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Genetic and Molecular Alterations Across Medulloblastoma Subgroups

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Abstract

Medulloblastoma is the most common malignant brain tumor diagnosed in children. Over the last few decades advances in radiation and chemotherapy have significantly improved the odds of survival. Nevertheless, one third of all patients still succumb to their disease, and many long-term survivors are afflicted with neurocognitive sequelae. Large scale multi-institutional efforts have provided insight into the transcriptional and genetic landscape of medulloblastoma. Four distinct subgroups of medulloblastoma have been identified, defined by distinct transcriptomes, genetics, demographics and outcomes. Integrated genomic profiling of each of these subgroups has revealed distinct genetic alterations, driving pathways and in some instances cells of origin. In this review we highlight, in a subgroup specific manner, our current knowledge of the genetic and molecular alterations in medulloblastoma and underscore the possible avenues for future therapeutic intervention.

Keywords

Medulloblastoma; molecular genetics; therapy; subgroups

INTRODUCTION

Medulloblastoma is the most common malignant paediatric brain cancer, having an incidence of approximately 0.74/100000 person-year [1, 2]. They are located in the cerebellum and 30% of cases present with metastatic dissemination over the cranial and spinal leptomeninges [3]. Initial treatment for medulloblastoma is maximal safe surgical resection followed by adjuvant craniospinal irradiation and/or high dose cytotoxic platinum based chemotherapy. Radiation as per current protocols in North America and Western Europe is risk adapted, in that, metastatic patients receive 36 Gy and non-metastatic patients

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receive 23.4 Gy of craniospinal irradiation with a boost to the tumour bed. With the current standard of care, overall patient survival has reached 70%, however, metastatic patients and infants are both high risk groups with poor survival [3–7]. Despite successful completion of treatment, patients frequently present with neurocognitive sequelae — long term neurological deficits in cognition [6, 7]. As such, there is an urgent need for more specific targeted therapies which minimize the impact on the developing brain.

Recent integrated genomic studies have now shown that medulloblastoma is not one single morphological entity, and is in fact at the molecular level, comprised of several different diseases. Large scale efforts focused on studying the transcriptional landscape have revealed 4 distinct subgroups (WNT, SHH, Group 3, Group 4), each with their own unique survival, age demographics, and genetic aberrations [8, 9]. These subgroups are stable at recurrence and across tumour compartments [4, 10] and are likely reminiscent of the cell of origin. The next generation of clinical trials is already taking subgroup into account to rationally stratify patients and tailor therapy. Accurate, robust, and inexpensive subgroup prediction methods are essential; molecular subgroups can be reliably assigned by either expression profiling or through the use of genome wide methylation arrays [13, 14]. Further investigation into the molecular genetics of medulloblastoma will hopefully pave the way for new targeted therapeutic strategies to cure this devastating childhood disease.

Familial Predisposition Syndromes

Initial insights into the pathways driving medulloblastoma were inferred from familial predispositions associated with medulloblastoma. The most common being Li-Framumeni syndrome with germline mutations in *TP53* [11]. These mutations can drive a variety of other cancers, but in medulloblastoma both somatic and germline *TP53* mutations are frequently present in childhood SHH patients and are known to facilitate catastrophic large scale rearrangements via chromothripsis [16–18]. Somatic *TP53* mutations can also occur in the WNT subgroup. Less frequent is Gorlin syndrome which is an autosomal dominate disease characterised by mutations of the transmembrane receptor Patched1 (*PTCH1*). The majority of these patients will acquire basal cell carcinoma, while about 5–20% will get medulloblastoma [13, 14]. Deletion of the *PTCH1* locus results in higher Smoothened (SMO) activity and upregulation of the Sonic Hedgehog (Shh) signalling pathway, a marker of the SHH subgroup. Less common predispositions are: i) Turcot Syndrome adenomatous polyposis coli (*APC*) germline mutations which are associated with a multitude of other central nervous system tumours and colorectal cancer [15, 16], and ii) autosomal dominant mutations in CREB binding protein (*CREBBP*) causing Rubinstein-Taybi syndrome [23]. These familial predispositions are not all encompassing and only account for a subset of patients. There are many other genetic factors which can lead to the development of medulloblastoma which will be covered in the sections that follow.

WNT SUBGROUP

Clinical Attributes

Of all the subgroups, the WNT subgroup has the most favourable prognosis with over 95% of patients surviving the disease (Table 1). WNT tumors exhibit classic histology, are rarely

metastatic and have an even gender predisposition. WNT is the least common subtype, with a rate of 10% among medulloblastoma patients. The hallmark alteration in WNT tumors is somatic activating mutations in exon 3 of β-catenin (*CTNNB1*). Monosomy 6 is the main recurrent structural alteration and is usually found in an otherwise balanced genome [17–19].

Molecular Biology

The Wnt signalling pathway plays an essential role in embryonic development, controlling cell fate specification, cell proliferation, cell migration and body axis patterning. In the developing brain, the Wnt pathway has broad regulatory effects on neuronal maturation and synapse formation. This pathway is activated through binding of WNT ligands to the Frizzled receptors, which relay signals into the nucleus through the release of *CTNNB1* (Fig. 1 Left). Important negative regulators of this pathway are *APC* and *SUFU* which normally prevent the accumulation of *CTNNB1* in the nucleus [20, 21]. Nearly all (90%) of WNT patients have somatic missense mutations in *CTNNB1* which promote protein stabilization. The next most common mutation is in *DDX3X*, with mutations clustering in its two helicase domains hypothesized to alter its RNA binding capacity rather than abolish it. *In vivo* and *in vitro* functional studies of *DDX3X* suggest that it enhances cellular and/or maintains proliferation of the WNT progenitor cells. It is also possible that these mutations help enhance transformation by β-catenin activation [22–24]. Also commonly found in WNT are missense mutations in *TP53*. Despite being a marker of high risk in the SHH subgroup and other cancers, *TP53* mutations confer no difference in survival for patients diagnosed with WNT subgroup medulloblastomas [25].

Models

The progenitors of the lower rhombic lip are the likely cell of origin for WNT tumours. CTNNB1 stabilization and nuclear localization is the most characteristic feature of the WNT subgroup and in mouse models its action is not sufficient to transform external granule cells, which are the SHH cells of origin. Furthermore, WNT tumours in humans are found adjacent to the brainstem unlike SHH which arise from within the cerebellum. During development, postmitotic mossy-fibre neuron precursors in the dorsal brainstem migrate into the central brainstem. Targeted expression of activated beta-catenin in mouse postmitotic mossy-fibre neuron precursors using a brain lipid-binding protein (Blbp) promoter, coupled with a knockout of *TP53* leads to the formation of a WNT tumour with high latency and low penetrance [26]. Subsequent work established that through the addition of a phosphoinositide 3-kinase (*PI3K*) catalytic-α polypeptide mutant allele ($Pik3ca^{E545K}$) identified in WNT medulloblastomas, the penetrance in the mouse model was increased to 100% with highly representative WNT tumours forming within 3 months [23, 27]

SHH SUBGROUP

Clinical attributes

The SHH subgroup accounts for a third of all medulloblastoma patients and has an intermediate prognosis with a five year survival ranging between 60–80%. The age distribution is bimodal with the majority of infant and adult medulloblastomas being SHH.

The histological classification can be any of the 5 described variants from the WHO classification system; however the desmoplastic variant is more common in children and adults. Large cell and/or anaplastic histology is common in children harbouring germline or somatic mutations in *TP53*. SHH patients commonly have focal amplifications of *GLI2*, and *MYCN*, as well as loss of 17p (Table 1) [11, 25, 34, 35].

Molecular Biology

During early cerebellar development, Purkinje cells release Shh glycoproteins and stimulate the proliferation and subsequent migration of granule cells into the internal granule cell layer. Excessive activation of the Shh pathway overdrives the expression of GLI2 transcription factor targets which induce uncontrolled proliferation of the granule cells and the formation of a tumour [28, 36]. Alterations in this subgroup most often fall within the Shh signalling pathway and less frequently in cooperating pathways such as PI3K and mTOR (Fig. 1 Right). The most common are somatic or germline inactivating alterations or loss of *PTCH1* and *SUFU*, or somatic missense mutations activating SMO [26, 29–31]. A subset of high-risk patients present with co-amplification of *MYCN* and *GLI2* accompanied by inactivation of *TP53*. Within the SHH subgroup there is also a difference in the molecular biology and risk factors for different age groups. *SUFU* mutations are found predominantly in infants, while the high risk *GLI2* amplifications are found in older children and teenagers [17, 37]. In adults, the most common are somatic mutations in *SMO* and C228T or C250T of the *TERT* promoter [21], which creates an E-twenty-six binding motif [22, 23].

Models

There are a large variety of mouse models that recapitulate SHH subgroup, and these function mainly through dysregulation of the hedgehog signalling pathway. The first medulloblastoma mouse model used a single allele knockout of the *PTCH1* gene, a negative inhibitor of the SMO pathway which drives tumorigenesis in granule cells [41]. Since then there have been other models that cross $Ptch1^{+/-}$ with other aberrations which confer a more aggressive phenotype, such as deletions of cyclin-dependant kinases *Ink4c* and *Kip1* [42, 43], or the master regulator *TP53* [44]. NeuroD2 dependant overexpression of mutant SMO in granule cells is also able to drive highly penetrant tumours with leptomeningeal metastasis [45, 46]. In addition, even though SHH medulloblastoma are traditionally thought to arise from granule cells, there have been mouse models that demonstrate that aberrant Shh signalling in cochlear nuclei and neural stem cells are capable of forming a tumour [47, 48].

A model that has shown great utility in screening for novel driver genes and cooperating events has been the medulloblastoma Sleeping Beauty (SB) mouse model [49] which utilizes random transposon integration to drive tumorigenesis. The transposons contain elements which are capable of overexpressing or truncating genes depending on the insertion location and orientation. Insertion events are mediated by a transposase, which is limited to granule cell precursors through the use of the *MATH1* promoter to drive expression of the transposase. Nearly all the mice develop tumours with a high rate of leptomeningeal metastasis by 3 months. The SB model has identified a large number of

primary tumour drivers such as MyoD [50] and Nfia [51] and has also revealed the large degree of divergence between primary and metastatic tumours (discussed below).

GROUP 3

Clinical Attributes

Group 3 medulloblastoma comprise about 20% of all medulloblastomas (Table 1). These patients have the worst survival and the highest rate of metastatic dissemination. Group 3 patients recur almost exclusively with metastatic dissemination with a clean tumour bed [4]. Patients diagnosed with this subgroup are commonly infants and younger children with a male to female discordance of 2:1. The histology of this tumour is commonly classic or large cell anaplastic (LCA) and the genome of these tumours is very unbalanced with a large number of broad alterations such as gain of chromosome 7 and isochromosome 17q; many of these alterations are also shared with Group 4.

Molecular Biology

There are several recurrent somatic copy-number alterations in Group 3, but unusually few recurrent single nucleotide variants and indels. The Group 3 transcriptome is dominated by photoreceptor and GABAergic expression signatures [10]. The most common event is amplification of *MYC* in 10–20% of patients, which in many cases occurs with a fusion between the promoter of *PVT1* and the second exon of *MYC* [27]. In many cancers the *MYC* locus is often co-amplified with the non-coding RNA *PVT1*, which is able to stabilize the MYC protein [28]. In medulloblastoma, it is likely that these fusions create a positive feedback loop since the *PVT1* promoter contains canonical E-boxes which are activated by the MYC protein [29]. Amplification of the transcription factor *OTX2* is another common copy number alteration occurring in 10% of patients, and mutually exclusive of *MYC* amplification. OTX2 is known to play an important role in controlling cell fate and differentiation of various progenitors in the developing brain and is able to repress the myogenic differentiation of medulloblastoma cells. It also plays a role in the TGF-B signalling pathway which contains numerous other less recurrent copy-number alterations indicating that deregulation of this pathway may be a driver event [54–56].

Models

Two orthotopic transplantation models of Group 3 have been created which couple the overexpression of MYC with the inactivation of *TP53* [57, 58]. MYC expression leads to a higher rate of proliferation as well as a higher rate of TP53 mediated apoptosis which necessitates the need to inactivate the *TP53* locus. In the first model, Pei *et al* isolated CD133-positive and glial lineage marker-negative neural stem cells from the postnatal cerebellum and infected these cells with *MYC* and dominate negative *TP53* (*DNp53*) [59]. These cells were shown to be unresponsive to Shh stimulation and capable of differentiating into neurons, astrocytes and oligodendrocytes. Infection of these cells with a stabilized *MYC* (Myc^{T58A}) followed by transplantation into a mouse led to the formation of a number of transient hyperplastic lesions with a high rate of apoptosis. By introducing DNp53, the apoptotic effects were abolished and tumours were formed with LCA histology, prominent necrosis, and nuclear molding. The second model was produced by isolating granule

progenitor cells from Atoh1-GFP mice that express GFP in external granule layer progenitor cells, from postnatal *TP53*$^{-/-}$ mice. Atoh1-GFP enriched cells transduced with *MYC* were able to form aggressive tumours with LCA histology even after multiple passages in mice. In Group 3, focal events in *TP53* are exceedingly rare but there is frequent loss of 17p (where *TP53* resides). The resistance to Shh pathway inhibition and the similarity in Group 3 signature genes suggest that these two models are highly representative of the human disease.

GROUP 4

Clinical Attributes

Group 4 is the most common of the medulloblastoma subgroups and has an intermediate overall survival of 75% (Table 1). The histology is most commonly classic. It has a high rate of metastasis and a 2:1 male to female discordance. The Group 4 genome is commonly tetraploid, and the most common structural alteration is isochromosome 17q, which is found in 80% of patients.

Molecular Biology

Group 4 has a neuronal and glutaminergic expression signature and, like Group 3, few recurrent single nucleotide variants and indels. The most frequently mutated somatic gene in Group 4 medulloblastoma is *KDM6A*, a histone H3 Lys27 (H3K27) demethylase; nonsense mutation of which are found in 13% of patients [60–62]. KDM6A belongs to the Jumonji C family of histone demethylases along with KDM6B, which is also found to be mutated in medulloblastoma. The proto-oncogenes *MYCN* and cyclin-dependant kinase *CDK6* are recurrently amplified in Group 4. More common are focal amplifications / tandem duplications of the alpha-synuclein interacting protein (SNCAIP) gene on chromosome 5q23 [26], which encodes a protein previously implicated in Parkinson's disease [63]. It is still unknown if *SNCAIP* is the driver for these patients, more research needs to be done to uncover its specific role in Group 4 medulloblastoma.

Models

Due to the low number of focal events and many broad rearrangements the search for a model of Group 4 has proven elusive. *MYCN* is commonly upregulated in medulloblastoma and is the site of one of the most recurrent focal amplifications in Group 4. Swartling *et al* created a mouse model of *MYCN* driven medulloblastoma by targeting its expression with Glt1, a brain specific promoter highly expressed in the cerebellum throughout development until adulthood. The tumours had a low latency and metastatic rate and were either a classic or LCA histology type. *MYCN* was required for both the initiation and maintenance of the tumour but was likely cooperating with other events since the genome was unbalanced and had a number of recurrent copy number alterations. This model is showing great promise as a Group 4 model, but additional studies need to be performed in order to characterize the expression signatures and identify the cell of origin [33].

EPIGENETICS

There have been a number of recurrent somatic single nucleotide and copy number variants within chromatin modifiers identified across all of the subgroups. Most common are truncating mutations in myeloid/lymphoid or mixed-lineage leukemia protein 2 (*MLL2*) and *MLL3* suggesting a role as a universal oncogenic driver. In Group 3 and Group 4 there are a large variety of recurrent somatic mutations in *SMARCA4* (exclusive to Group 3), and *KDM6A* (exclusive to Group 4), and less commonly in *CHD7, ARID1B, KDM4C*, and *ZMYM3* [26, 29–31]. There is also over-expression of EZH2, a H3K27 methyltransferase that is part of the polycomb repressive complex essential for regulating development and differentiation. These events are largely mutually exclusive of each other and the amplifications of *MYC* and *MYCN*. The mechanism of their pathogenesis is still a subject of intense investigation, but it is possible that these events preserve Group 3 and Group 4 tumours in a stem cell-like state by maintaining high levels of the repressive H3K27me3 mark (EZH2 upregulation or KDM6A inactivation) and/or disruption of H3K4me3 associated transcription (ZMYM3 and CHD7 inactivation) [55, 64, 65].

Enhancer-promoter interactions play an essential role in tissue specific regulation of genes and development [66]. The three-dimensional localization of active enhancers ultimately decides which genes can be activated by the enhancer and any disruption can lead to the aberrant regulation of genes. In Group 3 and Group 4 medulloblastoma it has recently been demonstrated that structural rearrangements are able to alter the intended targets of enhancers to drive tumorigenesis in medulloblastoma [67]. In particular, a series of seemingly unrelated deletion, duplication and translocation events were able to activate expression of the transcription factors *GFI1* or *GFI1B* through the repositioning of distal enhancers. These somatic events were highly recurrent, constituting a third of Group 3 and 10% of Group 4 tumours. When these genes were co-expressed with *MYC* in murine neural stem cells, they were also able to drive the formation of an aggressive tumour in recipient mice with a high rate of metastasis.

METASTASES

In medulloblastoma patients, metastasis is a sign of dismal prognosis. It is most common in Group 3 and Group 4 patients, both of which almost exclusively recur with metastatic dissemination [4]. Little is known about the genes driving dissemination and the context in which they operate since matching patient primary and metastatic samples are exceedingly rare. Studies with the SB mouse model have shown that there is a large genetic divergence between the primary and metastatic compartments with almost no overlap in common insertion sites [50] suggesting that the primary tumour is a poor indicator of the therapeutic targets present in the metastatic lesions. These *PTCH1*-driven SB models have revealed a number of possible drivers of metastasis such as Eras, Lhx1, Ccrk, and Gdi2 which potentially drive dissemination in SHH patients [70, 71]. Tumour-stromal interactions also appear to play an essential role in medulloblastoma tumorigenesis and metastatic dissemination. Tumour cell-induced expression of the placental growth factor (PIGF) in the stroma was shown to activate pro-survival pathways through the Nrp1 receptor [68] and promote tumour growth and metastasis.

THERAPIES

The WNT subgroup has the best prognosis out of all the subgroups; nearly all patients surviving after surgery, radiation and chemotherapy. For this subgroup there have been international efforts to de-escalate therapy to help reduce the long term cognitive deficits common in children after receiving radiotherapy.

There are a number of proposed therapies for SHH patients which all aim to lower the aberrant activity of the Shh pathway. One of the most promising class of drugs are SMO inhibitors, which are already in phase II clinical trials for a number of cancers including medulloblastoma [69–71]. Unfortunately, acquired resistance is inevitable in both animals and humans; a recurrent mutation in a conserved aspartic acid residue within the G protein–coupled receptor domain of SMO has been shown to disrupt the functionality of inhibitors, leaving Shh signalling intact [71, 72]. Furthermore, the drug is only effective for patients with alterations within or upstream of SMO, and therefore high-risk children with amplifications of *MYCN* and *GLI2* would not be able to benefit from this treatment [17]. Another class of drugs called bromodomain inhibitors may be able to circumvent this problem by inhibiting the Shh at the level of GLI2. BRD4 is a bromodomain protein which binds to ε-N-lysine acetylation motifs on open chromatin and is known to facilitate transcription at promoter regions of key transcription factors such as GlI2 and MYC. Treatment of SHH with BRD4 inhibitors has shown great promise in pre-clinical models even in the presence of *SMO* drug resistance mutations [72, 73].

The search for specific therapies for Group 3 and 4 patients has proven elusive. In Group 3, TGF-beta signaling is commonly dysregulated and pathway antagonists, to target this, are already being explored for a multitude of cancers, including glioblastoma; varying success has been observed [74]. MYC inhibition is another attractive but challenging therapeutic strategy. There are no known direct inhibitors of MYC — studies have focused on inhibiting the expression of MYC RNA [75] or inhibiting its heterodimer MAX [76, 77]. So far the most promising approach is inhibition of BRD4 using bromodomain inhibitor JQ1 to reduce the transcription of *MYC* [78]. There is also some evidence that JQ1 may be effective for treatment of MYCN driven neuroblastoma in pre-clinical models; suggesting that it could be effective for Group 4 patients [79]. In both Group 3 and Group 4, epigenetic alterations are a characteristic feature and there are a number of approved drugs in clinical trials for several adult and pediatric brain tumours. In particular, there are several specific inhibitors of the polycomb repressive complex 2 as well as EZH2 which act to antagonize the levels of H3K27me3 [80].

CONCLUSION

Large scale genomic and transcriptional studies have completely revolutionized our understanding of medulloblastoma pathogenesis. They have made sense of a diverse set of dysregulated pathways, and genetic alterations, and have presented a more rational way to stratify patients for targeted therapy. With these new paradigms in mind, clinical trials are already underway which address the tumour biology, particularly for the SHH and WNT subgroups. With so few recurrent genetic events, the focus is shifting towards the

relationship between epi- and molecular genetics. This is an exciting time, and research into the molecular genetics of medulloblastoma will no doubt help maximize survival while minimizing long term developmental defects.

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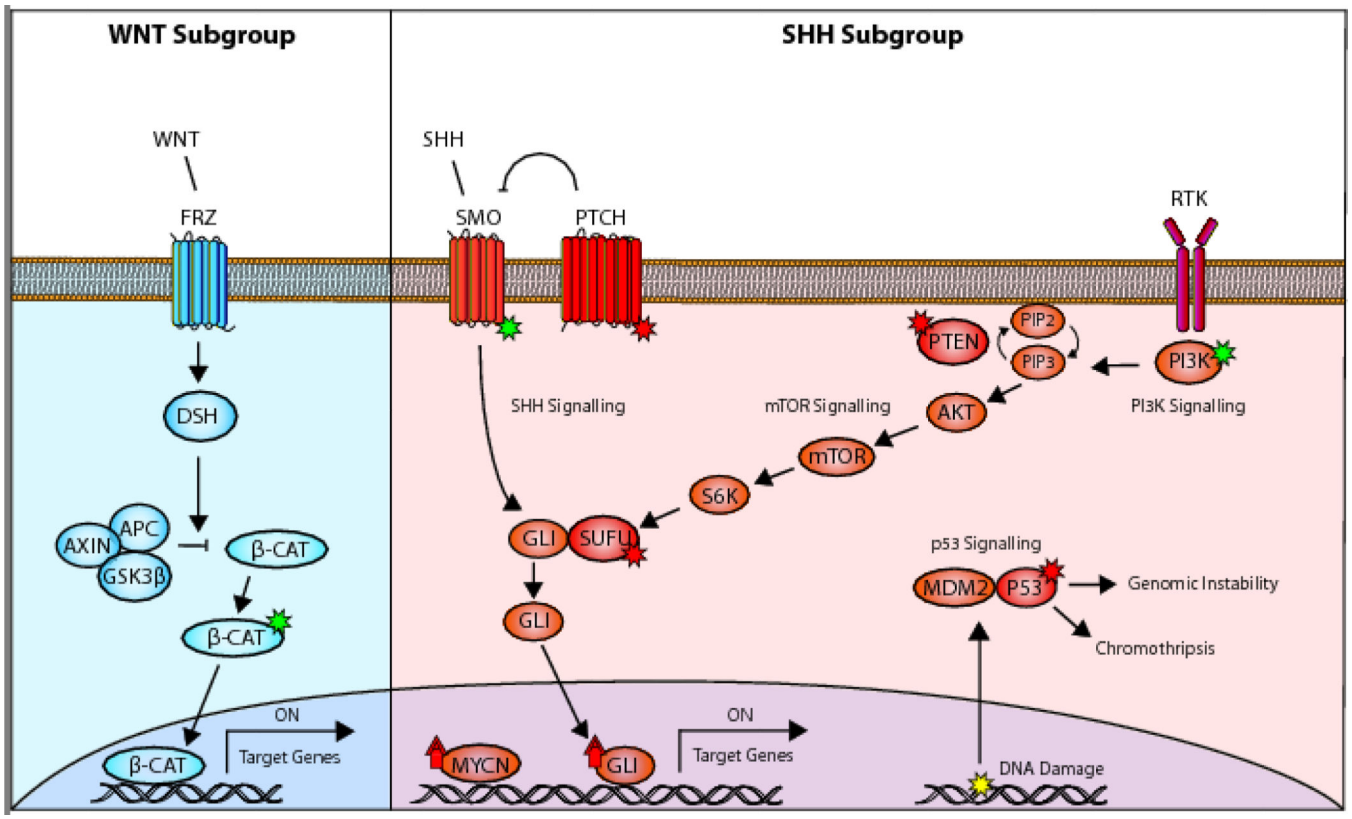
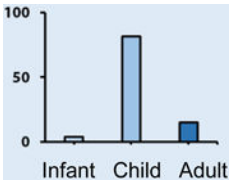
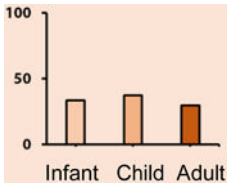
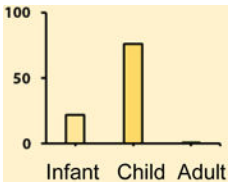
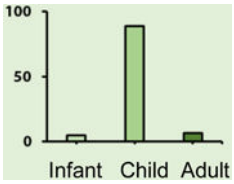


Figure 1.

Dysregulated pathways in WNT and SHH medulloblastoma. (a) WNT patients normally have activating alteration in β-cat which promote its stabilization and allow it to upregulate target genes. (b) Alterations in the SHH subgroup usually fall within the Shh signalling as well as cooperating PI3K/ mTOR pathways and converge on the upregulation of GLI. The most common are inactivating alterations in PTCH or SMO or activating mutations in SMO. High risk patients typically have co-amplifications of MYCN, GLI2 and mutations in P53 which results in genomic instability and/or chromothripsis [26, 29–31, 81]. Activating mutations (green star); inactivating mutations (red star); amplifications (red arrow); DNA damage (yellow star); amplification (up arrow).

Table 1

Clinical and Genomic Characteristics of Medulloblastoma Subgroups [4, 17, 26, 27, 30–32]

	WNT	SHH	GROUP 3	GROUP 4
AGE DISTRIBUTION				
GENDER (F|M)				
HISTOLOGY	Classic, Rarely LCA	Desmoplastic, Classic, LCA	Classic, LCA	Classic, LCA
METASTATIC RATE	Low	Low	High	High
PROGNOSIS	Excellent	Intermediate	Poor	Intermediate
SCNA	-	MYCN (12%) GLI2 (8%)	MYC (17%) PVT1 (12%) OTX2 (8%)	SNCAIP (10%) MYCN (6%) CDK6 (5%)
SNVS	CTNNB1 (91%) DDX3X (50%) SMARCA4 (26%) MLL2 (13%) TP53 (13%)	TERT (60%) PTCH1 (46%) SUFU (24%) MLL2 (16%) SMO (14%) TP53 (13%)	SMARCA4 (11%) MLL2 (4%)	KDM6A (13%) MLL3 (5%)
BROAD EVENTS	6 Loss	3q Gain 9q, 10q, 14q Loss	1q, 7, 17q, 18q Gain 8, 10q, 11, 16p, 17p Loss	7, 17q, 18q Gain 8, 11p, X Loss
EXPRESSION	WNT Signaling	SHH Signaling	MYC/Retinal Signature	Neuronal Signature
RECURRENCE	-	Local	Metastatic	Metastatic