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Review: Personalized mice: modelling the molecular heterogeneity of medulloblastoma

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Personalized mice: modelling the molecular heterogeneity of medulloblastoma

Medulloblastoma, the most common malignant paediatric brain tumour, is thought to arise from mutations in progenitors or stem cells in the cerebellum. Recent molecular analyses have highlighted the heterogeneity of these tumours, and demonstrated that they can be classified into at least four major subtypes that differ in terms of gene expression, genomic gains and losses, epidemiology and patient outcome. Along with analysis of human tumours, a variety of animal models of medulloblastoma have been developed using transgenic and knockout technology as well as somatic gene delivery. These models have provided valuable insight into the origins of the disease and the signalling pathways that control tumour growth. But the degree to which current models recapitulate the heterogeneity of the human disease remains unclear. Here we review the recent literature on the genomics of medulloblastoma and discuss the relationship of mouse models to the subtypes of the disease. Judicious use of existing models, and generation of additional models for poorly studied subtypes of medulloblastoma, will increase our understanding of tumour biology and allow evaluation of novel approaches to treatment of the disease.

Keywords: genomics, medulloblastoma, mouse models, preclinical testing, Sonic hedgehog, WNT

Medulloblastoma (MB), a tumour of the cerebellum, is the most common malignant brain tumour in children. The disease is usually treated by surgical resection followed by radiation and chemotherapy. While this regimen has been extremely effective at eradicating the tumour (with 5-year survival rates of up to 80% [1,2]), survivors suffer severe physical, cognitive and emotional deficits [3–5]. Improved therapeutic approaches are clearly necessary and are likely to come from a deeper understanding of the origins and development of the disease.

In recent years, it has become clear that tumours classified as MB can differ markedly from one another in terms of histology, genetics, prognosis and therapeutic responsiveness. This heterogeneity presents a challenge to investigators interested in studying the mechanisms of tumorigenesis or in developing therapies. Different subtypes of MB may arise from different progenitors that are regulated by distinct signalling pathways and are responsive to different modes of therapy. Thus, one key to understanding MB is an appreciation of the normal stem cells and progenitors that can give rise to the disease. Studying the signals that regulate growth and differentiation of these cells may point to pathways that can be targeted to promote tumour cell differentiation or death. In addition, identification of the cells from which MB arises facilitates generation of animal models that can be used to evaluate therapies. In this review, we discuss how our understanding of cerebellar development, coupled with knowledge of the common genomic alterations in the disease, is leading to more robust models of MB that can be used to study tumour biology and to test novel approaches to therapy.

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Germinal zones in the developing cerebellum

The cerebellum is composed of multiple cell types, including granule, Purkinje, Golgi, stellate and basket neurones,

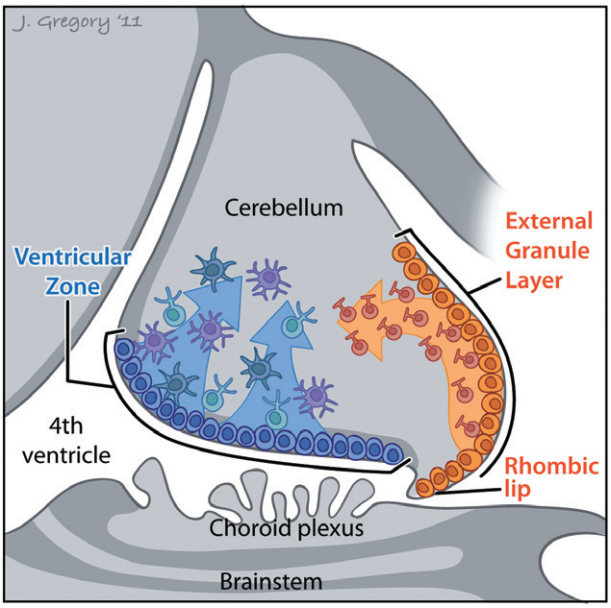

Figure 1. Germinal zones in the developing cerebellum. The cerebellum is composed of multiple types of neurones and glia, which are derived from two germinal zones: the ventricular zone (VZ) and the external granular layer (EGL). The VZ contains multipotent stem cells (round blue cells) that migrate outwards towards the surface of the cerebellum to generate differentiated neurones and glia (green, purple and violet cells). A subset of stem cells also moves laterally to the rhombic lip, and undergoes commitment to the granule neurone lineage. Granule neurone precursors (GNPs, round orange cells) stream around the outside of the cerebellum to form the external granule layer (EGL). In the EGL, GNPs proliferate in response to the mitogen Sonic hedgehog and then differentiate into granule neurones (pale orange cells) before migrating inwards to form the internal granule layer. Stem cells in the VZ and GNPs in the EGL are likely to be cells of origin for medulloblastoma. However, recent studies have suggested that progenitors in the brainstem may also give rise to some types of the disease (Illustration by Jill Gregory).

as well as several classes of astrocytes and oligodendrocytes. Each of these cell types is derived from one of two germinal zones in the embryonic cerebellum (see Figure 1): the ventricular zone (VZ) and the external granule layer (EGL) [6–8]. The VZ consists of a layer of stem cells within the neuroepithelium around the roof of the fourth ventricle. These cells proliferate during embryogenesis, and then differentiate and migrate outwards towards the surface of the cerebellum, giving rise to the majority of cerebellar neurones and glia. During postnatal development the VZ shrinks and disappears, but a population of stem cells persists in the white matter of the postnatal cerebellum [9]. These cells express Prominin1 (CD133), lack expression of neuronal and glial lineage markers, and can give rise to neurones, astrocytes and oligodendrocytes *in vitro* and following transplantation. Although these cells exhibit characteristics of multipotent neural stem cells, their contribution to neuronal and glial lineages in the mature cerebellum remains unknown.

While the majority of stem cells in the embryonic VZ migrates radially outwards to generate differentiated neurones and glia, a subset of these cells moves laterally to a structure called the upper rhombic lip (URL), turns on expression of the transcription factor Math1 (ATOH1), and commits to the granule neurone lineage [6–8]. Granule neurone precursors (GNPs) from the URL then stream around the outside of the cerebellum to form the EGL. During the first 2–3 weeks after birth, GNPs in the EGL proliferate rapidly in response to the mitogen Sonic hedgehog (SHH) [10,11], which is produced by neighbouring Purkinje cells. Then, in response to signals that remain poorly understood, GNPs exit the cell cycle, differentiate into post-mitotic neurones, and migrate inwards to form the internal granule layer (IGL).

The VZ and the EGL represent rich environments for initiation of MB, but are by no means the only possible sources of the tumour. Lineage-restricted progenitors that have migrated away from these germinal zones may retain sensitivity to transformation. In addition, cells outside the cerebellum, in the neighbouring brainstem, can become transformed and invade the boundaries of the cerebellum [12]. As stem cells and progenitors in each of these regions differ in terms of the signals that control their proliferation, differentiation and survival, it is easy to imagine that distinct types of mutations might be required for their transformation. It is the interaction between cells of origin and oncogenic mutations that gives rise to the diverse subtypes of MB.

Histological and molecular classification of medulloblastoma

Medulloblastoma is classified histopathologically into four major subtypes: desmoplastic/nodular, classic, large cell/anaplastic (LC/A), and MB with extensive nodularity (MBEN) [13,14]. The desmoplastic subtypes (desmoplastic/nodular and MBEN) represent approximately 25% of MBs and are characterized by nodules of sparsely distributed differentiated cells, often bounded by extracellular matrix (reticulin), and interspersed with regions of more densely packed proliferating cells [15]. Approximately 65% of MBs belong to the 'classic' subtype,

which is comprised of relatively uniform sheets of undifferentiated small round blue cells. LC/A tumours consist of large, pleomorphic cells with prominent nucleoli, nuclear wrapping and high mitotic and apoptotic indices [16–18]. These tumours represent approximately 10% of MBs and are associated with a poor prognosis. Finally, MBENs, which display greater nodularity and more advanced neuronal differentiation than desmoplastic/nodular tumours, tend to present in infancy and to have a favourable prognosis [19].

Histological classification of MB has long formed the basis of patient stratification, but more recent genomic data have significantly enhanced our ability to classify tumours, define key molecular alterations, and develop appropriate targeted therapies. Based on mRNA and miRNA expression profiling, DNA copy number analysis and mutational analysis conducted by multiple groups [20–24], MB can now be classified into at least four molecular subtypes: SHH-associated, WNT-associated, 'Group 3' (characterized by overexpression or amplification of the *MYC* oncogene) and 'Group 4' (a heterogeneous group of tumours for which a defining molecular event has not yet been identified). Importantly, these subtypes differ not only in terms of genetics, but also in terms of clinical features, such as age and gender distribution, likelihood of metastasis and patient outcome. There is some debate regarding the precise number of MB subtypes, with some investigators dividing tumours into four classes [21], and others describing five or six [22–24]. For simplicity, the discussion below will refer to the four-subtype scheme developed by Northcott *et al.*, which is based on data from 103 primary MB samples. Table 1 shows the relationship between this scheme and the classification systems proposed by other groups.

SHH-associated tumours

Approximately 20–30% of MBs are associated with mutation or activation of the SHH pathway [21–24]. This pathway, which is critical for the proliferation of GNPs in the EGL, is normally activated by binding of the SHH ligand to the transmembrane protein Patched1 (PTCH1) [25–27]. In the absence of ligand, PTCH1 functions as a repressor of signalling by blocking the activity of the 7-pass transmembrane protein Smoothened (SMO). Upon binding of SHH, this repression is relieved and SMO is activated, leading to release of GLI family transcription factors from a complex containing Suppressor of Fused (SUFU), and enabling translocation of GLI proteins to the nucleus where they can activate transcription of target genes. Thus, SHH, SMO, and GLI represent activators of the SHH pathway, while PTCH and SUFU represent repressors.

The importance of the SHH pathway in MB was first recognized when Gorlin syndrome, a hereditary disease associated with increased incidence of basal cell carcinoma and MB, was linked to germline mutations in *PTCH1* [28–30]. Since then, inactivating mutations in *PTCH1* and *SUFU*, activating mutations in *SMO*, and amplification of *GLI2* have also been observed in sporadic MBs [22,31–34]. SHH-associated tumours occur predominantly in infants and adults, but not in children, and are associated with a mixed prognosis [21,22]. Other common properties of SHH-associated tumours include amplification of *MYCN* and the microRNA miR-17/92, chromosomal gains of 9p, 3q, 20q, and 21q, and loss of chromosomes 9q (specifically the *PTCH1* locus at 9q22) and 10q [21,22,35]. The identities of most of the oncogenes and tumour suppressors that can cooperate with SHH signalling to promote tumorigenesis remain unkonwn and may be critical for improved treatment of this MB subgroup.

Historically, desmoplastic histology has been considered a surrogate for activation of the SHH pathway [31,36]. However, genomic data have indicated that this association is not entirely reliable [21,22,24]. Although some SHH-associated tumours display desmoplastic histology, other histologies (classic, LC/A and MBEN) have also been observed within this subtype. Thus, elucidation of the molecular properties of this subtype represents a significant advance that will enable more accurate diagnosis and implementation of appropriate treatments for individual patients.

WNT-associated tumours

Similar to SHH-associated tumours, the designation of a WNT-associated subtype of MB was foreshadowed by patients with hereditary tumour syndromes. A subset of patients with Turcot syndrome, a disease characterized by a predisposition to colon cancer and brain tumours, harbours mutations in the adenomatous polyposis coli gene (*APC*, a negative regulator of WNT signalling) [37]. In addition, mutations in WNT pathway components, including beta-catenin (*CTNNB1*), *APC* and *AXIN1/2*, have been shown to occur in approximately 15% of

Table 1. Classification and characteristics of medulloblastoma subgroups

Classification scheme Taylor *et al.* (2012) [20]; Northcott *et al.* (2010) [21]	WNT	SHH	Group 3 (Group C)		Group 4 (Group D)	
Cho *et al.* (2010) [22]	c6	c3	c1	c5	c4	c2
Kool *et al.* (2008) [23]	A	B	E		C	D
Thompson *et al.* (2006) [24]	B	C′, D	E, A		A, C	
Presumed origin	Brainstem	GNPs	Unknown	Unknown	Unknown	Unknown
Transcriptional programmes	WNT MYC	SHH MYCN	Photoreceptor GABAergic MYC	Photoreceptor GABAergic	Photoreceptor Neuronal/glutamatergic	Neuronal/glutamatergic
Gene/chromosome alterations (+, gain; –, loss; mut, mutated.)	CTNNB mut TP53 mut – Ch. 6	+ GLI2, MYCN PTCH, SMO, SUFU mut + Ch. 2, 3q – Ch. 9q, 10q, 20p	+ MYC, OTX2 + Ch. 1q, 8, 18 – Ch. X loss i(17q)	+ OTX2 + Ch. 1q, 14, 17, 18 – Ch. 8, 10, 11, 13, 16	+ MYCN, OTX2 + Ch. 7, 12q – Ch. 8, X (females) i(17q)	+ OTX2 + Ch. 18 – Ch. X (females) i(17q)
Immunohistochemical markers	CTNNB1 DKK1 FilaminA YAP1	GLI1 SFRP1 GAB1 FilaminA YAP1	NPR3		KCNA1	
Age distribution	Children & teenagers 25% of adults	Infants 50% of adults	Young children Not in adults	Children Not in adults	Children 25% of adults	
Metastasis	Rare	Variable	Very common	Rare	Variable	
Prognosis	Favourable	Infants favourable Others intermediate	Poor	Intermediate	Intermediate	

sporadic MBs [38–42]. Recent genomic data indicate that tumours within the WNT subgroup display elevated expression of WNT pathway target genes, including *WIF1*, *DKK1* and *DKK2*, as well as genes involved in axon guidance and O-glycan biosynthesis [21,24]. On a genetic level, loss of chromosome 6 has also been associated with this subtype [43], but does not occur in all tumours [21,44]. Furthermore, mutations in the tumour suppressor gene *TP53* occur most frequently in tumours of this subtype [45]. WNT-associated tumours occur almost always in children aged 5–15, rather than in infants or adults, and the prognosis for patients with these tumours is better than for other subtypes [44,46,47].

Non-WNT/non-SHH tumours

Genomic data indicate that Group 3 and Group 4 tumours are more similar to one another than to the other two subgroups [21]. For example, both groups express high levels of Otx2 and FoxG1B (known MB oncogenes), exhibit isochromosome 17q [a cytogenetic rearrangement in which the long arm (17q) is duplicated and the short arm is lost], gains of chromosomes 17q and 18, and loss of chromosome 11p. In addition, pathways involved in neuronal development display elevated expression in both Group 3 and Group 4 tumours, but overexpression of phototransduction and glutamate signalling pathways is specific to Group 3 tumours. Other genetic events that occur specifically in Group 3 include gain of chromosome 1q, distal loss of 5q, and loss of 16q; these events can help discriminate between Group 3 and Group 4 tumours.

Another extremely important difference between the Group 3 and Group 4 subtypes is overexpression, and in some cases focal amplification, of the *MYC* oncogene on chromosome 8q24. Overexpression of MYC and its target genes have previously been associated with treatment failure [16,48], and consistent with this, patients with Group 3 tumours have the worst prognosis among all subtypes of MB [21]. Although multiple histological types are represented within this subgroup, LC/A histology has been significantly associated with Group 3, and Group 3 tumours that also exhibit LC/A histology have the worst prognosis of all MBs. Furthermore, cerebrospinal fluid dissemination and metastasis occur much more frequently in Group 3 tumours than in the other subgroups. Based on these characteristics, it has been suggested that individuals with Group 3 tumours should be treated with the most aggressive regimens available.

Although the Group 4 subtype of MB shares many features with Group 3, several characteristics are specific for these tumours. Group 4 tumours express elevated levels of genes involved in semaphorin, cAMP, G-protein-coupled receptor, and β-adrenergic signalling [21]. Furthermore, genetic events that occur specifically in Group 4 tumours include losses of both 8p and 8q and loss of the X chromosome in tumours from female patients. Isochromosome 17q occurs more frequently in Group 4 than in Group 3. Group 4 tumours occur in patients of all ages, and the prognosis for these patients varies, mainly according to recognized clinicopathological factors such as metastatic disease and LC/A histology.

The four-group scheme simplifies our understanding of some of the molecular features of MB, but it may also mask some of the heterogeneity among MB patients. Focusing on this heterogeneity, Cho *et al*. [22] also recognize SHH-associated (designated 'c3') and WNT-associated ('c6') subgroups, but have suggested that Group 3 and Group 4 may be best represented as a greater range of molecular tumour types (denoted c2, c4, c5 and c1). At one end of the spectrum is Group c2, which is characterized by a 'neuronal/glutamatergic' signature. The next subgroup, c4, displays neuronal features as well as a photoreceptor signature. At the other end of the spectrum lie subgroups c5 and c1; both of these display photoreceptor/GABAergic signatures, but c1 also exhibits a MYC activation signature. Like the Group 3 tumours described by Northcott *et al*., the MYC-associated c1 tumours are the most aggressive and display the worst prognosis.

Regardless of the number of subgroups used to describe the disease, genomic analysis has increased our appreciation of the heterogeneity of MB, and allowed us to focus on the key molecular characteristics that drive each patient's tumour. This information will prove invaluable as we begin to develop models that can be used to elucidate tumour biology and to develop more effective approaches to therapy.

Genetically engineered models of medulloblastoma

Genomic data have generated numerous hypotheses regarding the biology and appropriate treatments for each of the MB subgroups. One approach to testing these hypotheses is using genetically engineered mice (GEM). Many existing GEM models were created to test the func-

tion of particular genes in development or cancer, and were unexpectedly found to develop MB. More recently, models have been created by specifically perturbing expression of putative MB oncogenes or tumour suppressors in stem cells or GNPs, with the express purpose of generating models of MB. In both scenarios, these models have yielded valuable information about the origins and biology of the disease, and have provided valuable platforms for evaluation of therapies. Models representing the different subtypes of MB are described below.

Models of SHH-associated MB

Among the first GEM models of MB was the *Ptch1*-knockout mouse [49]. These animals were created using conventional homologous recombination technology, which replaced a portion of the *Ptch1* gene with LacZ and neomycin reporter genes, leading to loss of expression of the Ptch1 protein. Because Ptch1 functions as a repressor of SHH signalling, the pathway is constitutively active in these mice. Homozygous ($Ptch1^{-/-}$) mice exhibit severe defects in the neural tube, heart and other tissues, and die during embryogenesis. In contrast, heterozygotes ($Ptch1^{+/-}$ mice) are viable, and 15–20% develop cerebellar tumours that resemble human MB [49,50]. These mice have been used extensively to examine properties of MB driven by SHH pathway activation, such as the early stages of tumorigenesis [51,52], cooperating oncogenic pathways [50,53–56], the role of cancer stem cells [57,58], the mechanisms of radioresistance [59] and the effects of targeted therapies [60,61].

More recently, conditional *Ptch1*-knockout models of MB have been developed [62]. In these animals, loxP-flanked alleles of *Ptch1* ($Ptch1^{flox}$) are deleted by the Cre recombinase, whose expression is controlled by the Math1 or glial fibrillary acidic protein (GFAP) promoters. The resulting mice develop MB with an incidence of 100% and a much shorter average latency than conventional $Ptch1^{+/-}$ mice. Unlike *Math1*-Cre or *Math1*-CreER, which delete *Ptch1* specifically in GNPs, *GFAP*-Cre deletes *Ptch1* in stem cells. Analysis of the early stages of tumorigenesis in *GFAP*-Cre;$Ptch1^{fl/fl}$ mice indicates that activation of the SHH pathway in stem cells only results in tumour formation once cells enter the rhombic lip and commit to the granule lineage. These data suggest that GNPs represent critical targets of transformation for SHH-associated tumours. This model represents a valuable tool for understanding the early stages of tumorigenesis, and given the high tumour incidence and short latency, will likely prove useful for preclinical studies as well.

Activation of other components of the SHH pathway has also been shown to cause MB in mice. Evidence that SHH ligand can promote tumorigenesis comes from an elegant series of studies using the RCAS/TVA transduction system [63–67]. In this system, transgenic mice expressing the avian retrovirus receptor (TVA) in specific cell types are inoculated with RCAS-based avian retroviruses encoding genes of interest, and only cells expressing the TVA receptor become infected. Fults and colleagues have demonstrated that infection of neonatal Nestin-TVA mice with viruses encoding SHH results in MB in 10–15% of mice; co-infection with viruses encoding other oncogenes (see below) markedly increases the incidence of tumours.

Models of MB have also been generated by targeting downstream elements in the SHH pathway. In the most widely studied of these models, constitutive expression of an activated *Smo* transgene (*SmoA1*) is controlled by the *NeuroD2* (ND2) promoter, which is active in GNPs [68]. In mice hemizygous for *ND2-SmoA1*, approximately 50% of the animals develop tumours, with a median latency of 6 months. A homozygous *ND2-SmoA1* model has also been created; this model has a dramatically higher tumour incidence (>90%) and a much shorter latency (2 months) [69]. Furthermore, this model is one of the few models in which animals develop leptomeningeal metastases, which is a common feature of human MB. In light of the high incidence, short latency and metastatic phenotype, the '*Smo-Smo*' mouse represents another important and valuable tool for preclinical studies. Activation of Smo in earlier neural progenitors or stem cells (using *GFAP*, *Math1*, *Tlx3* or *Olig2*-driven Cre) also results in MB [70], and as with the conditional *Ptch1*-knockout mice, these tumours appear to result from expansion of GNPs. Finally, animals lacking Sufu or overexpressing an activated form of Gli2 also develop MB; however, in each case additional genes (*Tp53* and *Kif3a*, respectively) must be mutated for tumours to develop [71–73].

Indeed, one important use of SHH-associated models has been in defining genes that can cooperate with SHH signalling to promote MB formation. For example, crossing $Ptch1^{+/-}$ mice with $Tp53^{-/-}$ mice leads to a dramatic increase in tumour incidence, from 15% in $Ptch1^{+/-}$ mice to 100% in $Ptch1^{+/-}Tp53^{-/-}$ mice [53]. This penetrance has made the $Ptch1^{+/-}Tp53^{-/-}$ model particularly useful for preclinical studies [60,61,74,75]. Although there have been some questions about the relevance of this model to

human MB, recent studies have clearly shown that a subset of human SHH-associated tumours also harbours mutations in *TP53* [45,76].

Analogous to Tp53, other cell cycle regulators are also dysfunctional in some cases of human MB. To determine whether these alterations can cooperate with SHH signalling in tumorigenesis, mice deficient in the cell cycle regulators p18-Ink4c or p27-Kip1 were crossed with *Ptch1*$^{+/-}$ mice [54,77,78]. Both heterozygous and homozygous loss of these genes led to increased tumour incidence and shortened latency in combination with loss of *Ptch1*, suggesting that alterations in these cell cycle regulators can indeed affect development of SHH-associated tumours. Interestingly, loss of Ink4c, Kip1 or p19-Ink4d can also synergize with loss of p53 to generate MB [54,77–79]; although these tumours arise in mice that are wild-type for *Ptch1*, the tumours themselves lack expression of Ptch1 and resemble SHH-associated MB at a molecular level [54,80], suggesting that mutations in these genes also result from aberrant activation of the SHH pathway.

The RCAS/TVA system has also been used to define oncogenic pathways that can cooperate with SHH signalling to cause MB. *Nestin-TVA* mice infected with viruses encoding SHH develop MB with an incidence of 15–30%. Co-infection with viruses encoding C-myc, N-myc, insulin-like growth factor 2 (Igf-1), Akt, Bcl-2, or hepatocyte growth factor (HGF) leads to increased incidence of MB, suggesting that each of these genes is capable of cooperating with SHH pathway activation to promote tumour development [63,64,66,67,81]. In addition to identifying genes that synergize with SHH signalling, this model has also been used for preclinical studies to identify therapeutic approaches that may be broadly applicable in SHH-associated MB. For example, mice bearing tumours initiated by SHH + HGF were treated with control antibodies, HGF neutralizing antibodies, or SHH neutralizing antibodies [82]. Tumour development was significantly delayed in animals treated with either HGF antibody or SHH antibodies; interestingly, the combination of these antibodies was no more effective than anti-HGF alone. These studies suggest that targeting cooperating pathways may represent an effective approach for treatment of SHH-associated MB.

Another class of genes that has been shown to be important in MB formation includes regulators of DNA damage sensing or repair [83–88]. For example, mice deficient in DNA Ligase 4 (Lig4), an enzyme involved in repair of DNA double-strand breaks as well as Tp53 (*Lig4*$^{-/-}$*Tp53*$^{-/-}$ mice) develop tumours with 100% penetrance and an extremely short latency (3–9 weeks). Although this model causes loss of expression of these proteins throughout the animal, the mice specifically develop MB and B-cell lymphomas. Deletion of Xrcc4 (a non-homologous end joining repair protein), Xrcc2 (a protein involved in repair of double-strand breaks by homologous recombination), Parp1 (a DNA single-strand repair protein), or Brca2 (a double-strand break repair protein) on a *Tp53*$^{-/-}$ background also results in MB [84,86–88]. Surprisingly, these tumours all appear to exhibit SHH pathway activation and resemble *Ptch1*$^{+/-}$ tumours at a molecular level. Subsequent studies have demonstrated that the majority have deletions or translocations involving mouse chromosome 13 (which contains *Ptch1*), and inactivating mutations in the remaining *Ptch1* allele [88]. The fact that such mutations are so strongly selected for highlights the importance of the SHH pathway in MB formation, and raises the possibility that some human SHH-associated tumours may not be initiated by mutations in the SHH pathway.

Models of WNT-associated MB

The models described above have been extremely valuable for understanding SHH-associated tumours, but somewhat frustrating for investigators interested in other tumour subtypes. Initial attempts to create models of WNT-associated tumours – by driving expression of β-catenin (Ctnnb1, an activator of the canonical WNT pathway) in granule neurones or by transducing *Nestin-TVA* mice with β-catenin retroviruses – were unsuccessful [89,90]. Infection of *GFAP-TVA* mice with viruses encoding β-catenin and c-myc (in a *Tp53*$^{-/-}$ background) was shown to result in primitive neuroectodermal tumours in the cerebrum and cerebellum [91]. However, these tumours do not resemble human WNT-associated MB histologically; moreover, tumours can also be induced in the absence of β-catenin (that is, with c-myc viruses alone), raising questions about the role of WNT signalling in this model.

Recently, a model of WNT-driven MB was established by targeting a distinct population of progenitors [12]. Gibson *et al*. crossed *Blbp-Cre* mice, which express the Cre recombinase in the cerebellar VZ and in the dorsal brainstem (lower rhombic lip), with mice expressing a conditional allele of *Ctnnb1* (*Ctnnb1*$^{lox(ex3)}$) that can be activated by Cre. In the progeny of this cross, aberrant cell masses were

observed in the brainstem, but these masses never progressed to MB. However, when these mice were crossed with *Tp53*^{flx} mice to delete p53 (which is often mutated in human WNT-associated tumours), the resulting mice developed MB in 15% of cases. Gene expression analysis indicated that the mouse tumours were distinct from SHH-associated tumours and closely resembled human WNT-associated MB.

Unlike SHH-associated tumours, which arise on the surface of the cerebellum, tumours in the *Blbp-Cre;Ctnnb1*$^{lox(ex3)}$*;Tp53*^{flx} mice appear to develop below the cerebellum in association with the brainstem [12]. Interestingly, radiological analysis of human MB also reveals a difference in tumour location between SHH- and WNT-associated MB, with SHH-associated tumours primarily in the cerebellar cortex and WNT-associated tumours in the fourth ventricle infiltrating the brainstem. In light of these findings, the authors speculate that SHH- and WNT-associated tumours arise from distinct cells of origin: the former from GNPs in the EGL and the latter from progenitors in the brainstem. To determine whether WNT-associated tumours could also arise from GNPs, they utilized *Math1-Cre* (instead of *Blbp-Cre*) to activate β-catenin and delete *Tp53*. These animals did not develop tumours, suggesting that activation of the WNT pathway must occur in a specific cell type – brainstem progenitors – to initiate tumour formation. Assuming that different subtypes of MB arise from distinct cells of origin, it will be important to consider the properties of these cells in developing subtype-specific therapies.

Models of other subtypes of MB

In addition to models of WNT-associated tumours, several models of non-SHH-associated tumours have recently been developed. Like the model for the WNT-associated subtype, the tumours that develop in these models can be derived from cells other than GNPs in the EGL. In one series of studies, Cre-mediated deletion of conditional alleles of *Tp53* and *Rb* has been performed in both GFAP+ cells *in vivo* and in cerebellar stem cells grown as neurospheres *in vitro* [92–94]. Upon deletion of these genes *in vivo* by GFAP-Cre, tumours formed on the surface of the cerebellum and exhibited amplification of *N-myc*, *Gli2*, and *Ptch2* [93]. The increased expression of these genes suggests that these tumours might represent a model for human SHH-associated MB. But unlike previous models, which display classic histology, these tumours exhibit characteristics of LC/A MB, which occurs in a subset of human SHH-associated tumours.

In contrast to tumours induced by GFAP-Cre-mediated deletion of *Rb* and *Tp53*, tumours resulting from infection of *Rb*$^{flox/flox}$*; p53*$^{flox/flox}$ cerebellar stem cells with viral Cre (followed by injection of these cells into the flank) lack activation of the SHH pathway and express stem cell markers, such as Nestin, Sox2 and Sox9 [94]. Although it is not clear which subtype of human MB these tumours most closely resemble, the authors note that a subset of human MBs expresses stem cell markers and is associated with poor outcome. The distinct tumour types resulting from deletion of *Rb* and *Tp53* in different cell types provide further support for the notion that the cell of origin can play a critical role in determining tumour phenotype.

Another elegant model of MB was recently generated by expressing MYCN in the developing cerebellum [95]. In this system, mice in which the glutamate transporter Glt1 drives expression of the tetracycline transactivator (tTA) were crossed to animals in which a tetracycline response element (TRE) controls expression of both MYCN and luciferase (the latter allowing for bioluminescent tumour monitoring). Dosing the progeny of this cross (Glt1-tTA;TRE-MYCN-Luc, or GTML) with doxycycline activates expression of MYCN and luciferase in Glt1-expressing cells. Glt1 is expressed by multiple progenitors within the cerebellum, so multiple cell types can potentially be targeted using this transgene. Interestingly, GTML mice develop MB with two separate histologies. Approximately half the animals develop tumours with a classic histology, while the remainder exhibits LC/A histology. Furthermore, metastatic dissemination of tumours into the spinal column is observed in some mice, suggesting that these animals might represent a model for some of the most aggressive forms of MB. Among human MBs, the highest levels of MYCN expression are found in SHH-associated tumours, but elevated levels (compared with normal cerebellum) are found in other subtypes as well [95]. Notably, the majority of tumours in GTML mice do not exhibit a SHH pathway activation profile (as indicated by expression of Gli1 and Math1) and have high levels of Otx2 (a marker associated with non-SHH/non-WNT tumours). Thus, GTML mice represent a novel model for non-SHH-associated MB, although the precise relationship of these tumours to the human MB subgroups described above remains to be determined.

Summary

Mouse models have proven to be critical for understanding the biology of MB, elucidating the pathways that can lead to tumour formation and highlighting the influence of the cell of origin on MB subtype. Moreover, GEM models have allowed preclinical studies to be performed *in vivo* in syngeneic, immunocompetent hosts, facilitating assessment of the influence of tumour microenvironment on responsiveness to therapy. For example, hedgehog antagonists have been developed by several pharmaceutical companies [96–98], and GEM models of SHH-associated MB have been instrumental in testing the efficacy of these compounds [60,61,74]. In the wake of promising results in these preclinical studies, patients are now being treated with SHH antagonists, and at least in some cases, exhibiting tumour regression [98,99]. However, as with many targeted therapies, resistance has also been observed [100,101]. Mouse models are now being used to determine the mechanisms of this resistance [61,75], and to test approaches to preventing or overcoming it. Continual integration of genomic data, information from mouse models and results of clinical trials will undoubtedly lead to improved treatment outcomes for patients with MB.

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References

1 Crawford JR, MacDonald TJ, Packer RJ. Medulloblastoma in childhood: new biological advances. *Lancet Neurol* 2007; **6**: 1073–85
2 Rossi A, Caracciolo V, Russo G, Reiss K, Giordano A. Medulloblastoma: from molecular pathology to therapy. *Clin Cancer Res* 2008; **14**: 971–6
3 Saury JM, Emanuelson I. Cognitive consequences of the treatment of medulloblastoma among children. *Pediatr Neurol* 2011; **44**: 21–30
4 Wolfe-Christensen C, Mullins LL, Scott JG, McNall-Knapp RY. Persistent psychosocial problems in children who develop posterior fossa syndrome after medulloblastoma resection. *Pediatr Blood Cancer* 2007; **49**: 723–6
5 Xu W, Janss A, Packer RJ, Phillips P, Goldwein J, Moshang T Jr. Endocrine outcome in children with medulloblastoma treated with 18 Gy of craniospinal radiation therapy. *Neuro Oncol* 2004; **6**: 113–18
6 Wang VY, Zoghbi HY. Genetic regulation of cerebellar development. *Nat Rev Neurosci* 2001; **2**: 484–91
7 ten Donkelaar HJ, Lammens M, Wesseling P, Thijssen HO, Renier WO. Development and developmental disorders of the human cerebellum. *J Neurol* 2003; **250**: 1025–36
8 Hatten ME, Roussel MF. Development and cancer of the cerebellum. *Trends Neurosci* 2011; **34**: 134–42
9 Lee A, Kessler JD, Read TA, Kaiser C, Corbeil D, Huttner WB, Johnson JE, Wechsler-Reya RJ. Isolation of neural stem cells from the postnatal cerebellum. *Nat Neurosci* 2005; **8**: 723–9
10 Wechsler-Reya RJ, Scott MP. Control of neuronal precursor proliferation in the cerebellum by Sonic Hedgehog. *Neuron* 1999; **22**: 103–14
11 Wallace VA. Purkinje-cell-derived Sonic hedgehog regulates granule neuron precursor cell proliferation in the developing mouse cerebellum. *Curr Biol* 1999; **9**: 445–8
12 Gibson P, Tong Y, Robinson G, Thompson MC, Currle DS, Eden C, Kranenburg TA, Hogg T, Poppleton H, Martin J, Finkelstein D, Pounds S, Weiss A, Patay Z, Scoggins M, Ogg R, Pei Y, Yang ZJ, Brun S, Lee Y, Zindy F, Lindsey JC, Taketo MM, Boop FA, Sanford RA, Gajjar A, Clifford SC, Roussel MF, McKinnon PJ, Gutmann DH, Ellison DW, Wechsler-Reya R, Gilbertson RJ. Subtypes of medulloblastoma have distinct developmental origins. *Nature* 2010; **468**: 1095–9
13 Gulino A, Arcella A, Giangaspero F. Pathological and molecular heterogeneity of medulloblastoma. *Curr Opin Oncol* 2008; **20**: 668–75
14 Lamont JM, McManamy CS, Pearson AD, Clifford SC, Ellison DW. Combined histopathological and molecular cytogenetic stratification of medulloblastoma patients. *Clin Cancer Res* 2004; **10**: 5482–93
15 McManamy CS, Pears J, Weston CL, Hanzely Z, Ironside JW, Taylor RE, Grundy RG, Clifford SC, Ellison DW. Nodule formation and desmoplasia in medulloblastomas-defining the nodular/desmoplastic variant and its biological behavior. *Brain Pathol* 2007; **17**: 151–64
16 von Hoff K, Hartmann W, von Bueren AO, Gerber NU, Grotzer MA, Pietsch T, Rutkowski S. Large cell/anaplastic medulloblastoma: outcome according to myc status, histopathological, and clinical risk factors. *Pediatr Blood Cancer* 2010; **54**: 369–76
17 Eberhart CG, Burger PC. Anaplasia and grading in medulloblastomas. *Brain Pathol* 2003; **13**: 376–85
18 Leonard JR, Cai DX, Rivet DJ, Kaufman BA, Park TS, Levy BK, Perry A. Large cell/anaplastic medulloblastomas and medullomyoblastomas: clinicopathological and genetic features. *J Neurosurg* 2001; **95**: 82–8

19 Giangaspero F, Perilongo G, Fondelli MP, Brisigotti M, Carollo C, Burnelli R, Burger PC, Garre ML. Medulloblastoma with extensive nodularity: a variant with favorable prognosis. *J Neurosurg* 1999; **91**: 971–7
20 Taylor MD, Northcott PA, Korshunov A, Remke M, Cho YJ, Clifford SC, Eberhart CG, Parsons DW, Rutkowski S, Gajjar A, Ellison D, Lichter P, Gilbertson RJ, Pomeroy SL, Kool M, Pfister SM. Molecular subgroups of medulloblastoma: the current consensus. *Acta Neuropathol* 2012 (in press)
21 Northcott PA, Korshunov A, Witt H, Hielscher T, Eberhart CG, Mack S, Bouffet E, Clifford SC, Hawkins CE, French P, Rutka JT, Pfister S, Taylor MD. Medulloblastoma comprises four distinct molecular variants. *J Clin Oncol* 2010; **29**: 1408–14
22 Cho YJ, Tsherniak A, Tamayo P, Santagata S, Ligon A, Greulich H, Berhoukim R, Amani V, Goumnerova L, Eberhart CG, Lau CC, Olson JM, Gilbertson RJ, Gajjar A, Delattre O, Kool M, Ligon K, Meyerson M, Mesirov JP, Pomeroy SL. Integrative genomic analysis of medulloblastoma identifies a molecular subgroup that drives poor clinical outcome. *J Clin Oncol* 2010; **29**: 1424–30
23 Kool M, Koster J, Bunt J, Hasselt NE, Lakeman A, van Sluis P, Troost D, Meeteren NS, Caron HN, Cloos J, Mrsic A, Ylstra B, Grajkowska W, Hartmann W, Pietsch T, Ellison D, Clifford SC, Versteeg R. Integrated genomics identifies five medulloblastoma subtypes with distinct genetic profiles, pathway signatures and clinicopathological features. *PLoS ONE* 2008; **3**: e3088
24 Thompson MC, Fuller C, Hogg TL, Dalton J, Finkelstein D, Lau CC, Chintagumpala M, Adesina A, Ashley DM, Kellie SJ, Taylor MD, Curran T, Gajjar A, Gilbertson RJ. Genomics identifies medulloblastoma subgroups that are enriched for specific genetic alterations. *J Clin Oncol* 2006; **24**: 1924–31
25 Lum L, Beachy PA. The Hedgehog response network: sensors, switches, and routers. *Science* 2004; **304**: 1755–9
26 Cohen MM Jr. Hedgehog signaling update. *Am J Med Genet A* 2010; **152A**: 1875–914
27 Hui CC, Angers S. Gli proteins in development and disease. *Annu Rev Cell Dev Biol* 2011; **27**: 513–37
28 Evans DG, Farndon PA, Burnell LD, Gattamaneni HR, Birch JM. The incidence of Gorlin syndrome in 173 consecutive cases of medulloblastoma. *Br J Cancer* 1991; **64**: 959–61
29 Johnson RL, Rothman AL, Xie J, Goodrich LV, Bare JW, Bonifas JM, Quinn AG, Myers RM, Cox DR, Epstein EH Jr, Scott MP. Human homolog of patched, a candidate gene for the basal cell nevus syndrome. *Science* 1996; **272**: 1668–71
30 Hahn H, Wicking C, Zaphiropoulous PG, Gailani MR, Shanley S, Chidambaram A, Vorechovsky I, Holmberg E, Unden AB, Gillies S, Negus K, Smyth I, Pressman C, Leffell DJ, Gerrard B, Goldstein AM, Dean M, Toftgard R, Chenevix-Trench G, Wainwright B, Bale AE. Mutations of the human homolog of Drosophila patched in the nevoid basal cell carcinoma syndrome. *Cell* 1996; **85**: 841–51
31 Pietsch T, Waha A, Koch A, Kraus J, Albrecht S, Tonn J, Sorensen N, Berthold F, Henk B, Schmandt N, Wolf HK, von Deimling A, Wainwright B, Chenevix-Trench G, Wiestler OD, Wicking C. Medulloblastomas of the desmoplastic variant carry mutations of the human homologue of Drosophila patched. *Cancer Res* 1997; **57**: 2085–8
32 Raffel C, Jenkins RB, Frederick L, Hebrink D, Alderete B, Fults DW, James CD. Sporadic medulloblastomas contain PTCH mutations. *Cancer Res* 1997; **57**: 842–5
33 Taylor MD, Liu L, Raffel C, Hui CC, Mainprize TG, Zhang X, Agatep R, Chiappa S, Gao L, Lowrance A, Hao A, Goldstein AM, Stavrou T, Scherer SW, Dura WT, Wainwright B, Squire JA, Rutka JT, Hogg D. Mutations in SUFU predispose to medulloblastoma. *Nat Genet* 2002; **31**: 306–10
34 Lam CW, Xie J, To KF, Ng HK, Lee KC, Yuen NW, Lim PL, Chan LY, Tong SF, McCormick F. A frequent activated smoothened mutation in sporadic basal cell carcinomas. *Oncogene* 1999; **18**: 833–6
35 Uziel T, Karginov FV, Xie S, Parker JS, Wang YD, Gajjar A, He L, Ellison D, Gilbertson RJ, Hannon G, Roussel MF. The miR-17~92 cluster collaborates with the Sonic Hedgehog pathway in medulloblastoma. *Proc Natl Acad Sci U S A* 2009; **106**: 2812–17
36 Pomeroy SL, Tamayo P, Gaasenbeek M, Sturla LM, Angelo M, McLaughlin ME, Kim JY, Goumnerova LC, Black PM, Lau C, Allen JC, Zagzag D, Olson JM, Curran T, Wetmore C, Biegel JA, Poggio T, Mukherjee S, Rifkin R, Califano A, Stolovitzky G, Louis DN, Mesirov JP, Lander ES, Golub TR. Prediction of central nervous system embryonal tumour outcome based on gene expression. *Nature* 2002; **415**: 436–42
37 Hamilton SR, Liu B, Parsons RE, Papadopoulos N, Jen J, Powell SM, Krush AJ, Berk T, Cohen Z, Tetu B, Burger PC, Wood PA, Taqi F, Booker SV, Petersen GM, Offerhaus GJA, Tersmette AC, Giardiello FM, Vogelstein B, Kinzler KW. The molecular basis of Turcot's syndrome. *N Engl J Med* 1995; **332**: 839–47
38 Dahmen RP, Koch A, Denkhaus D, Tonn JC, Sorensen N, Berthold F, Behrens J, Birchmeier W, Wiestler OD, Pietsch T. Deletions of AXIN1, a component of the WNT/wingless pathway, in sporadic medulloblastomas. *Cancer Res* 2001; **61**: 7039–43
39 Huang H, Mahler-Araujo BM, Sankila A, Chimelli L, Yonekawa Y, Kleihues P, Ohgaki H. APC mutations in sporadic medulloblastomas. *Am J Pathol* 2000; **156**: 433–7
40 Koch A, Waha A, Tonn JC, Sorensen N, Berthold F, Wolter M, Reifenberger J, Hartmann W, Friedl W, Reifenberger G, Wiestler OD, Pietsch T. Somatic mutations of WNT/wingless signaling pathway components in

primitive neuroectodermal tumors. *Int J Cancer* 2001; **93**: 445–9

41 Zurawel RH, Chiappa SA, Allen C, Raffel C. Sporadic medulloblastomas contain oncogenic beta-catenin mutations. *Cancer Res* 1998; **58**: 896–9

42 Koch A, Hrychyk A, Hartmann W, Waha A, Mikeska T, Schuller U, Sorensen N, Berthold F, Goodyer CG, Wiestler OD, Birchmeier W, Behrens J, Pietsch T. Mutations of the Wnt antagonist AXIN2 (Conductin) result in TCF-dependent transcription in medulloblastomas. *Int J Cancer* 2007; **121**: 284–91

43 Clifford SC, Lusher ME, Lindsey JC, Langdon JA, Gilbertson RJ, Straughton D, Ellison DW. Wnt/Wingless pathway activation and chromosome 6 loss characterize a distinct molecular sub-group of medulloblastomas associated with a favorable prognosis. *Cell Cycle* 2006; **5**: 2666–70

44 Ellison DW, Dalton J, Kocak M, Nicholson SL, Fraga C, Neale G, Kenney AM, Brat DJ, Perry A, Yong WH, Taylor RE, Bailey S, Clifford SC, Gilbertson RJ. Medulloblastoma: clinicopathological correlates of SHH, WNT, and non-SHH/WNT molecular subgroups. *Acta Neuropathol* 2011; **121**: 381–96

45 Pfaff E, Remke M, Sturm D, Benner A, Witt H, Milde T, von Bueren AO, Wittmann A, Schottler A, Jorch N, Graf N, Kulozik AE, Witt O, Scheurlen W, von Deimling A, Rutkowski S, Taylor MD, Tabori U, Lichter P, Korshunov A, Pfister SM. TP53 mutation is frequently associated with CTNNB1 mutation or MYCN amplification and is compatible with long-term survival in medulloblastoma. *J Clin Oncol* 2010; **28**: 5188–96

46 Ellison DW, Onilude OE, Lindsey JC, Lusher ME, Weston CL, Taylor RE, Pearson AD, Clifford SC. beta-Catenin status predicts a favorable outcome in childhood medulloblastoma: the United Kingdom Children's Cancer Study Group Brain Tumour Committee. *J Clin Oncol* 2005; **23**: 7951–7

47 Fattet S, Haberler C, Legoix P, Varlet P, Lellouch-Tubiana A, Lair S, Manie E, Raquin MA, Bours D, Carpentier S, Barillot E, Grill J, Doz F, Puget S, Janoueix-Lerosey I, Delattre O. Beta-catenin status in paediatric medulloblastomas: correlation of immunohistochemical expression with mutational status, genetic profiles, and clinical characteristics. *J Pathol* 2009; **218**: 86–94

48 Eberhart CG, Kratz J, Wang Y, Summers K, Stearns D, Cohen K, Dang CV, Burger PC. Histopathological and molecular prognostic markers in medulloblastoma: c-myc, N-myc, TrkC, and anaplasia. *J Neuropathol Exp Neurol* 2004; **63**: 441–9

49 Goodrich LV, Milenkovic L, Higgins KM, Scott MP. Altered neural cell fates and medulloblastoma in mouse *patched* mutants. *Science* 1997; **277**: 1109–13

50 Hahn H, Wojnowski L, Specht K, Kappler R, Calzada-Wack J, Potter D, Zimmer A, Muller U, Samson E, Quintanilla-Martinez L. Patched target Igf2 is indispensable for the formation of medulloblastoma and rhabdomyosarcoma. *J Biol Chem* 2000; **275**: 28341–4

51 Oliver TG, Read TA, Kessler JD, Mehmeti A, Wells JF, Huynh TT, Lin SM, Wechsler-Reya RJ. Loss of patched and disruption of granule cell development in a pre-neoplastic stage of medulloblastoma. *Development* 2005; **132**: 2425–39

52 Kessler JD, Hasegawa H, Brun SN, Yang ZJ, Dutton JW, Wang F, Wechsler-Reya RJ. N-myc alters the fate of preneoplastic cells in a mouse model of medulloblastoma. *Genes Dev* 2009; **23**: 157–70

53 Wetmore C, Eberhart DE, Curran T. Loss of p53 but not ARF accelerates medulloblastoma in mice heterozygous for patched. *Cancer Res* 2001; **61**: 513–16

54 Uziel T, Zindy F, Xie S, Lee Y, Forget A, Magdaleno S, Rehg JE, Calabrese C, Solecki D, Eberhart CG, Sherr SE, Plimmer S, Clifford SC, Hatten ME, McKinnon PJ, Gilbertson RJ, Curran T, Sherr CJ, Roussel MF. The tumor suppressors Ink4c and p53 collaborate independently with Patched to suppress medulloblastoma formation. *Genes Dev* 2005; **19**: 2656–67

55 Briggs KJ, Corcoran-Schwartz IM, Zhang W, Harcke T, Devereux WL, Baylin SB, Eberhart CG, Watkins DN. Cooperation between the Hic1 and Ptch1 tumor suppressors in medulloblastoma. *Genes Dev* 2008; **22**: 770–85

56 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**: 722–7

57 Read TA, Fogarty MP, Markant SL, McLendon RE, Wei Z, Ellison DW, Febbo PG, Wechsler-Reya RJ. Identification of CD15 as a marker for tumor-propagating cells in a mouse model of medulloblastoma. *Cancer Cell* 2009; **15**: 1–13

58 Ward RJ, Lee L, Graham K, Satkunendran T, Yoshikawa K, Ling E, Harper L, Austin R, Nieuwenhuis E, Clarke ID, Hui CC, Dirks PB. Multipotent CD15+ cancer stem cells in patched-1-deficient mouse medulloblastoma. *Cancer Res* 2009; **69**: 4682–90

59 Hambardzumyan D, Becher OJ, Rosenblum MK, Pandolfi PP, Manova-Todorova K, Holland EC. PI3K pathway regulates survival of cancer stem cells residing in the perivascular niche following radiation in medulloblastoma in vivo. *Genes Dev* 2008; **22**: 436–48

60 Romer JT, Kimura H, Magdaleno S, Sasai K, Fuller C, Baines H, Connelly M, Stewart CF, Gould S, Rubin LL, Curran T. Suppression of the Shh pathway using a small molecule inhibitor eliminates medulloblastoma in Ptc1(+/–)p53(–/–) mice. *Cancer Cell* 2004; **6**: 229–40

61 Buonamici S, Williams J, Morrissey M, Wang A, Guo R, Vattay A, Hsiao K, Yuan J, Green J, Ospina B, Yu Q, Ostrom L, Fordjour P, Anderson DL, Monahan JE, Kelleher JF, Peukert S, Pan S, Wu X, Maira SM, Garcia-Echeverria C, Briggs KJ, Watkins DN, Yao YM, Lengauer C, Warmuth M, Sellers WR, Dorsch M. Interfering with

resistance to smoothened antagonists by inhibition of the PI3K pathway in medulloblastoma. *Sci Transl Med* 2010; **2**: 51ra70

62 Yang ZJ, Ellis T, Markant SL, Read TA, Kessler JD, Bourboulas M, Schuller U, Machold R, Fishell G, Rowitch DH, Wainwright BJ, Wechsler-Reya RJ. Medulloblastoma can be initiated by deletion of Patched in lineage-restricted progenitors or stem cells. *Cancer Cell* 2008; **14**: 135–45

63 Binning MJ, Niazi T, Pedone CA, Lal B, Eberhart CG, Kim KJ, Laterra J, Fults DW. Hepatocyte growth factor and sonic Hedgehog expression in cerebellar neural progenitor cells costimulate medulloblastoma initiation and growth. *Cancer Res* 2008; **68**: 7838–45

64 Browd SR, Kenney AM, Gottfried ON, Yoon JW, Walterhouse D, Pedone CA, Fults DW. N-myc can substitute for insulin-like growth factor signaling in a mouse model of sonic hedgehog-induced medulloblastoma. *Cancer Res* 2006; **66**: 2666–72

65 Broderick DK, Di C, Parrett TJ, Samuels YR, Cummins JM, McLendon RE, Fults DW, Velculescu VE, Bigner DD, Yan H. Mutations of PIK3CA in anaplastic oligodendrogliomas, high-grade astrocytomas, and medulloblastomas. *Cancer Res* 2004; **64**: 5048–50

66 Rao G, Pedone CA, Coffin CM, Holland EC, Fults DW. c-Myc enhances sonic hedgehog-induced medulloblastoma formation from nestin-expressing neural progenitors in mice. *Neoplasia* 2003; **5**: 198–204

67 Rao G, Pedone CA, Valle LD, Reiss K, Holland EC, Fults DW. Sonic hedgehog and insulin-like growth factor signaling synergize to induce medulloblastoma formation from nestin-expressing neural progenitors in mice. *Oncogene* 2004; **23**: 6156–62

68 Hallahan AR, Pritchard JI, Hansen S, Benson M, Stoeck J, Hatton BA, Russell TL, Ellenbogen RG, Bernstein ID, Beachy PA, Olson JM. The SmoA1 mouse model reveals that notch signaling is critical for the growth and survival of sonic hedgehog-induced medulloblastomas. *Cancer Res* 2004; **64**: 7794–800

69 Hatton BA, Villavicencio EH, Tsuchiya KD, Pritchard JI, Ditzler S, Pullar B, Hansen S, Knoblaugh SE, Lee D, Eberhart CG, Hallahan AR, Olson JM. The Smo/Smo model: hedgehog-induced medulloblastoma with 90% incidence and leptomeningeal spread. *Cancer Res* 2008; **68**: 1768–76

70 Schuller U, Heine VM, Mao J, Kho AT, Dillon AK, Han YG, Huillard E, Sun T, Ligon AH, Qian Y, Ma Q, Alvarez-Buylla A, McMahon AP, Rowitch DH, Ligon KL. Acquisition of granule neuron precursor identity is a critical determinant of progenitor cell competence to form Shh-induced medulloblastoma. *Cancer Cell* 2008; **14**: 123–34

71 Lee Y, Kawagoe R, Sasai K, Li Y, Russell HR, Curran T, McKinnon PJ. Loss of suppressor-of-fused function promotes tumorigenesis. *Oncogene* 2007; **26**: 6442–7

72 Heby-Henricson K, Bergstrom A, Rozell B, Toftgard R, Teglund S. Loss of Trp53 promotes medulloblastoma development but not skin tumorigenesis in Sufu heterozygous mutant mice. *Mol Carcinog* 2011; doi: 10.1002/mc.20852

73 Han YG, Kim HJ, Dlugosz AA, Ellison DW, Gilbertson RJ, Alvarez-Buylla A. Dual and opposing roles of primary cilia in medulloblastoma development. *Nat Med* 2009; **15**: 1062–5

74 Berman DM, Karhadkar SS, Hallahan AR, Pritchard JI, Eberhart CG, Watkins DN, Chen JK, Cooper MK, Taipale J, Olson JM, Beachy PA. Medulloblastoma growth inhibition by hedgehog pathway blockade. *Science* 2002; **297**: 1559–61

75 Dijkgraaf GJ, Alicke B, Weinmann L, Januario T, West K, Modrusan Z, Burdick D, Goldsmith R, Robarge K, Sutherlin D, Scales SJ, Gould SE, Yauch RL, de Sauvage FJ. Small molecule inhibition of GDC-0449 refractory smoothened mutants and downstream mechanisms of drug resistance. *Cancer Res* 2011; **71**: 435–44

76 Lindsey JC, Hill RM, Megahed H, Lusher ME, Schwalbe EC, Cole M, Hogg TL, Gilbertson RJ, Ellison DW, Bailey S, Clifford SC. TP53 mutations in favorable-risk Wnt/Wingless-subtype medulloblastomas. *J Clin Oncol* 2011; **29**: e344–6; (author reply e7–8)

77 Uziel T, Zindy F, Sherr CJ, Roussel MF. The CDK inhibitor p18Ink4c is a tumor suppressor in medulloblastoma. *Cell Cycle* 2006; **5**: 363–5

78 Ayrault O, Zindy F, Rehg J, Sherr CJ, Roussel MF. Two tumor suppressors, p27Kip1 and patched-1, collaborate to prevent medulloblastoma. *Mol Cancer Res* 2009; **7**: 33–40

79 Zindy F, Nilsson LM, Nguyen L, Meunier C, Smeyne RJ, Rehg JE, Eberhart C, Sherr CJ, Roussel MF. Hemangiosarcomas, medulloblastomas, and other tumors in Ink4c/p53-null mice. *Cancer Res* 2003; **63**: 5420–7

80 Lee Y, Miller HL, Jensen P, Hernan R, Connelly M, Wetmore C, Zindy F, Roussel MF, Curran T, Gilbertson RJ, McKinnon PJ. A molecular fingerprint for medulloblastoma. *Cancer Res* 2003; **63**: 5428–37

81 McCall TD, Pedone CA, Fults DW. Apoptosis suppression by somatic cell transfer of Bcl-2 promotes Sonic hedgehog-dependent medulloblastoma formation in mice. *Cancer Res* 2007; **67**: 5179–85

82 Coon V, Laukert T, Pedone CA, Laterra J, Kim KJ, Fults DW. Molecular therapy targeting Sonic hedgehog and hepatocyte growth factor signaling in a mouse model of medulloblastoma. *Mol Cancer Ther* 2010; **9**: 2627–36

83 Lee Y, McKinnon PJ. DNA ligase IV suppresses medulloblastoma formation. *Cancer Res* 2002; **62**: 6395–9

84 Tong WM, Ohgaki H, Huang H, Granier C, Kleihues P, Wang ZQ. Null mutation of DNA strand break-binding molecule poly(ADP-ribose) polymerase causes medulloblastomas in p53(–/–) mice. *Am J Pathol* 2003; **162**: 343–52

85 Holcomb VB, Vogel H, Marple T, Kornegay RW, Hasty P. Ku80 and p53 suppress medulloblastoma that arise independent of Rag-1-induced DSBs. *Oncogene* 2006; **25**: 7159–65
86 Yan CT, Kaushal D, Murphy M, Zhang Y, Datta A, Chen C, Monroe B, Mostoslavsky G, Coakley K, Gao Y, Mills KD, Fazeli AP, Tepsuporn S, Hall G, Mulligan R, Fox E, Bronson R, De Girolami U, Lee C, Alt FW. XRCC4 suppresses medulloblastomas with recurrent translocations in p53-deficient mice. *Proc Natl Acad Sci U S A* 2006; **103**: 7378–83
87 Frappart PO, Lee Y, Lamont J, McKinnon PJ. BRCA2 is required for neurogenesis and suppression of medulloblastoma. *EMBO J* 2007; **26**: 2732–42
88 Frappart PO, Lee Y, Russell HR, Chalhoub N, Wang YD, Orii KE, Zhao J, Kondo N, Baker SJ, McKinnon PJ. Recurrent genomic alterations characterize medulloblastoma arising from DNA double-strand break repair deficiency. *Proc Natl Acad Sci U S A* 2009; **106**: 1880–5
89 Kratz JE, Stearns D, Huso DL, Slunt HH, Price DL, Borchelt DR, Eberhart CG. Expression of stabilized beta-catenin in differentiated neurons of transgenic mice does not result in tumor formation. *BMC Cancer* 2002; **2**: 33
90 Fults D, Pedone C, Dai C, Holland EC. MYC expression promotes the proliferation of neural progenitor cells in culture and in vivo. *Neoplasia* 2002; **4**: 32–9
91 Momota H, Shih AH, Edgar MA, Holland EC. c-Myc and beta-catenin cooperate with loss of p53 to generate multiple members of the primitive neuroectodermal tumor family in mice. *Oncogene* 2008; **27**: 4392–401
92 Marino S, Vooijs M, van Der Gulden H, Jonkers J, Berns A. Induction of medulloblastomas in p53-null mutant mice by somatic inactivation of Rb in the external granular layer cells of the cerebellum. *Genes Dev* 2000; **14**: 994–1004
93 Shakhova O, Leung C, van Montfort E, Berns A, Marino S. Lack of Rb and p53 delays cerebellar development and predisposes to large cell anaplastic medulloblastoma through amplification of N-Myc and Ptch2. *Cancer Res* 2006; **66**: 5190–200
94 Sutter R, Shakhova O, Bhagat H, Behesti H, Sutter C, Penkar S, Santuccione A, Bernays R, Heppner FL, Schuller U, Grotzer M, Moch H, Schraml P, Marino S. Cerebellar stem cells act as medulloblastoma-initiating cells in a mouse model and a neural stem cell signature characterizes a subset of human medulloblastomas. *Oncogene* 2010; **29**: 1845–56
95 Swartling FJ, Grimmer MR, Hackett CS, Northcott PA, Fan QW, Goldenberg DD, Lau J, Masic S, Nguyen K, Yakovenko S, Zhe XN, Gilmer HC, Collins R, Nagaoka M, Phillips JJ, Jenkins RB, Tihan T, Vandenberg SR, James CD, Tanaka K, Taylor MD, Weiss WA, Chesler L. Pleiotropic role for MYCN in medulloblastoma. *Genes Dev* 2010; **24**: 1059–72
96 Tremblay MR, Nesler M, Weatherhead R, Castro AC. Recent patents for Hedgehog pathway inhibitors for the treatment of malignancy. *Expert Opin Ther Pat* 2009; **19**: 1039–56
97 Gould A, Missailidis S. Targeting the hedgehog pathway: the development of cyclopamine and the development of anti-cancer drugs targeting the hedgehog pathway. *Mini Rev Med Chem* 2011; **11**: 200–13
98 Low JA, de Sauvage FJ. Clinical experience with Hedgehog pathway inhibitors. *J Clin Oncol* 2010; **28**: 5321–6
99 Rudin CM, Hann CL, Laterra J, Yauch RL, Callahan CA, Fu L, Holcomb T, Stinson J, Gould SE, Coleman B, LoRusso PM, Von Hoff DD, de Sauvage FJ, Low JA. Treatment of medulloblastoma with hedgehog pathway inhibitor GDC-0449. *N Engl J Med* 2009; **361**: 1173–8
100 Yauch RL, Dijkgraaf GJ, Alicke B, Januario T, Ahn CP, Holcomb T, Pujara K, Stinson J, Callahan CA, Tang T, Bazan JF, Kan Z, Seshagiri S, Hann CL, Gould SE, Low JA, Rudin CM, de Sauvage FJ. Smoothened mutation confers resistance to a Hedgehog pathway inhibitor in medulloblastoma. *Science* 2009; **326**: 572–4
101 Metcalfe C, de Sauvage FJ. Hedgehog fights back: mechanisms of acquired resistance against Smoothened antagonists. *Cancer Res* 2011; **71**: 5057–61

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