflecting more nuanced subtypes within the SHH subgroup. As the cellular compositions of both NES cells and hbNES cells are currently unknown, oncogenic events were likely introduced and retained in specific subpopulations, rendering the models inconsistent given the heterogeneity of the cultures. This underscores the importance of perturbation of the developmentally informed tumor cell of origin for accurate disease modeling.

Cerebellar organoids

Human cerebellar organoids closely mimic physiological cerebellar development and provide an attractive, 3D ecosystem for MB subgroup modeling (Muguruma et al. 2015; Nayler et al. 2021; Atamian et al. 2024). With physiological properties surpassing monolayer cultures and recapitulating diverse cerebellar cell types that reflect the developmental trajectory in vivo, cerebellar organoids provide a promising human system for generating isogenic MB models to study tumor initiation and progression. *PTCH1*$^{-/-}$ cerebellar organoids differentiated from CRISPR-edited human iPSCs failed to acquire cerebellar cell fate, consistent with failure of neural tube closure observed in *Ptch1*$^{-/-}$ mice (Goodrich et al. 1997). In the same study, *PTCH1*$^{+/-}$ cerebellar organoids were highly proliferative and displayed gradual loss of PTCH1 expression over time, mimicking *PTCH1* loss frequently observed in SHH-MB (van Essen et al. 2024). Although this study effectively mimicked genetic predisposition in the context of cerebellar development, *PTCH1*$^{+/-}$ cerebellar organoids only displayed phenotypes comparable with preneoplastic stages of SHH-MB despite expression of SHH-MB signature genes.

To acquire complete neoplastic transformation of cerebellar organoids, orthotopic transplants involving mice is likely unavoidable. Vasculature, oxygenation, and other components of the microenvironment remain essential, as substantiated in first-generation cerebellar organoid models of group 3-MB. Combined *GFI1/MYC* and *OTX2/MYC* overexpression induced regions of hyperproliferation in iPSC-derived cerebellar organoids. Upon orthotopic implantation, these organoids gave rise to tumors reminiscent of group 3-MB that classified as subtypes 2 and 4, respectively, based on DNA methylation profiling (Ballabio et al. 2020). A follow-up study from the same group aimed to trace the specific cell of origin that gave rise to *GFI1/MYC*-induced tumors in an attempt to deduce the developmental origins of group 3-MB. Rather than implicating a specific cell lineage vulnerable to *GFI1/MYC* overexpression, the investigators identified Notch1 pathway activation as a crucial step for tumor initiation (Ballabio et al. 2021). Although these group 3-MB models pioneer the reproduction of human MB subtypes, it is important to note that these driver gene combinations account for only a subset of group 3-MB. Future studies testing additional, patient-informed driver gene combinations while incorporating cellular lineage and state into the experimental approach will be essential to leverage this technology and effectively mirror the intertumoral and intratumoral heterogeneity of MB (Fig. 4).

There is little doubt that human-specific developmental models hold immense potential to unveil the multifaceted molecular and phenotypic properties of MB subgroups. In vitro and ex vivo cultures are attractive alternatives for otherwise scarce human-derived resources (e.g., hbNES cells) and overcome the obvious inability to perform experiments in developing human hindbrains. Current knowledge of cerebellar development gleaned from single-cell studies are valuable references for generating model systems that better recapitulate the cellular hierarchies and heterogeneity of MB.

Concluding remarks

Tremendous efforts over the past decades have collectively discovered and defined MB subgroups, retracing diverse subgroup identities to lineage-specific developmental origins. Our understanding of MB cellular origins lays the foundation for a multitude of future MB research and applications. These include improved disease modeling of all molecular subgroups and subtypes in the relevant developmental lineage(s) and engineering next-generation cellular therapies that target cell surface antigens conserved between cell of origin and MB cells. Going forward, more nuanced molecular comparisons between cell of origin and MB are needed to further dissect the oncogenic mechanisms that arrest normal developmental trajectories and drive dysfunctional differentiation. These efforts will require higher-resolution cerebellar atlases that include a broader range of developmental stages than those included in first-generation references. In addition, the incorporation of spatial transcriptomics and proteomics, coupled with multimodal single-cell and single-nucleus data sets that include DNA methylation, accessible chromatin, and histone modifications from both tumor and normal samples will undoubtedly further enhance knowledge of the developmental biology underlying MB subgroups and subtypes. Together, these analyses will facilitate the discovery of novel therapeutic targets, leading to advancements in treatment that are required to improve outcomes and cure affected children of this devastating developmental malignancy.

Competing interest statement

The authors declare no competing interests.

Acknowledgments

This work was principally supported by the American Lebanese Syrian Associated Charities and St. Jude (P.A.N. and M.F.R.), the Mark Foundation for Cancer Research (Emerging Leader

Award to P.A.N.), and the National Cancer Institute (P01CA096832 to P.A.N. and M.F.R., and 1R01CA270785 to P.A.N.).

References

Aldinger KA, Thomson Z, Phelps IG, Haldipur P, Deng M, Timms AE, Hirano M, Santpere G, Roco C, Rosenberg AB, et al. 2021. Spatial and cell type transcriptional landscape of human cerebellar development. *Nat Neurosci* **24:** 1163–1175. doi:10.1038/s41593-021-00872-y

Atamian A, Birtele M, Hosseini N, Nguyen T, Seth A, Del Dosso A, Paul S, Tedeschi N, Taylor R, Coba MP, et al. 2024. Human cerebellar organoids with functional Purkinje cells. *Cell Stem Cell* **31:** 39–51.e6. doi:10.1016/j.stem.2023.11.013

Ayrault O, Zindy F, Rehg J, Sherr CJ, Roussel MF. 2009. Two tumor suppressors, p27Kip1 and patched-1, collaborate to prevent medulloblastoma. *Mol Cancer Res* **7:** 33–40. doi:10.1158/1541-7786.MCR-08-0369

Bagnall MW, Zingg B, Sakatos A, Moghadam SH, Zeilhofer HU, Du Lac S. 2009. Glycinergic projection neurons of the cerebellum. *J Neurosci* **29:** 10104–10110. doi:10.1523/JNEUROSCI.2087-09.2009

Bai R-Y, Staedtke V, Lidov HG, Eberhart CG, Riggins GJ. 2012. OTX2 represses myogenic and neuronal differentiation in medulloblastoma cells. *Cancer Res* **72:** 5988–6001. doi:10.1158/0008-5472.CAN-12-0614

Ballabio C, Anderle M, Gianesello M, Lago C, Miele E, Cardano M, Aiello G, Piazza S, Caron D, Gianno F, et al. 2020. Modeling medulloblastoma in vivo and with human cerebellar organoids. *Nat Commun* **11:** 583. doi:10.1038/s41467-019-13989-3

Ballabio C, Gianesello M, Lago C, Okonechnikov K, Anderle M, Aiello G, Antonica F, Zhang T, Gianno F, Giangaspero F, et al. 2021. Notch1 switches progenitor competence in inducing medulloblastoma. *Sci Adv* **7:** eabd2781. doi:10.1126/sciadv.abd2781

Bigner SH, Friedman HS, Vogelstein B, Oakes WJ, Bigner DD. 1990. Amplification of the c-myc gene in human medulloblastoma cell lines and xenografts. *Cancer Res* **50:** 2347–2350.

Boulay G, Awad ME, Riggi N, Archer TC, Iyer S, Boonseng WE, Rossetti NE, Naigles B, Rengarajan S, Volorio A, et al. 2017. OTX2 activity at distal regulatory elements shapes the chromatin landscape of group 3 medulloblastoma. *Cancer Discov* **7:** 288–301. doi:10.1158/2159-8290.CD-16-0844

Brabetz S, Leary SE, Gröbner SN, Nakamoto MW, Şeker-Cin H, Girard EJ, Cole B, Strand AD, Bloom KL, Hovestadt V, et al. 2018. A biobank of patient-derived pediatric brain tumor models. *Nat Med* **24:** 1752–1761. doi:10.1038/s41591-018-0207-3

Bunt J, Hasselt NE, Zwijnenburg DA, Hamdi M, Koster J, Versteeg R, Kool M. 2012. OTX2 directly activates cell cycle genes and inhibits differentiation in medulloblastoma cells. *Int J Cancer* **131:** E21–E32. doi:10.1002/ijc.26474

Čančer M, Hutter S, Holmberg KO, Rosén G, Sundström A, Tailor J, Bergström T, Garancher A, Essand M, Wechsler-Reya RJ, et al. 2019. Humanized stem cell models of pediatric medulloblastoma reveal an Oct4/mTOR axis that promotes malignancy. *Cell Stem Cell* **25:** 855–870.e11. doi:10.1016/j.stem.2019.10.005

Carter RA, Bihannic L, Rosencrance C, Hadley JL, Tong Y, Phoenix TN, Natarajan S, Easton J, Northcott PA, Gawad C. 2018. A single-cell transcriptional atlas of the developing murine cerebellum. *Curr Biol* **28:** 2910–2920.e2. doi:10.1016/j.cub.2018.07.062

Cavalli FMG, Remke M, Rampasek L, Peacock J, Shih DJH, Luu B, Garzia L, Torchia J, Nor C, Morrissy AS, et al. 2017. Intertumoral heterogeneity within medulloblastoma subgroups. *Cancer Cell* **31:** 737–754.e6. doi:10.1016/j.ccell.2017.05.005

Cho YJ, Tsherniak A, Tamayo P, Santagata S, Ligon A, Greulich H, Berhoukim R, Amani V, Goumnerova L, Eberhart CG, et al. 2011. Integrative genomic analysis of medulloblastoma identifies a molecular subgroup that drives poor clinical outcome. *J Clin Oncol* **29:** 1424–1430. doi:10.1200/JCO.2010.28.5148

Clifford SC, Lusher ME, Lindsey JC, Langdon JA, Gilbertson RJ, Straughton D, Ellison DW. 2006. Wnt/wingless pathway activation and chromosome 6 loss characterise a distinct molecular sub-group of medulloblastomas associated with a favourable prognosis. *Cell cycle* **5:** 2666–2670. doi:10.4161/cc.5.22.3446

Clifford SC, Lannering B, Schwalbe EC, Hicks D, O'Toole K, Nicholson SL, Goschzik T, Zur Mühlen A, Figarella-Branger D, Doz F, et al. 2015. Biomarker-driven stratification of disease-risk in non-metastatic medulloblastoma: results from the multi-center HIT–SIOP–PNET4 clinical trial. *Oncotarget* **6:** 38827–38839. doi:10.18632/oncotarget.5149

Diede SJ, Yao ZZ, Keyes CC, Tyler AE, Dey J, Hackett CS, Elsaesser K, Kemp CJ, Neiman PE, Weiss WA, et al. 2013. Fundamental differences in promoter CpG island DNA hypermethylation between human cancer and genetically engineered mouse models of cancer. *Epigenetics* **8:** 1254–1260. doi:10.4161/epi.26486

Eberhart CG, Tihan T, Burger PC. 2000. Nuclear localization and mutation of β-catenin in medulloblastomas. *J Neuropathol Exp Neurol* **59:** 333–337. doi:10.1093/jnen/59.4.333

Ellison DW, Onilude OE, Lindsey JC, Lusher ME, Weston CL, Taylor RE, Pearson AD, Clifford SC. 2005. β-Catenin status predicts a favorable outcome in childhood medulloblastoma: the United Kingdom Children's Cancer Study Group Brain Tumour Committee. *J Clin Oncol* **23:** 7951–7957. doi:10.1200/JCO.2005.01.5479

Falk A, Koch P, Kesavan J, Takashima Y, Ladewig J, Alexander M, Wiskow O, Tailor J, Trotter M, Pollard S, et al. 2012. Capture of neuroepithelial-like stem cells from pluripotent stem cells provides a versatile system for in vitro production of human neurons. *PLoS One* **7:** e29597. doi:10.1371/journal.pone.0029597

Gajjar A, Robinson GW, Smith KS, Lin T, Merchant TE, Chintagumpala M, Mahajan A, Su J, Bouffet E, Bartels U, et al. 2021. Outcomes by clinical and molecular features in children with medulloblastoma treated with risk-adapted therapy: results of an international phase III trial (SJMB03). *J Clin Oncol* **39:** 822–835. doi:10.1200/JCO.20.01372

Garcia-Lopez J, Kumar R, Smith KS, Northcott PA. 2021. Deconstructing sonic hedgehog medulloblastoma: molecular subtypes, drivers, and beyond. *Trends Genet* **37:** 235–250. doi:10.1016/j.tig.2020.11.001

Gerrard L, Rodgers L, Cui W. 2005. Differentiation of human embryonic stem cells to neural lineages in adherent culture by blocking bone morphogenetic protein signaling. *Stem Cells* **23:** 1234–1241. doi:10.1634/stemcells.2005-0110

Ghasemi DR, Okonechnikov K, Rademacher A, Tirier S, Maass KK, Schumacher H, Joshi P, Gold MP, Sundheimer J, Statz B, et al. 2024. Compartments in medulloblastoma with extensive nodularity are connected through differentiation along the granular precursor lineage. *Nat Commun* **15:** 269. doi:10.1038/s41467-023-44117-x

Gibson P, Tong Y, Robinson G, Thompson MC, Currle DS, Eden C, Kranenburg TA, Hogg T, Poppleton H, Martin J, et al. 2010.

Subtypes of medulloblastoma have distinct developmental origins. *Nature* **468:** 1095–1099. doi:10.1038/nature09587

Gilbertson RJ, Ellison DW. 2008. The origins of medulloblastoma subtypes. *Annu Rev Pathol* **3:** 341–365. doi:10.1146/annurev.pathmechdis.3.121806.151518

Gold MP, Ong W, Masteller AM, Ghasemi DR, Galindo JA, Park NR, Huynh NC, Donde A, Pister V, Saurez RA, et al. 2024. Developmental basis of SHH medulloblastoma heterogeneity. *Nat Commun* **15:** 270. doi:10.1038/s41467-023-44300-0

Goodrich LV, Milenković L, Higgins KM, Scott MP. 1997. Altered neural cell fates and medulloblastoma in mouse *patched* mutants. *Science* **277:** 1109–1113. doi:10.1126/science.277.5329.1109

Haldipur P, Aldinger KA, Bernardo S, Deng M, Timms AE, Overman LM, Winter C, Lisgo SN, Razavi, Silvestri E, et al. 2019. Spatiotemporal expansion of primary progenitor zones in the developing human cerebellum. *Science* **366:** 454–460. doi:10.1126/science.aax7526

Haldipur P, Millen KJ, Aldinger KA. 2022. Human cerebellar development and transcriptomics: implications for neurodevelopmental disorders. *Annu Rev Neurosci* **45:** 515–531. doi:10.1146/annurev-neuro-111020-091953

Hendrikse LD, Haldipur P, Saulnier O, Millman J, Sjoboen AH, Erickson AW, Ong W, Gordon V, Coudière-Morrison L, Mercier AL, et al. 2022. Failure of human rhombic lip differentiation underlies medulloblastoma formation. *Nature* **609:** 1021–1028. doi:10.1038/s41586-022-05215-w

Hill RM, Kuijper S, Lindsey JC, Petrie K, Schwalbe EC, Barker K, Boult JK, Williamson D, Ahmad Z, Hallsworth A, et al. 2015. Combined MYC and P53 defects emerge at medulloblastoma relapse and define rapidly progressive, therapeutically targetable disease. *Cancer Cell* **27:** 72–84. doi:10.1016/j.ccell.2014.11.002

Hovestadt V, Smith KS, Bihannic L, Filbin MG, Shaw ML, Baumgartner A, DeWitt JC, Groves A, Mayr L, Weisman HR, et al. 2019. Resolving medulloblastoma cellular architecture by single-cell genomics. *Nature* **572:** 74–79. doi:10.1038/s41586-019-1434-6

Hovestadt V, Ayrault O, Swartling FJ, Robinson GW, Pfister SM, Northcott PA. 2020. Medulloblastomics revisited: biological and clinical insights from thousands of patients. *Nat Rev Cancer* **20:** 42–56. doi:10.1038/s41568-019-0223-8

Huang M, Tailor J, Zhen Q, Gillmor AH, Miller ML, Weishaupt H, Chen J, Zheng T, Nash EK, McHenry LK, et al. 2019. Engineering genetic predisposition in human neuroepithelial stem cells recapitulates medulloblastoma tumorigenesis. *Cell Stem Cell* **25:** 433–446.e7. doi:10.1016/j.stem.2019.05.013

Ikemoto Y, Miyashita T, Nasu M, Hatsuse H, Kajiwara K, Fujii K, Motojima T, Kokido I, Toyoda M, Umezawa A. 2020. Gorlin syndrome-induced pluripotent stem cells form medulloblastoma with loss of heterozygosity in PTCH1. *Aging* **12:** 9935–9947. doi:10.18632/aging.103258

Ivanov D, Walker D, Coyle B, Grabowska A. 2016a. Data on the number and frequency of scientific literature citations for established medulloblastoma cell lines. *Data Brief* **9:** 696–698. doi:10.1016/j.dib.2016.10.004

Ivanov DP, Coyle B, Walker DA, Grabowska AM. 2016b. In vitro models of medulloblastoma: choosing the right tool for the job. *J Biotechnol* **236:** 10–25. doi:10.1016/j.jbiotec.2016.07.028

Jessa S, Blanchet-Cohen A, Krug B, Vladoiu M, Coutelier M, Faury D, Poreau B, De Jay N, Hebert S, Monlong J, et al. 2019. Stalled developmental programs at the root of pediatric brain tumors. *Nat Genet* **51:** 1702–1713. doi:10.1038/s41588-019-0531-7

Jones DT, Jäger N, Kool M, Zichner T, Hutter B, Sultan M, Cho YJ, Pugh TJ, Hovestadt V, Stütz AM, et al. 2012. Dissecting the genomic complexity underlying medulloblastoma. *Nature* **488:** 100–105. doi:10.1038/nature11284

Jones DT, Northcott PA, Kool M, Pfister SM. 2013. The role of chromatin remodeling in medulloblastoma. *Brain Pathol* **23:** 193–199. doi:10.1111/bpa.12019

Kawauchi D, Robinson G, Uziel T, Gibson P, Rehg J, Gao C, Finkelstein D, Qu C, Pounds S, Ellison DW, et al. 2012. A mouse model of the most aggressive subgroup of human medulloblastoma. *Cancer Cell* **21:** 168–180. doi:10.1016/j.ccr.2011.12.023

Kawauchi D, Ogg RJ, Liu L, Shih DJH, Finkelstein D, Murphy BL, Rehg JE, Korshunov A, Calabrese C, Zindy F, et al. 2017. Novel MYC-driven medulloblastoma models from multiple embryonic cerebellar cells. *Oncogene* **36:** 5231–5242. doi:10.1038/onc.2017.110

Koch P, Opitz T, Steinbeck JA, Ladewig J, Brüstle O. 2009. A rosette-type, self-renewing human ES cell-derived neural stem cell with potential for in vitro instruction and synaptic integration. *Proc Natl Acad Sci* **106:** 3225–3230. doi:10.1073/pnas.0808387106

Kool M, Koster J, Bunt J, Hasselt NE, Lakeman A, van Sluis P, Troost D, Meeteren NS, Caron HN, Cloos J, et al. 2008. Integrated genomics identifies five medulloblastoma subtypes with distinct genetic profiles, pathway signatures and clinicopathological features. *PLoS One* **3:** e3088. doi:10.1371/journal.pone.0003088

Kool M, Korshunov A, Remke M, Jones DT, Schlanstein M, Northcott PA, Cho Y-J, Koster J, Schouten-van Meeteren A, Van Vuurden D, et al. 2012. Molecular subgroups of medulloblastoma: an international meta-analysis of transcriptome, genetic aberrations, and clinical data of WNT, SHH, group 3, and group 4 medulloblastomas. *Acta Neuropathol* **123:** 473–484. doi:10.1007/s00401-012-0958-8

Kool M, Jones DT, Jäger N, Northcott PA, Pugh TJ, Hovestadt V, Piro RM, Esparza LA, Markant SL, Remke M, et al. 2014. Genome sequencing of SHH medulloblastoma predicts genotype-related response to smoothened inhibition. *Cancer Cell* **25:** 393–405. doi:10.1016/j.ccr.2014.02.004

Kozareva V, Martin C, Osorno T, Rudolph S, Guo C, Vanderburg C, Nadaf N, Regev A, Regehr WG, Macosko E. 2021. A transcriptomic atlas of mouse cerebellar cortex comprehensively defines cell types. *Nature* **598:** 214–219. doi:10.1038/s41586-021-03220-z

Landsberg RL, Awatramani RB, Hunter NL, Farago AF, DiPietrantonio HJ, Rodriguez CI, Dymecki SM. 2005. Hindbrain rhombic lip is comprised of discrete progenitor cell populations allocated by Pax6. *Neuron* **48:** 933–947. doi:10.1016/j.neuron.2005.11.031

Lee C, Rudneva VA, Erkek S, Zapatka M, Chau LQ, Tacheva-Grigorova SK, Garancher A, Rusert JM, Aksoy O, Lea R, et al. 2019. Lsd1 as a therapeutic target in Gfi1-activated medulloblastoma. *Nat Commun* **10:** 332. doi:10.1038/s41467-018-08269-5

Leto K, Arancillo M, Becker EB, Buffo A, Chiang C, Ding B, Dobyns WB, Dusart I, Haldipur P, Hatten ME, et al. 2016. Consensus paper: cerebellar development. *Cerebellum* **15:** 789–828. doi:10.1007/s12311-015-0724-2

Li P, Du F, Yuelling LW, Lin T, Muradimova RE, Tricarico R, Wang J, Enikolopov G, Bellacosa A, Wechsler-Reya RJ, et al. 2013. A population of Nestin-expressing progenitors in the cerebellum exhibits increased tumorigenicity. *Nat Neurosci* **16:** 1737–1744. doi:10.1038/nn.3553

Lin CY, Erkek S, Tong Y, Yin L, Federation AJ, Zapatka M, Haldipur P, Kawauchi D, Risch T, Warnatz HJ, et al. 2016. Active

medulloblastoma enhancers reveal subgroup-specific cellular origins. *Nature* **530:** 57–62. doi:10.1038/nature16546

Louis DN, Perry A, Reifenberger G, Von Deimling A, Figarella-Branger D, Cavenee WK, Ohgaki H, Wiestler OD, Kleihues P, Ellison DW. 2016. The 2016 world health organization classification of tumors of the central nervous system: a summary. *Acta Neuropathol* **131:** 803–820. doi:10.1007/s00401-016-1545-1

Luo W, Lin GN, Song W, Zhang Y, Lai H, Zhang M, Miao J, Cheng X, Wang Y, Li W, et al. 2021. Single-cell spatial transcriptomic analysis reveals common and divergent features of developing postnatal granule cerebellar cells and medulloblastoma. *BMC Biol* **19:** 135. doi:10.1186/s12915-021-01071-8

Mainwaring OJ, Weishaupt H, Zhao M, Rosén G, Borgenvik A, Breinschmid L, Verbaan AD, Richardson S, Thompson D, Clifford SC, et al. 2023. ARF suppression by MYC but not MYCN confers increased malignancy of aggressive pediatric brain tumors. *Nat Commun* **14:** 1221. doi:10.1038/s41467-023-36847-9

Milde T, Lodrini M, Savelyeva L, Korshunov A, Kool M, Brueckner LM, Antunes AS, Oehme I, Pekrun A, Pfister SM, et al. 2012. HD-MB03 is a novel group 3 medulloblastoma model demonstrating sensitivity to histone deacetylase inhibitor treatment. *J Neurooncol* **110:** 335–348. doi:10.1007/s11060-012-0978-1

Mugnaini E, Sekerková G, Martina M. 2011. The unipolar brush cell: a remarkable neuron finally receiving deserved attention. *Brain Res Rev* **66:** 220–245. doi:10.1016/j.brainresrev.2010.10.001

Muguruma K, Nishiyama A, Kawakami H, Hashimoto K, Sasai Y. 2015. Self-organization of polarized cerebellar tissue in 3D culture of human pluripotent stem cells. *Cell Rep* **10:** 537–550. doi:10.1016/j.celrep.2014.12.051

Nagao K, Kato C, Ikemoto Y, Motojima T, Fujii K, Umezawa A, Miyashita T. 2022. PTCH1-null induced pluripotent stem cells exclusively differentiate into immature ectodermal cells with large areas of medulloblastoma-like tissue. *Discov Oncol* **13:** 36. doi:10.1007/s12672-022-00498-x

Nayler S, Agarwal D, Curion F, Bowden R, Becker EBE. 2021. High-resolution transcriptional landscape of xeno-free human induced pluripotent stem cell-derived cerebellar organoids. *Sci Rep* **11:** 12959. doi:10.1038/s41598-021-91846-4

Northcott PA, Korshunov A, Witt H, Hielscher T, Eberhart CG, Mack S, Bouffet E, Clifford SC, Hawkins CE, French P, et al. 2011. Medulloblastoma comprises four distinct molecular variants. *J Clin Oncol* **29:** 1408–1414. doi:10.1200/JCO.2009.27.4324

Northcott PA, Jones DT, Kool M, Robinson GW, Gilbertson RJ, Cho YJ, Pomeroy SL, Korshunov A, Lichter P, Taylor MD, et al. 2012a. Medulloblastomics: the end of the beginning. *Nat Rev Cancer* **12:** 818–834. doi:10.1038/nrc3410

Northcott PA, Shih DJ, Peacock J, Garzia L, Morrissy AS, Zichner T, Stutz AM, Korshunov A, Reimand J, Schumacher SE, et al. 2012b. Subgroup-specific structural variation across 1,000 medulloblastoma genomes. *Nature* **488:** 49–56. doi:10.1038/nature11327

Northcott PA, Lee C, Zichner T, Stutz AM, Erkek S, Kawauchi D, Shih DJ, Hovestadt V, Zapatka M, Sturm D, et al. 2014. Enhancer hijacking activates GFI1 family oncogenes in medulloblastoma. *Nature* **511:** 428–434. doi:10.1038/nature13379

Northcott PA, Buchhalter I, Morrissy AS, Hovestadt V, Weischenfeldt J, Ehrenberger T, Gröbner S, Segura-Wang M, Zichner T, Rudneva VA, et al. 2017. The whole-genome landscape of medulloblastoma subtypes. *Nature* **547:** 311–317. doi:10.1038/nature22973

Northcott PA, Robinson GW, Kratz CP, Mabbott DJ, Pomeroy SL, Clifford SC, Rutkowski S, Ellison DW, Malkin D, Taylor MD, et al. 2019. Medulloblastoma. *Nat Rev Dis Primers* **5:** 11. doi:10.1038/s41572-019-0063-6

Okonechnikov K, Joshi P, Sepp M, Leiss K, Sarropoulos I, Murat F, Sill M, Beck P, Chan KC, Korshunov A, et al. 2023. Mapping pediatric brain tumors to their origins in the developing cerebellum. *Neuro Oncol* **25:** 1895–1909. doi:10.1093/neuonc/noad124

Orr BA. 2020. Pathology, diagnostics, and classification of medulloblastoma. *Brain Pathol* **30:** 664–678. doi:10.1111/bpa.12837

Patay Z, DeSain LA, Hwang SN, Coan A, Li Y, Ellison DW. 2015. MR imaging characteristics of wingless-type-subgroup pediatric medulloblastoma. *AJNR Am J Neuroradiol* **36:** 2386–2393. doi:10.3174/ajnr.A4495

Pei Y, Moore CE, Wang J, Tewari AK, Eroshkin A, Cho YJ, Witt H, Korshunov A, Read TA, Sun JL, et al. 2012. An animal model of MYC-driven medulloblastoma. *Cancer Cell* **21:** 155–167. doi:10.1016/j.ccr.2011.12.021

Phoenix TN, Patmore DM, Boop S, Boulos N, Jacus MO, Patel YT, Roussel MF, Finkelstein D, Goumnerova L, Perreault S, et al. 2016. Medulloblastoma genotype dictates blood brain barrier phenotype. *Cancer Cell* **29:** 508–522. doi:10.1016/j.ccell.2016.03.002

Poli V, Fagnocchi L, Fasciani A, Cherubini A, Mazzoleni S, Ferrillo S, Miluzio A, Gaudioso G, Vaira V, Turdo A, et al. 2018. MYC-driven epigenetic reprogramming favors the onset of tumorigenesis by inducing a stem cell-like state. *Nat Commun* **9:** 1024. doi:10.1038/s41467-018-03264-2

Pöschl J, Stark S, Neumann P, Gröbner S, Kawauchi D, Jones DT, Northcott PA, Lichter P, Pfister SM, Kool M, et al. 2014. Genomic and transcriptomic analyses match medulloblastoma mouse models to their human counterparts. *Acta Neuropathol* **128:** 123–136. doi:10.1007/s00401-014-1297-8

Pugh TJ, Weeraratne SD, Archer TC, Pomeranz Krummel DA, Auclair D, Bochicchio J, Carneiro MO, Carter SL, Cibulskis K, Erlich RL, et al. 2012. Medulloblastoma exome sequencing uncovers subtype-specific somatic mutations. *Nature* **488:** 106–110. doi:10.1038/nature11329

Ramaswamy V, Nör C, Taylor MD. 2016. p53 and medulloblastoma. *Cold Spring Harb Perspect Med* **6:** a026278. doi:10.1101/cshperspect.a026278

Read TA, Fogarty MP, Markant SL, McLendon RE, Wei Z, Ellison DW, Febbo PG, Wechsler-Reya RJ. 2009. Identification of CD15 as a marker for tumor-propagating cells in a mouse model of medulloblastoma. *Cancer Cell* **15:** 135–147. doi:10.1016/j.ccr.2008.12.016

Remke M, Hielscher T, Northcott PA, Witt H, Ryzhova M, Wittmann A, Benner A, Von Deimling A, Scheurlen W, Perry A, et al. 2011. Adult medulloblastoma comprises three major molecular variants. *J Clin Oncol* **29:** 2717–2723. doi:10.1200/JCO.2011.34.9373

Riemondy KA, Venkataraman S, Willard N, Nellan A, Sanford B, Griesinger AM, Amani V, Mitra S, Hankinson TC, Handler MH, et al. 2022. Neoplastic and immune single-cell transcriptomics define subgroup-specific intra-tumoral heterogeneity of childhood medulloblastoma. *Neuro Oncol* **24:** 273–286. doi:10.1093/neuonc/noab135

Robinson G, Parker M, Kranenburg TA, Lu C, Chen X, Ding L, Phoenix TN, Hedlund E, Wei L, Zhu X, et al. 2012. Novel mutations target distinct subgroups of medulloblastoma. *Nature* **488:** 43–48. doi:10.1038/nature11213

Robinson GW, Rudneva VA, Buchhalter I, Billups CA, Waszak SM, Smith KS, Bowers DC, Bendel A, Fisher PG, Partap S, et al. 2018. Risk-adapted therapy for young children with

medulloblastoma (SJYC07): therapeutic and molecular outcomes from a multicentre, phase 2 trial. *Lancet Oncol* **19:** 768–784. doi:10.1016/S1470-2045(18)30204-3

Roussel MF, Hatten ME. 2011. Cerebellum development and medulloblastoma. *Curr Top Dev Biol* **94:** 235–282. doi:10.1016/B978-0-12-380916-2.00008-5

Roussel MF, Stripay JL. 2020. Modeling pediatric medulloblastoma. *Brain Pathol* **30:** 703–712. doi:10.1111/bpa.12803

Rusert JM, Juarez EF, Brabetz S, Jensen J, Garancher A, Chau LQ, Tacheva-Grigorova SK, Wahab S, Udaka YT, Finlay D, et al. 2020. Functional precision medicine identifies new therapeutic candidates for medulloblastoma. *Cancer Res* **80:** 5393–5407. doi:10.1158/0008-5472.CAN-20-1655

Sarropoulos I, Sepp M, Frömel R, Leiss K, Trost N, Leushkin E, Okonechnikov K, Joshi P, Giere P, Kutscher LM, et al. 2021. Developmental and evolutionary dynamics of *cis*-regulatory elements in mouse cerebellar cells. *Science* **373:** eabg4696. doi:10.1126/science.abg4696

Schüller U, Heine VM, Mao J, Kho AT, Dillon AK, Han YG, Huillard E, Sun T, Ligon AH, Qian Y, et al. 2008. Acquisition of granule neuron precursor identity is a critical determinant of progenitor cell competence to form Shh-induced medulloblastoma. *Cancer Cell* **14:** 123–134. doi:10.1016/j.ccr.2008.07.005

Schwalbe EC, Lindsey JC, Nakjang S, Crosier S, Smith AJ, Hicks D, Rafiee G, Hill RM, Iliasova A, Stone T, et al. 2017. Novel molecular subgroups for clinical classification and outcome prediction in childhood medulloblastoma: a cohort study. *Lancet Oncol* **18:** 958–971. doi:10.1016/S1470-2045(17)30243-7

Selvadurai HJ, Luis E, Desai K, Lan X, Vladoiu MC, Whitley O, Galvin C, Vanner RJ, Lee L, Whetstone H, et al. 2020. Medulloblastoma arises from the persistence of a rare and transient $Sox2^+$ granule neuron precursor. *Cell Rep* **31:** 107511. doi:10.1016/j.celrep.2020.03.075

Sepp M, Leiss K, Murat F, Okonechnikov K, Joshi P, Leushkin E, Spänig L, Mbengue N, Schneider C, Schmidt J, et al. 2024. Cellular development and evolution of the mammalian cerebellum. *Nature* **625:** 788–796. doi:10.1038/s41586-023-06884-x

Sharma T, Schwalbe EC, Williamson D, Sill M, Hovestadt V, Mynarek M, Rutkowski S, Robinson GW, Gajjar A, Cavalli F, et al. 2019. Second-generation molecular subgrouping of medulloblastoma: an international meta-analysis of group 3 and group 4 subtypes. *Acta Neuropathol* **138:** 309–326. doi:10.1007/s00401-019-02020-0

Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, Dirks PB. 2003. Identification of a cancer stem cell in human brain tumors. *Cancer Res* **63:** 5821–5828.

Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. 2004. Identification of human brain tumour initiating cells. *Nature* **432:** 396–401. doi:10.1038/nature03128

Smith KS, Xu K, Mercer KS, Boop F, Klimo P, DeCupyere M, Grenet J, Robinson S, Dunphy P, Baker SJ, et al. 2020. Patient-derived orthotopic xenografts of pediatric brain tumors: a St. Jude resource. *Acta Neuropathol* **140:** 209–225. doi:10.1007/s00401-020-02171-5

Smith KS, Bihannic L, Gudenas BL, Haldipur P, Tao R, Gao Q, Li Y, Aldinger KA, Iskusnykh IY, Chizhikov VV, et al. 2022. Unified rhombic lip origins of group 3 and group 4 medulloblastoma. *Nature* **609:** 1012–1020. doi:10.1038/s41586-022-05208-9

Sullivan DK, Deutzmann A, Yarbrough J, Krishnan MS, Gouw AM, Bellovin DI, Adam SJ, Liefwalker DF, Dhanasekaran R, Felsher DW. 2022. MYC oncogene elicits tumorigenesis associated with embryonic, ribosomal biogenesis, and tissue-lineage dedifferentiation gene expression changes. *Oncogene* **41:** 4960–4970. doi:10.1038/s41388-022-02458-9

Susanto E, Navarro AM, Zhou LL, Sundström A, van Bree N, Stantic M, Moslem M, Tailor J, Rietdijk J, Zubillaga V, et al. 2020. Modeling SHH-driven medulloblastoma with patient iPS cell-derived neural stem cells. *Proc Natl Acad Sci* **117:** 20127–20138. doi:10.1073/pnas.1920521117

Swartling FJ, Grimmer MR, Hackett CS, Northcott PA, Fan QW, Goldenberg DD, Lau J, Masic S, Nguyen K, Yakovenko S, et al. 2010. Pleiotropic role for *MYCN* in medulloblastoma. *Genes Dev* **24:** 1059–1072. doi:10.1101/gad.1907510

Swartling FJ, Savov V, Persson AI, Chen J, Hackett CS, Northcott PA, Grimmer MR, Lau J, Chesler L, Perry A, et al. 2012. Distinct neural stem cell populations give rise to disparate brain tumors in response to N-MYC. *Cancer Cell* **21:** 601–613. doi:10.1016/j.ccr.2012.04.012

Tailor J, Kittappa R, Leto K, Gates M, Borel M, Paulsen O, Spitzer S, Karadottir RT, Rossi F, Falk A, et al. 2013. Stem cells expanded from the human embryonic hindbrain stably retain regional specification and high neurogenic potency. *J Neurosci* **33:** 12407–12422. doi:10.1523/JNEUROSCI.0130-13.2013

Tao R, Murad N, Xu Z, Zhang P, Okonechnikov K, Kool M, Rivero-Hinojosa S, Lazarski C, Zheng P, Liu Y, et al. 2019. *MYC* drives group 3 medulloblastoma through transformation of $Sox2^+$ astrocyte progenitor cells. *Cancer Res* **79:** 1967–1980. doi:10.1158/0008-5472.CAN-18-1787

Taylor MD, Northcott PA, Korshunov A, Remke M, Cho YJ, Clifford SC, Eberhart CG, Parsons DW, Rutkowski S, Gajjar A, et al. 2012. Molecular subgroups of medulloblastoma: the current consensus. *Acta Neuropathol* **123:** 465–472. doi:10.1007/s00401-011-0922-z

Thompson MC, Fuller C, Hogg TL, Dalton J, Finkelstein D, Lau CC, Chintagumpala M, Adesina A, Ashley DM, Kellie SJ, et al. 2006. Genomics identifies medulloblastoma subgroups that are enriched for specific genetic alterations. *J Clin Oncol* **24:** 1924–1931. doi:10.1200/JCO.2005.04.4974

van Essen MJ, Nayler S, Becker EB, Jacob J. 2020. Deconstructing cerebellar development cell by cell. *PLoS Genet* **16:** e1008630. doi:10.1371/journal.pgen.1008630

van Essen MJ, Apsley EJ, Riepsaame J, Xu R, Northcott PA, Cowley SA, Jacob J, Becker EBE. 2024. *PTCH1*-mutant human cerebellar organoids exhibit altered neural development and recapitulate early medulloblastoma tumorigenesis. *Dis Model Mech* **17:** dmm050323. doi:10.1242/dmm.050323

Vanner RJ, Remke M, Gallo M, Selvadurai HJ, Coutinho F, Lee L, Kushida M, Head R, Morrissy S, Zhu X, et al. 2014. Quiescent $sox2^+$ cells drive hierarchical growth and relapse in sonic hedgehog subgroup medulloblastoma. *Cancer Cell* **26:** 33–47. doi:10.1016/j.ccr.2014.05.005

Visvanathan A, Saulnier O, Chen C, Haldipur P, Orisme W, Delaidelli A, Shin S, Millman J, Bryant A, Abeysundara N, et al. 2024. Early rhombic lip protogenin^{+ve} stem cells in a human-specific neurovascular niche initiate and maintain group 3 medulloblastoma. *Cell* S0092-8674(24)00651-2. doi:10.1016/j.cell.2024.06.011

Vladoiu MC, El-Hamamy I, Donovan LK, Farooq H, Holgado BL, Sundaravadanam Y, Ramaswamy V, Hendrikse LD, Kumar S, Mack SC, et al. 2019. Childhood cerebellar tumours mirror conserved fetal transcriptional programs. *Nature* **572:** 67–73. doi:10.1038/s41586-019-1158-7

Vo BT, Li C, Morgan MA, Theurillat I, Finkelstein D, Wright S, Hyle J, Smith SMC, Fan Y, Wang YD, et al. 2017. Inactivation of Ezh2 upregulates Gfi1 and drives aggressive Myc-driven group 3 medulloblastoma. *Cell Rep* **18:** 2907–2917. doi:10.1016/j.celrep.2017.02.073

Vo BT, Kwon JA, Li C, Finkelstein D, Xu B, Orr BA, Sherr CJ, Roussel MF. 2018. Mouse medulloblastoma driven by CRISPR activation of cellular Myc. *Sci Rep* **8:** 8733. doi:10.1038/s41598-018-24956-1

Ward RJ, Lee L, Graham K, Satkunendran T, Yoshikawa K, Ling E, Harper L, Austin R, Nieuwenhuis E, Clarke ID, et al. 2009. Multipotent CD15$^+$ cancer stem cells in *patched-1*-deficient mouse medulloblastoma. *Cancer Res* **69:** 4682–4690. doi:10.1158/0008-5472.CAN-09-0342

Wefers AK, Warmuth-Metz M, Pöschl J, von Bueren AO, Monoranu CM, Seelos K, Peraud A, Tonn JC, Koch A, Pietsch T, et al. 2014. Subgroup-specific localization of human medulloblastoma based on pre-operative MRI. *Acta Neuropathol* **127:** 931–933. doi:10.1007/s00401-014-1271-5

Wetmore C, Eberhart DE, Curran T. 2001. Loss of p53 but not ARF accelerates medulloblastoma in mice heterozygous for patched. *Cancer Res* **61:** 513–516.

Williamson D, Schwalbe EC, Hicks D, Aldinger KA, Lindsey JC, Crosier S, Richardson S, Goddard J, Hill RM, Castle J, et al. 2022. Medulloblastoma group 3 and 4 tumors comprise a clinically and biologically significant expression continuum reflecting human cerebellar development. *Cell Rep* **40:** 111162. doi:10.1016/j.celrep.2022.111162

Wu X, Northcott PA, Croul S, Taylor MD. 2011. Mouse models of medulloblastoma. *Chin J Cancer* **30:** 442–449. doi:10.5732/cjc.011.10040

Yamada M, Seto Y, Taya S, Owa T, Inoue YU, Inoue T, Kawaguchi Y, Nabeshima Y-i, Hoshino M. 2014. Specification of spatial identities of cerebellar neuron progenitors by Ptf1a and Atoh1 for proper production of GABAergic and glutamatergic neurons. *J Neurosci* **34:** 4786–4800. doi:10.1523/JNEUROSCI.2722-13.2014

Yang ZJ, Ellis T, Markant SL, Read TA, Kessler JD, Bourboulas M, Schüller U, Machold R, Fishell G, Rowitch DH, et al. 2008. Medulloblastoma can be initiated by deletion of Patched in lineage-restricted progenitors or stem cells. *Cancer Cell* **14:** 135–145. doi:10.1016/j.ccr.2008.07.003

Yang F, Zhao Z, Zhang D, Xiong Y, Dong X, Wang Y, Yang M, Pan T, Liu C, Liu K, et al. 2024. Single-cell multi-omics analysis of lineage development and spatial organization in the human fetal cerebellum. *Cell Discov* **10:** 22. doi:10.1038/s41421-024-00656-1

Yao M, Ventura PB, Jiang Y, Rodriguez FJ, Wang L, Perry JSA, Yang Y, Wahl K, Crittenden RB, Bennett ML, et al. 2020. Astrocytic *trans*-differentiation completes a multicellular paracrine feedback loop required for medulloblastoma tumor growth. *Cell* **180:** 502–520.e19. doi:10.1016/j.cell.2019.12.024

Yeung J, Ha TJ, Swanson DJ, Choi K, Tong Y, Goldowitz D. 2014. *Wls* provides a new compartmental view of the rhombic lip in mouse cerebellar development. *J Neurosci* **34:** 12527–12537. doi:10.1523/JNEUROSCI.1330-14.2014

Zhang SC, Wernig M, Duncan ID, Brüstle O, Thomson JA. 2001. In vitro differentiation of transplantable neural precursors from human embryonic stem cells. *Nat Biotechnol* **19:** 1129–1133. doi:10.1038/nbt1201-1129

Zhang L, He X, Liu X, Zhang F, Huang LF, Potter AS, Xu L, Zhou W, Zheng T, Luo Z, et al. 2019. Single-cell transcriptomics in medulloblastoma reveals tumor-initiating progenitors and oncogenic cascades during tumorigenesis and relapse. *Cancer Cell* **36:** 302–318.e7. doi:10.1016/j.ccell.2019.07.009

Zhao X, Liu Z, Yu L, Zhang Y, Baxter P, Voicu H, Gurusiddappa S, Luan J, Su JM, Leung H-CE, et al. 2012. Global gene expression profiling confirms the molecular fidelity of primary tumor-based orthotopic xenograft mouse models of medulloblastoma. *Neuro-Oncology* **14:** 574–583. doi:10.1093/neuonc/nos061

Zhong S, Wang M, Huang L, Chen Y, Ge Y, Zhang J, Shi Y, Dong H, Zhou X, Wang B, et al. 2023. Single-cell epigenomics and spatiotemporal transcriptomics reveal human cerebellar development. *Nat Commun* **14:** 7613. doi:10.1038/s41467-023-43568-6

Zhukova N, Ramaswamy V, Remke M, Pfaff E, Shih DJ, Martin DC, Castelo-Branco P, Baskin B, Ray PN, Bouffet E, et al. 2013. Subgroup-specific prognostic implications of *TP53* mutation in medulloblastoma. *J Clin Oncol* **31:** 2927–2935. doi:10.1200/JCO.2012.48.5052

Arrested development: the dysfunctional life history of medulloblastoma

Ran Tao, Katie Han, Stephanie C. Wu, et al.

Genes Dev. published online September 4, 2024
Access the most recent version at doi:10.1101/gad.351936.124