Review: Personalized mice: modelling the molecular heterogeneity of medulloblastoma

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Medulloblastoma (MB), 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, the most common malignant paediatric brain tumour, is thought to arise from mutations in progenitors or stem cells in the cerebellum. 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 sub-types 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.

Germinal zones in the developing cerebellum

The cerebellum is composed of multiple cell types, including granule, Purkinje, Golgi, stellate and basket neurones.
as well as several classes of astrocytes and oligodendrocytes. Each of these cell types is derived from one of 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).

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 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 unknown 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
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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-
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 \textit{Ptch1}-knockout mouse [49]. These animals were created using conventional homologous recombination technology, which replaced a portion of the \textit{Ptch1} gene with LacZ and neomycin reporter genes, leading to loss of expression of the \textit{Ptch1} protein. Because \textit{Ptch1} functions as a repressor of SHH signalling, the pathway is constitutively active in these mice. Homozygous (\textit{Ptch1}\textsuperscript{-/-}) mice exhibit severe defects in the neural tube, heart and other tissues, and die during embryogenesis. In contrast, heterozygotes (\textit{Ptch1}\textsuperscript{+/−}) 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 \textit{Ptch1}-knockout models of MB have been developed [62]. In these animals, loxP-flanked alleles of \textit{Ptch1} (\textit{Ptch1}\textsubscript{floxed}) are deleted by the Cre recombinase, whose expression is controlled by the \textit{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 \textit{Ptch1}\textsuperscript{-/-} mice. Unlike \textit{Math1-Cre} or \textit{Math1-CreER}, which delete \textit{Ptch1} specifically in GNPs, GFAP-Cre deletes \textit{Ptch1} in stem cells. Analysis of the early stages of tumorigenesis in GFAP-Cre:Ptch1\textsubscript{floxed} 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/TV A transduction system [63–67]. In this system, transgenic mice expressing the avian retrovirus receptor (TV A) in specific cell types are inoculated with RCAS-based avian retroviruses encoding genes of interest, and only cells expressing the TV A receptor become infected. Fults and colleagues have demonstrated that infection of neonatal Nestin-TV A 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 \textit{Smo} transgene (\textit{SmoA1}) is controlled by the \textit{NeuroD2} (ND2) promoter, which is active in GNPs [68]. In mice hemizygous for \textit{ND2-SmoA1}, approximately 50% of the animals develop tumours, with a median latency of 6 months. A homozygous \textit{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 ‘\textit{Smo-Smo}’ mouse represents another important and valuable tool for preclinical studies. Activation of \textit{Smo} in earlier neural progenitors or stem cells (using GFAP, \textit{Math1}, \textit{Tlx3} or \textit{Olig2-driven Cre}) also results in MB [70], and as with the conditional \textit{Ptch1}-knockout mice, these tumours appear to result from expansion of GNPs. Finally, animals lacking \textit{Sufu} or overexpressing an activated form of \textit{Gli2} also develop MB; however, in each case additional genes (\textit{Tp53} and \textit{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 \textit{Ptch1}\textsuperscript{+/−} mice with \textit{Tp53}\textsuperscript{-/-} mice leads to a dramatic increase in tumour incidence, from 15% in \textit{Ptch1}\textsuperscript{+/−} mice to 100% in \textit{Ptch1}\textsuperscript{+/−}\textit{Tp53}\textsuperscript{-/-} mice [53]. This penetrance has made the \textit{Ptch1}\textsuperscript{+/−}\textit{Tp53}\textsuperscript{-/-} 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/TV A system has also been used to define oncogenic pathways that can cooperate with SHH signalling to cause MB. Nestin-TV A 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-TV A mice with β-catenin retroviruses – were unsuccessful [89,90]. Infection of GFAP-TV A 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 (Ctnnb1LoxPlox) that can be activated by Cre. In the progeny of this cross, aberrant cell masses were

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observed in the brainstem, but these masses never progressed to MB. However, when these mice were crossed with Tp53\textsuperscript{3lox} 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;Cntnb1\textsuperscript{loxp/loxp};Tp53\textsuperscript{3lox} 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 \(\beta\)-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 Ptc2 [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\textsuperscript{lacZ/loxp}; p53\textsuperscript{3lox/lox} 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 host, 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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