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Medulloblastoma: Clinical Challenges and Emerging Molecular Discoveries

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Abstract

Medulloblastoma is the most common type of malignant brain tumor in children, responsible for 25% of pediatric brain cancers. Conventional treatment methods, which include surgery, radiotherapy, and chemotherapy, have improved overall survival rates for patients with medulloblastoma to over 50%. A majority of survivors, however, suffer serious long-term side effects, including developmental, neurological, and psychosocial deficits. Now entering clinical trials for sonic hedgehog-driven medulloblastomas, Smoothened inhibitors have been FDA approved for the treatment of basal cell carcinomas. However, treatment efficacy endures only for a few months before lesion relapses and drug resistance occurs. Therefore, there is an urgent need for new therapies to reduce the significant problems associated with current drug-resistant treatments. In this chapter, we will illustrate the clinical presentation and current treatment methods for medulloblastoma and detail the molecular pathways within each of the four molecular subgroups of medulloblastoma, with an eye for possible candidates for novel combination therapies.

Keywords: medulloblastoma, Sonic hedgehog (SHH) pathway, pediatric tumors, CNS tumors, smoothened

1. Introduction: medulloblastoma as a clinical problem

Medulloblastoma is the most common malignant pediatric brain tumor, accounting for about 25% of pediatric brain tumor cases [1]. However, it is found in infants and adults as well. Arising from embryonal cells in the cerebellum, medulloblastoma is a highly invasive cancer which unfortunately manifests initially with subtle clinical symptoms [2]. While conventional treatments are able to control the tumor in the majority of patients, debilitating side effects along

with drug resistance remain significant concerns. For the clinician, one of the challenges to treating medulloblastoma is its complexity as it may be grouped either histologically or molecularly. Currently, there are four molecular subgroups of medulloblastoma, each of which contains specific genetic or cytological backgrounds which may impact prognosis [3].

1.1. Origin and epidemiology of medulloblastoma

Medulloblastoma is classified as a primitive neuroectodermal tumor, typically occurring in the cerebellar vermis which is located in the posterior fossa of the skull (**Figure 1**) [1]. This tumor accounts for 40% of those arising from the posterior fossa [4]. Medulloblastoma is the most common malignant central nervous system (CNS) tumor of childhood, comprising 15–30% of pediatric CNS tumors and 1–3% of adult CNS tumors [5]. Medulloblastomas can affect any age group, although the majority (85%) occurs in childhood, and of those half occur between the ages of 4–9 [6]. Tumors most often arise sporadically, although they may arise as part of inherited disorders such as Li-Fraumeni, Turcot, or Gorlin syndrome [7]. The cellular origins of medulloblastoma differ by the tumor subgroup (described below). For example, medulloblastomas of the sonic hedgehog (SHH) subgroup arise from granule neuron progenitors (GNPs) that reside in the outermost layer of the cerebellum [8]. Wnt-subgroup medulloblastomas, on the other hand, arise from lower rhombic lip precursors in the brain-stem [9].
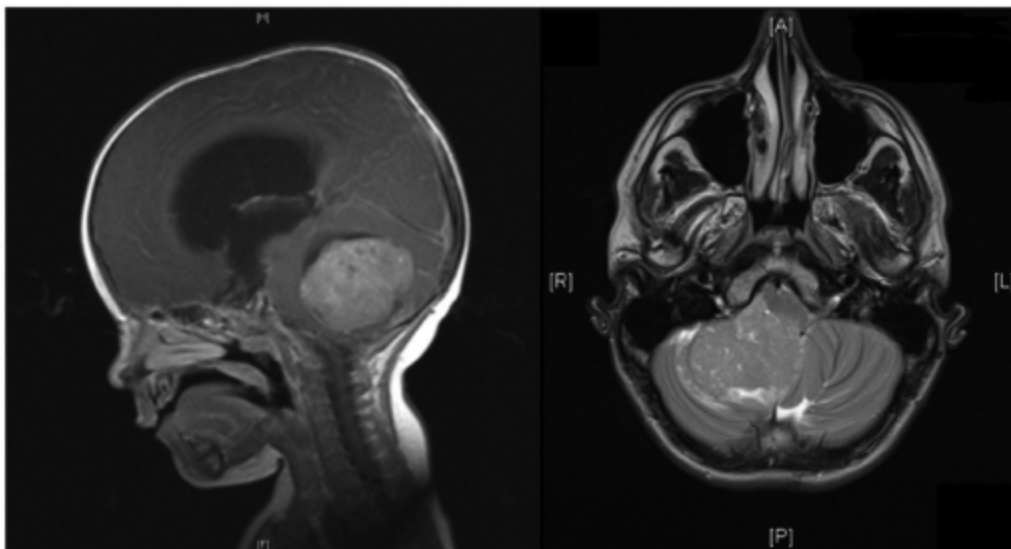

Figure 1. Medulloblastoma is a primitive neuroectodermal tumor commonly arising in the cerebellar vermis. The left image is a sagittal view of an MRI for a pediatric patient. The right image is a horizontal view of an MRI showing tumor growth towards the right cerebellar hemisphere, with displacement of the vermis. Copyright © 2014 from *Pediatric medulloblastoma—update on molecular classification driving targeted therapies* (DeSouza, Jones, Lowis and Kurian, Front. Oncol. 2014).

1.2. Clinical presentation and diagnosis

Given that the cerebellum is located against the fourth ventricle, tumors arising from it result in mass effect and hydrocephalus. Consequently, patients initially diagnosed with medullo-blastoma present most commonly with symptoms of elevated intracranial pressure—chronic progressive nausea, vomiting, and headache [10]. These symptoms can progress to altered mental status, sensorimotor symptoms, and cerebellar symptoms if left untreated [10]. Children and infants may present instead with nonspecific lethargy and weakness. Neuro-logical signs, often subtle, may be present for 3 or more months before diagnosis [11].

Medulloblastoma metastasizes most commonly to the spinal cord. In an international meta-analysis of medulloblastoma, metastatic disease was identified in 103 of 432 patients (24%) on initial diagnosis [6], although the incidence was much lower in adults (2%). Metastatic disease was most common in Group 3 and Group 4 medulloblastomas (30 and 31%, respectively), while much lower in the Wnt group (9% of children) [6].

Although a biopsy specimen is required for definitive diagnosis of medulloblastoma, brain magnetic resonance imaging (MRI) with gadolinium is the preferred imaging modality to best characterize lesions suspected to be medulloblastoma. Brain MRIs allow for greater resolu-tion of soft tissue with less interference from bone compared to computed tomography [12]. MRI findings associated with medulloblastoma can have varying enhancement patterns and intensities. Imaging can also identify areas of hemorrhage, calcification, or findings that suggest leptomeningeal metastasis [12]. It has been suggested that certain MRI findings may be more associated with certain histopathological subtypes [12].

1.3. Current conventional treatments and treatment considerations

Once identification of suspected medulloblastoma has been made on imaging, a decision needs to be as to how tissue sample should be accessed. The current standard of care is to resect as much of the lesion as possible if able to do so in a safe manner [13]. If it is deemed unsafe to do so, a stereotactic biopsy of the suspected lesion would allow for a confirmatory patholog-ic diagnosis. Once tissue has been obtained, the patient must be reassessed and assigned to the standard-risk group or high-risk group which informs subsequent patient treatment regi-men. The goal of this treatment regimen, which includes chemotherapy with or without chemoradiation, is to treat disease that may not have been fully resected by surgery.

In order to place patients into one of these groups, additional imaging is required postopera-tively, as well as cerebrospinal fluid (CSF) analysis and adequate pathologic specimen. Specifically, these two risk classifications are defined by size of residual tumor following resection and status of metastasis [14]. Standard-risk groups are less likely to have tumor recurrence following resection, while high-risk groups are more likely to have tumor recur-rence.

Standard-risk medulloblastoma occurs in 70% of patients [15]. Although prospective random-ized trials comparing radiotherapy alone to combined chemoradiation for treatment of standard-risk medulloblastoma have not been performed, combined therapy is currently the standard of care of standard risk medulloblastoma [16]. Patients in this risk group are typically

treated with a combination of chemotherapy followed by radiation, although radiation therapy alone has been used [15, 17, 18]. Multiple protocols exist for the chemotherapeutic treatment of medulloblastoma. One chemotherapeutic regimen includes treatment with a combination of vincristine, cisplastin, lomustine, and cyclophosphamide alongside radiation therapy over about a 1-year period [15]. High-risk or unresectable tumors are also treated with chemoradiation. Infants (<3 years old) are typically not treated with radiation owing to intolerability of side effects.

Risk stratification of medulloblastoma patients has improved cure rates for high-risk cases and limited radiation therapy exposure in treatment regimen for standard-risk patients, thereby reducing side effects. Nevertheless, even with improved cure rates for patients, long-term sequelae of treatment remain a concern. Radiation therapy has been associated with long-term neurocognitive deficits, cytopenias, opportunistic infections, and secondary malignancies [15, 19]. Children are especially sensitive to the adverse effects of radiation therapy, and as such radiation doses for treatment are lower for pediatric than for adult patients [15].

Long-term chemotherapy too has known side effects that have been described extensively elsewhere and include neurocognitive impairment, hearing loss, endocrine perturbations, cardiac and respiratory conditions, and secondary malignancies [15, 20]. Moving forward, further studies need to be performed to optimize current treatment or to identify new therapeutics to minimize side effect profile. Classification of medulloblastoma subgroups, for instance, focuses research toward drug targets within molecular pathways driving these subgroups. These subgroups are described in detail below.

1.4. Prognosis

In one trial of pediatric medulloblastoma, 10-year event free survival (EFS) and overall survival (OS) rates were 75 and 80%, respectively, for kids with standard-risk medulloblastoma treated with radiation followed by chemotherapy [21]. In another trial, 5-year EFS ranged from 65 to 70% in patients who received both chemotherapy and radiation following tumor resection [13]. Treatment with radiation therapy alone had survival rates 50–65% even with higher dose radiation [21, 22].

In comparison to pediatric medulloblastoma literature, studies assessing the treatment of adult medulloblastoma are rare. One retrospective study of adult medulloblastoma treated with chemotherapy and craniospinal radiation identified a 4-year EFS of 68% [18]. Other studies have identified survival rates of 40–80% [23].

Relapses most likely occur within the first 2 years of diagnosis, with one-third occurring within the first 3–5 years [21]. Earlier relapses are more likely to be associated with metastatic disease [21], while later relapses (>5 years after diagnosis) were more likely to be related to local disease. The posterior fossa is the most common site of relapse. Relapses must be distinguished from secondary tumors. Secondary tumors can occur following radiation, either at sites of prior irradiation or at extracranial sites near sites of primary radiation (thyroid, bone, etc.). One study identified a 4.2% 10-year cumulative incidence of secondary tumors follow-

ing treatment with chemoradiation [21]. Increased use of mutagenic chemotherapy has also been suggested to play a role in the increasing incidence of secondary tumors following treatment of medulloblastoma.

Molecular subgrouping of medulloblastoma plays an important role in prognosis. In brief, the Wnt subgroup demonstrates the most favorable prognosis, whereas Group 3 medulloblastomas present the worst. Other factors that may affect prognosis include stage and complete or incomplete resection of tumors [18].

2. Molecular subgroups of medulloblastoma

The World Health Organization has subdivided medulloblastoma into five distinct histopathologic categories [24]: classic, desmoplastic/nodular, medulloblastoma with excessive nodularity, anaplastic medulloblastoma, and large cell medulloblastoma (**Figures 2** and **3**). Certain histological subtypes predominate patient age groups: 71% of pediatric cases classify as classic medulloblastoma, whereas 57% of infant cases exhibit desmoplastic/nodular histology [25]. Large cell and anaplastic medulloblastomas are associated with a poor prognosis, whereas desmoplastic/nodular medulloblastomas usually demonstrate an excellent outcome [25].
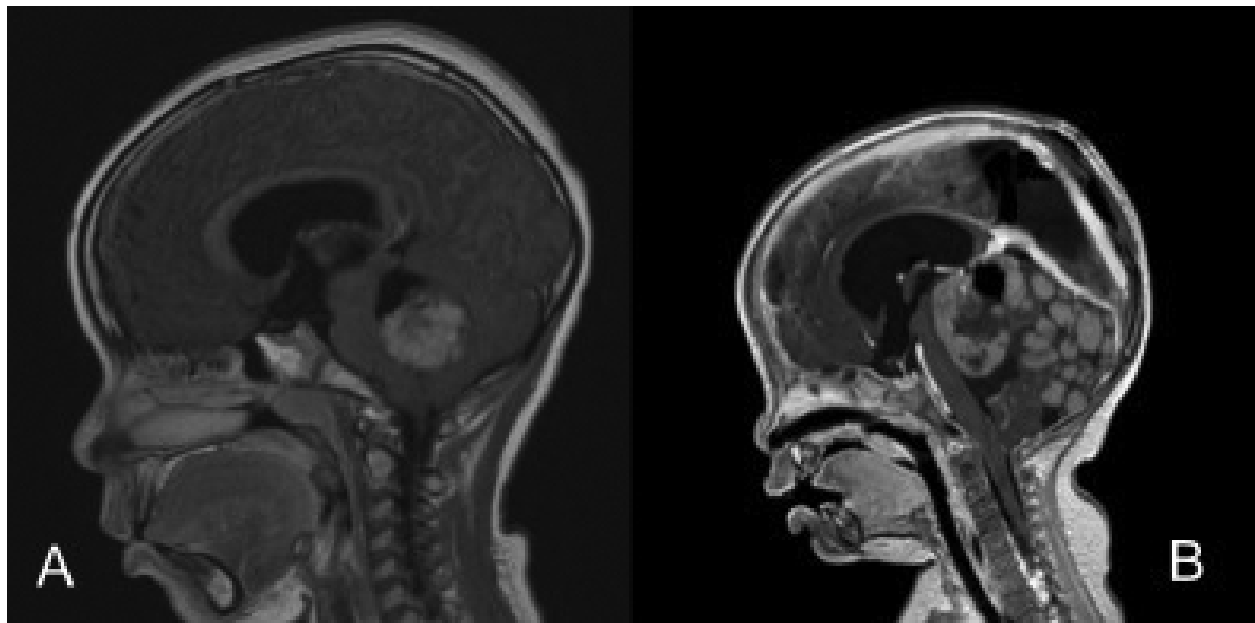

Figure 2. Medulloblastomas are grouped histologically or molecularly. Left image shows MRI of a pediatric patient with a classical medulloblastoma. Right image shows MRI of an infant with medulloblastoma with extensive nodularity. Copyright © 2014 Faculty of 1000 Ltd, from *Advances in managing medulloblastoma and intracranial primitive neuroectodermal tumors* (Adamski, Ramaswamy, Huang and Bouffet, F1000Prime Rep. 2014).

In addition to histological categories, retrospective molecular diagnostics have additionally allowed for medulloblastoma to be subdivided into four molecular subgroups (**Table 1**). The most well understood of these four subgroups are those medulloblastoma variants that involve the sonic hedgehog pathway (30% of patients with medulloblastoma and 60% of adults) and those involving the Wnt pathway (10% of all patients with medulloblastomas and 15% of adults) [26]. Molecular subgrouping may inform chemotherapy regimen, especially in light of emerging research about potential drug targets within involved molecular pathways.
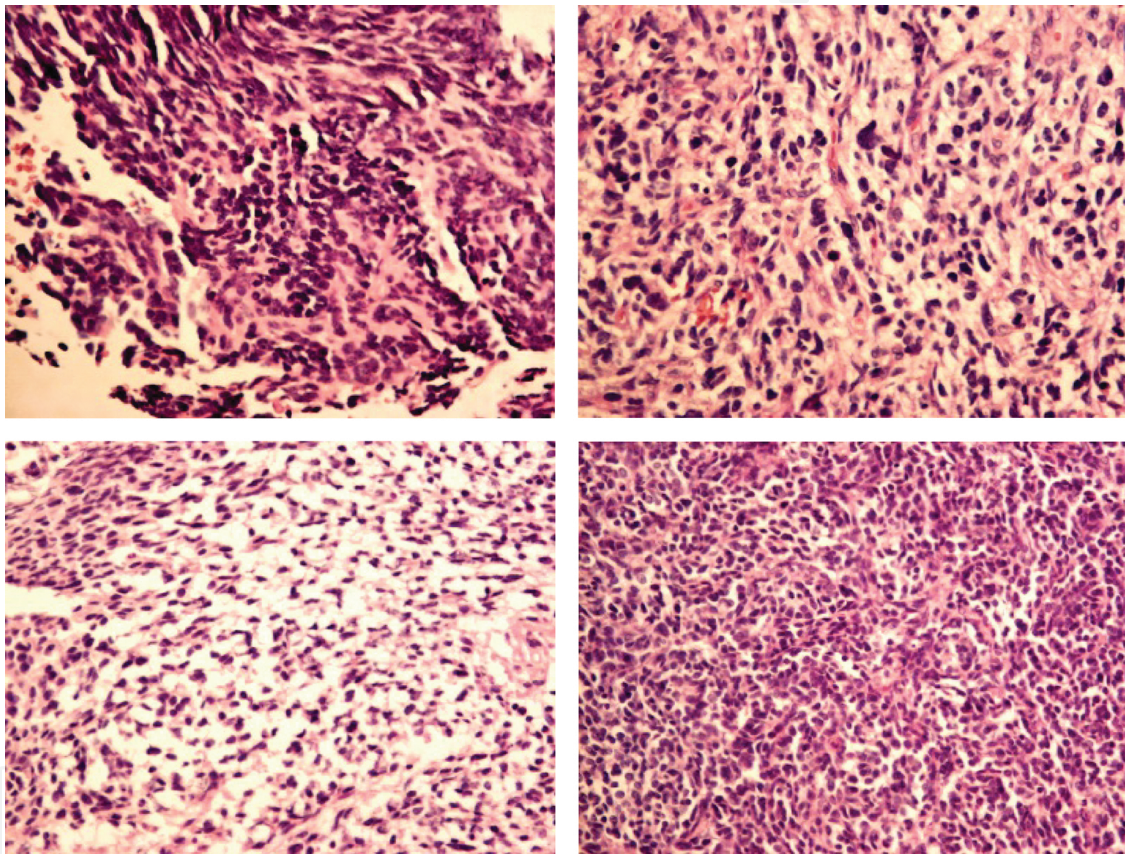

Figure 3. Histological slides stained with hematoxylin and eosin of medulloblastomas showing heterogeneity across patient tissue samples. Images obtained with permission from Dr. Kay Ka Wai Li (Prince of Wales Hospital, Department of Anatomical and Cellular Pathology, The Chinese University of Hong Kong).

2.1. Wnt pathway medulloblastoma

Wnt-type medulloblastoma is characterized by enhanced Wnt-β-catenin pathway activation [5] and tends to show classic histology rather than the poorer prognoses anaplastic or large cell type histology [6]. Among the medulloblastoma molecular subgroups, Wnt medulloblastoma is the least common, occurring in 10–15% of medulloblastomas [27]. It affects males 1.5 times more than females [6] and occurs rarely in infants (<3 years old).

For reasons that have yet to be elucidated, medulloblastoma tumors carrying Wnt mutations carry a better prognosis than other subtypes. In fact, meta-analysis of medulloblastoma subgroups found an overall 10-year survival rate of 95% in children with Wnt medulloblastoma and 100% 5-year survival rate among adult Wnt medulloblastoma [6].

2.1.1. Molecular basis of Wnt medulloblastomas

All medulloblastomas with heightened nuclear staining of β-catenin are grouped into Wnt-type. β-Catenin is a key promoter of the Wnt pathway, an evolutionarily conserved pathway involved in cellular homeostasis and embryogenesis. The pathway is involved in central nervous system development; indeed, derangements of Wnt signaling have been described in diseases of the CNS, including neural tube defects, Williams syndrome, Alzheimer's disease, and schizophrenia [28].

The Wnt pathway classifies into the canonical pathway and two separate noncanonical pathways. The noncanonical Wnt pathways appear to be independent of β-catenin. The canonical pathway is β-catenin dependent and is characterized by interaction of a Wnt ligand with the extracellular domain of Frizzled, a G-protein-coupled receptor. This interaction results in accumulation of intracellular β-catenin, promoting downstream gene activation [29, 30]. Multiple genes and proteins have been identified as regulatory factors for this pathway. β-Catenin is an unstable protein, and in the absence of Wnt ligand, it is broken down by a degradation complex composed of multiple proteins, the tumor suppressor protein APC and the scaffolding protein AXIN [31] are among them.

Ninety percent of the time, Wnt medulloblastoma is driven by mutation of β-catenin (*CTNNB1*), resulting in increased activation of MYC and MYCN oncogenes [5, 27]. A number of other frequently mutated genes have been identified in Wnt medulloblastoma [5, 27].

Alongside other evolutionarily conserved pathways [31] including the SHH and Notch pathways, the Wnt pathway has also been implicated in the development of cancer stem cells (CSCs), a subgroup of cancer cells defined by their pluripotency and capacity for self-renewal [29, 31]. The identification of cancer stem cells as a subgroup of pluripotent self-renewing cancer cells has led to the theory that they may be necessary for tumorigenesis. Aberrations in evolutionary conserved pathways, including the Wnt pathway, are frequently identified in cancer stem cells. The Wnt pathway therefore is an attractive means for targeting cancer stem cells, particularly in malignancies that are known to overexpress Wnt.

2.1.2. Drug targets in Wnt medulloblastoma

A number of molecules that interact with the Wnt pathway are currently being investigated as potential antitumor therapies in both preclinical studies and clinical trials. Tankyrase inhibitors have been identified that lead to downstream degradation of β-catenin [29]. JW55, a novel tankyrase inhibitor, has been shown in mice studies to reduce tumor development and colorectal cancer cell growth [32]. Inhibitors of Dishevelled, a protein that promotes downstream Wnt signal transduction, have also been shown to inhibit downstream Wnt signaling [33].

Interestingly, known nonsteroidal anti-inflammatory drugs (NSAIDs) have been found to have anti-Wnt pathway activity, possibly explaining in part their antineoplastic properties [27, 34, 35]. *In vitro* studies of colon cancer cells have shown that the NSAID sulindac inhibits canonical Wnt pathway activity via inhibition of cGMP hydrolysis [27]. Sulindac may also affect the Wnt pathway by affecting Dishevelled [34]. Celecoxib and diclofenac have been shown to decrease Wnt pathway signaling in *in vitro* glioblastoma cells [36]. Aspirin too affects the Wnt pathway [37]; in one study, aspirin diminished tumorigenesis in intestinal cells. The possible mechanism for aspirin in this study was downregulation of the expression of *PPAR-δ*, a growth and antiapoptotic promoting transcription factor that is a direct product of the Wnt pathway [38].

There are a number of ongoing trials using novel agents targeting the Wnt pathway. These agents include PRI-724, designed by Prism BioLab and which blocks the interaction of β-catenin with cotranscriptional coactivator CBP [29, 31]. A Phase I clinical on the molecule LGK-794, a porcupine inhibitor that inhibits Wnt protein secretion, is currently recruiting patients and will assess the safety profile in patients who carry malignancies that are dependent on Wnt ligands [29, 31]. It is important to note that these Wnt pathway-targeting compounds have not been tested in medulloblastomas, which would be the next direction for assessing their efficacy in Wnt medulloblastoma. However, although the Wnt pathway is a potential target for future medulloblastoma therapies, some authors have described potential theoretical barriers to the utilization of Wnt-targeted therapy in malignancy [28]. First, the Wnt pathway is crucial to organogenesis and homeostasis, begging the question as to whether altering the Wnt pathway may be detrimental to these processes. Second, some have contested the assumption that Wnt pathway antagonism would be desirable as anticancer therapy, given that the Wnt pathway is involved in neural regeneration after brain injury (such as surgery). The ongoing clinical trials using therapies targeting the Wnt pathway will help to better elucidate the safety and viability of targeting this pathway.

Clinical and molecular overview of medulloblastoma subgroups			
Group	Patient epidemiology	Prognosis	Associated genetic aberration
SHH	Frequent in infants and in adults but not in pediatric and teenage patients	75% 5-year survival	*Ptch1, Smo, Gli1, Gli2*, and/or *SUFU*—hyperactivation of sonic hedgehog signaling
WNT	Rare in infants More common in males than in females	Best prognosis of all subgroups: 95% 10-year survival in children; 100% 5-year survival in adults	β-Catenin—increased MYC expression
Group 3	Infants and pediatric patients but rare in adults	Worst prognosis of all subgroups: 40–60% 5-year survival	MYC Photoreceptor-associated pathways
Group 4	Most prevalent subgroup, 34% of cases Found in all age groups	75% 5-year survival	17q chromosome Loss of X chromosome in female patients

Table 1. Summary of key aspects of the four molecular subgroups of medulloblastoma.

2.2. Sonic hedgehog (SHH) pathway medulloblastomas

Activation of the sonic hedgehog (SHH) pathway drives tumorigenesis in the SHH group of medulloblastomas. SHH medulloblastomas are frequently found in infant (ages 0–3) and adult (>16 years) but occur less commonly in pediatric cases [25]. The prognosis is similar to Group 4 medulloblastomas.

2.2.1. Molecular basis of SHH medulloblastomas

In sonic hedgehog signaling, the receptor Patch (specifically Ptch1) inhibits a G-protein-coupled receptor called Smoothened (Smo) in the absence of Hedgehog ligand. Hedgehog ligand binding to Patch results in disinhibition of Patch from Smo, allowing downstream signaling transduction and the activation of the Gli transcription factors, Gli1, Gli2, and Gli3 (**Figure 4**) [39, 40]. Mutations in Patch, Smo, Gli1, and Gli2 have been shown to initiate medulloblastoma in a variety of models [41–44]. Mutation in SUFU, a negative regulator of SHH signaling, is another initiating mutation [45].
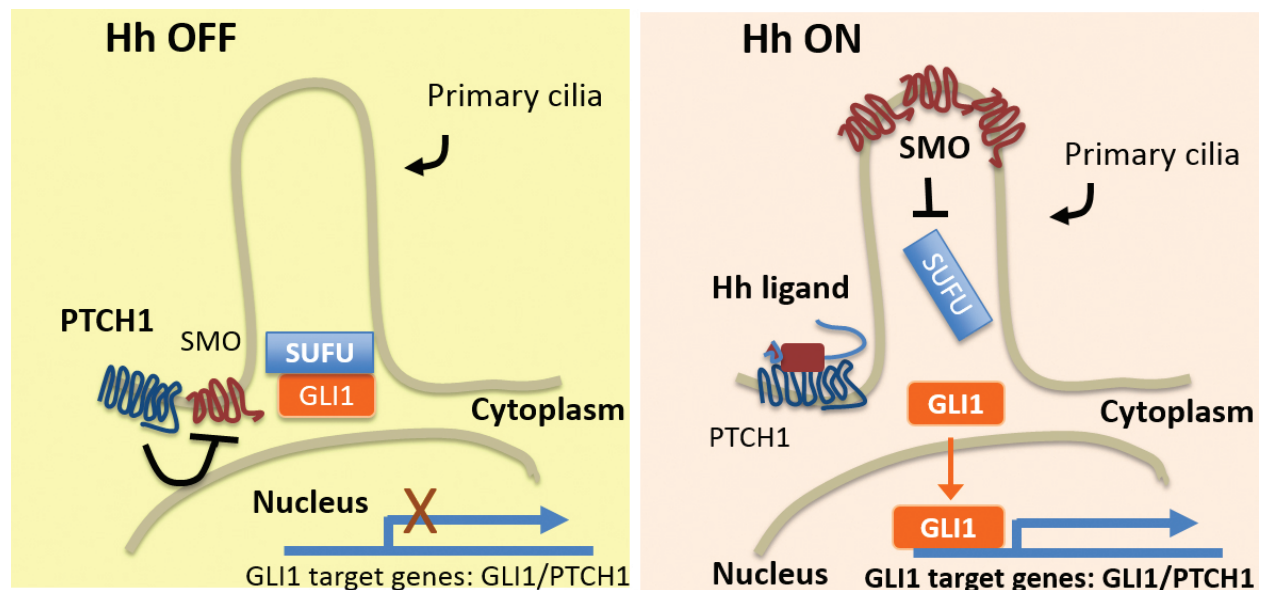

Figure 4. Schematic showing sonic hedgehog (SHH) signaling: in the absence of Hedgehog ligand, the Patch receptor (Ptch1) inhibits Smoothened (Smo). Hedgehog ligand binding to Patch results in the disinhibition of Smoothened, leading to downstream activation of the Gli transcription factors. Schematic illustrated by author JYY of this book chapter.

2.2.2. Drug targets in SHH medulloblastomas

Alkylating agents have long since served in chemotherapy for medulloblastoma, but for the SHH subgroup, inhibitors of Smo are also popular. The compound cyclopamine launched initial interest in targeting SHH signaling which was responsible for the developmental defects

found in sheep that ingested corn lilies in which cyclopamine was originally discovered [46]. 2004 marked the year that Genentech identified the drug vismodegib in a screen for compounds that inhibit the SHH pathway [46]. Studies assessed vismodegib initially in advanced basal cell carcinoma and were also launched to assess the drug for other cancers [46]. Vismodegib was approved in 2012 by the Food and Drug Administration (FDA) for the treatment of metastatic or recurring BCC [46]. A Phase I study has been undertaken to assess the safety, safe dosing range, and side effects of vismodegib in a population of children with recurrent or refractory medulloblastoma [47]. Out of the 20 patients enrolled for flat-dosage testing (150 mg for smaller body area and 300 mg for larger), only two dose-limiting toxicities were observed. The study concluded that vismodegib is well tolerated in pediatric patients with recurrent or refractory medulloblastoma and recommended 150 or 300 mg dosage for Phase II trials.

Consequently, a Phase II trial was conducted at this recommended dosage with adult and pediatric patient groups. The study found that vismodegib increased progression-free survival in SHH medulloblastoma group but not in the non-SHH medulloblastoma group. Vismodegib exhibited activity against adult SHH medulloblastoma. However, inadequate sampling size for the pediatric group precluded conclusions about vismodegib efficacy in this group [48]. Therefore, vismodegib appears promising for adult medulloblastoma patients but remains to be further examined for pediatric patients.

In 2015, the FDA approved another Smo inhibitor, sonidegib (also known as LDE225), for use in treating basal cell carcinoma [49]. Sonidegib has been tested in a variety of cancers, including medulloblastoma [50]. Other Smo inhibitors are being tested in other cancers. GANT61 has been tested in a prostate cancer model [51], while BMS-833923 was tested in a gastric and esophageal cancer model [52]. Both remain to be tested in medulloblastoma.

For SHH medulloblastoma, targeting SHH signaling is a more direct therapeutic approach than the use of alkylating agents; however, drug resistance may pose a realistic concern. For example, it has been found that drug resistance can arise from amino acids changes in Smo which leads to a deficiency in drug binding to vismodegib [53]. With the approval of sonidegib, researchers then investigated whether its usage might improve tumor response in patients with basal cell carcinoma who were resistant to vismodegib. They concluded that, unfortunately, patients with advanced basal cell carcinoma, who were previously resistant to vismodegib, also experienced resistance with sonidegib treatment [54]. So, drug resistance with novel Smo inhibitors remains an ongoing concern.

Toward the goal of developing combination therapies and limiting drug resistance, recent research has progressed to investigating the molecular regulation of proteins within the SHH pathway as potential drug targets. For example, several kinases have been shown to control the activity of Gli1: ribosomal protein S6 activates Gli1 through phosphorylation on its serine 84 [55], while protein kinase A phosphorylation inhibits Gli1's activity [56].

AMP kinase (AMPK), a regulator of cell energy allocation during stress conditions, has been shown to modulate Gli1 activity. Specifically, overexpression of AMPK leads to a decrease in Gli1 expression, while downregulation of AMPK activity increases Gli1 expression [57].

Therefore, suppression of SHH signaling through downregulation of Gli1 may serve as a venue of targeting SHH medulloblastomas. Our group has demonstrated how direct regulation of SHH signaling through AMPK function impacts tumorigenesis. We found that AMPK regulates Gli1 activity by phosphorylating the transcription factor at serines 102 and 408 and threonine 1074. Mutation of these phosphorylation sites to nonphosphorylatable alanine increases Gli1 protein stability, transcriptional activity, and oncogenic potency, suggesting that AMPK phosphorylation reduces Gli1 activity (**Figure 5**). Another group has supported our finding that AMPK phosphorylates and may regulate Gli1 through serine 408. This group found that AMPK promotes Gli1 degradation upon its phosphorylation of serine 408 on Gli1 [58]. Further studies illustrating the effect of modulating the activity of Gli1 regulators on medulloblastoma tumorigenesis in *in vivo* systems will inform whether they are potential drug targets.
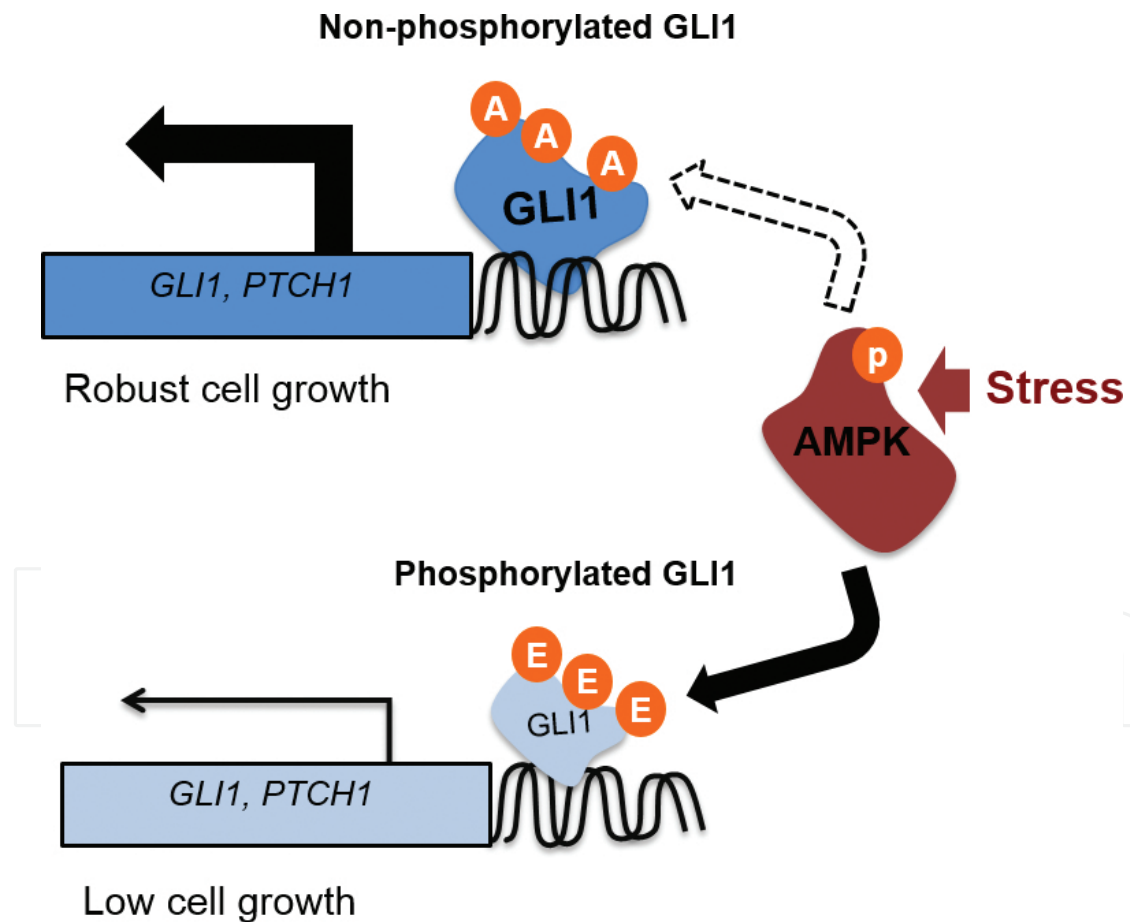

Figure 5. AMPK phosphorylation on Gli1 reduces Gli1 activity. During stress conditions, AMPK phosphorylation on Gli1 results in decreased cell growth. Uncontrolled Gli1 activity, which can arise from downregulating AMPK, leads to uncontrolled cell growth such as in medulloblastoma. Schematic adapted from author JYY's work, *AMP-activated protein kinase directly phosphorylates and destabilizes hedgehog pathway transcription factor GLI1 in medulloblastoma* (Li et al., Cell Rep. 2015).

Another approach to developing combination drug therapies has been to identify additional signaling pathways that impact SHH-driven medulloblastoma. Research has demonstrated that these pathways play a role in medulloblastoma development:

• p53: Tumor suppressor p53 is highly mutated in pediatric medulloblastomas and is a significant factor in determining prognosis [6]. A cohort study found that 5-year survival rates differed between 41 and 82%, respectively, for SHH medulloblastoma cases with and without p53 mutations [59]. In mice, the incidence of medulloblastoma increases to nearly 100% with p53 loss [60]. Therefore, regulators of p53 activity might serve as highly attractive drug candidates for combination therapy with Smo inhibitors. For example, driving down levels of MDM2, a negative regulator of p53, has been shown to decrease expression of Gli1 and Gli2 [61].

• cAMP: In general, researchers have discovered that the levels of second messenger cAMP are inversely correlated with tumor grade and growth. Ablation of the G protein Gαs is sufficient to initiate SHH medulloblastoma, and mice harboring the *GNAS* mutation demonstrate decreased tumor proliferation when cAMP levels are elevated [62].

• TGF-β: Expression analysis of Ptch1 heterozygous and Smo/Smo mouse medulloblastoma tumors of varying clinical severities found a correlation between TGF-β expression levels and medulloblastoma progression. In general, it was found that activation of the TGF-β pathway correlated with better prognosis with patients [63]. For instance, positive nuclear staining of SMAD3, a downstream component of TGF-β signaling, was associated with longer patient survival [63]. Therefore, regulation of the TGF-β signaling pathway in conjunction with SHH signaling may be another venue of combination therapy.

• Basic FGF: Overall, basic FGF (bFGF) signaling appears to have an inhibitory role on SHH-induced proliferation. The addition of bGFG to tumor cultures has been shown to limit tumor formation and proliferation and to inhibit expression of the transcriptional products of SHH signaling, namely *Gli1, Nmyc*, and *cyclin D1* [64].

While these intersecting pathways contain possible targets, determining the exact mechanism by which they impact SHH medulloblastoma is the limiting step to uncovering the best candidates to target.

2.3. Group 3 medulloblastomas

While Wnt and SHH medulloblastomas have been identified by mutations within these pathways, more comprehensive biological pathways have not been delineated for Group 3 and Group 4 medulloblastomas. Hence, these have been so named until the underlying biology is further elucidated.

Conventional diagnosis of Group 3 medulloblastomas is accomplished through transcriptional profiling [3]. Group 3 medulloblastoma is associated with increased MYC expression and enrichment for photoreceptor pathway-associated genes; these genes are overexpressed in Group 3 [3]. In addition, Group 3 can be divided into subtype based on MYC expression. In Group 3α subtype, all patients contain MYC amplification and this is associated with poor

prognosis with increased recurrence and mortality, while the Group 3β subtype contains no MYC amplification and has a prognosis similar to Group 4 medulloblastomas [3]. Medulloblastomas of this group are found in both infants and children, but rarely in adults, and are found more in males than in females [3]. Histologically, Group 3 medulloblastomas frequently have large anaplastic cell pathology [3].

2.3.1. Molecular basis of Group 3 medulloblastomas

While many details about the molecular makeup of Group 3 medulloblastomas remain unresolved, recent literature therapeutically targeting Group 3 medulloblastoma may reveal clues to the molecular pathways driving this subgroup. The folate synthesis inhibitor pemetrexed and nucleoside analog gemcitabine demonstrated a synergistic effect in increasing the survival of mice bearing MYC-overexpressing tumors [65]. The same drug combination had little effect on mice medulloblastomas of the SHH subgroup [65]. These observations are supported by gene set enrichment analysis showing that Group 3 medulloblastomas are enriched in the folate and purine metabolism pathways compared to Group 4 and SHH medulloblastoma [65].

The antihelmintic drug, mebendazole, has been shown to inhibit angiogenesis in medulloblastoma [66]. While it acts as a microtubule synthesis inhibitor in worms, studies with medulloblastoma models suggest that it can inhibit vascular endothelial growth factor receptor 2 (VEGFR2) [66]. Targeting class I histone deacetylase 2 has also been shown to impact Group 3 medulloblastoma tumor cell viability [67].

The International Cancer Genome Consortium (ICGC) PedBrain Tumor Project published in 2014 the analyses of deep sequencing of Group 3 and Group 4 tumors. This study uncovered novel information about the biology between this subgroup. Tetraploidy was a common event for both Group 3 and Group 4 tumors, respectively, and tetraploid tumors displayed signs of genomic instability [68]. With Group 3, the most frequently mutated gene was SMARCA4 [68]. Together, both *in vitro* drug assays and genome-wide mining of Wnt medulloblastomas introduce molecular pathways for further exploration in uncovering Group 3 medulloblastoma biology and which may reveal possible drug targets.

2.4. Group 4 medulloblastomas

Group 4 is the most prevalent medulloblastoma subgroup, accounting for about 34% of all medulloblastomas [6]. A high frequency (66%) of isochromosome 17q is associated with Group 4 medulloblastomas [6]. Strikingly, 80% of women with Group 4 medulloblastoma also have X chromosome loss [6]. Group 4 medulloblastomas have a prognosis comparable to SHH group medulloblastomas [6].

2.4.1. Molecular basis of Group 4 medulloblastomas

The ICGC PedBrain Project found that KDM64, a histone 3 lysine 27 demethylase, was mutated in 10% of Group 4 tumors [68]. These mutations reveal the genetic and molecular pathways that go awry in Group 3 and Group 4 tumors. For example, the ICGC PedBrain uncovered an

association between TBR1 and Group 4 medulloblastomas [68]. TBR1 is a T-box transcription factor shown to play a role in brain development. Of particular interest is the gene CTDNEP1, found mutated in 10% of Group 4 tumors and which is located on 17q [68]. CTDNEP1 encodes a nuclear membrane phosphatase and in mammals is shown to play a role in nuclear membrane biogenesis and in lipid activation. As 66% of Group 4 medulloblastomas contain 17q, mutations found on this isochromosome are particularly important for study.

2.5. Future clinical and basic science directions for medulloblastoma

Clearly, with respect to Group 3 and Group 4 medulloblastomas, further studies about the molecular basis for these subgroups are needed. These two subgroups pose great clinical challenges: Group 4 is the most prevalent group, while Group 3 has the poorest diagnosis. Yet a dearth of knowledge about the molecular basis behind each group limits drug targeting. The growing body of studies which include genome-wide mining for enrichments within each subgroup along with *in vitro* studies for Group 3 and Group 4 may soon intersect to reveal a broader picture of the molecular pathways behind these subgroups.

Currently, there are a number of clinical trials evaluating the safety and efficacy of Wnt-targeted therapies in patients with other malignancies that overexpress the Wnt pathway; however, none of these are being tested in medulloblastomas. The efficacy of these agents in treating Wnt medulloblastoma remains to be assessed. Additionally, in light of the high survival rates of standard risk patients with Wnt medulloblastoma, additional studies would be helpful to identify optimal treatment regimens that will maintain these high survival rates while minimizing treatment side effects. With respect to SHH-driven medulloblastoma, identification of novel targets especially for combination drug therapy will address the concern for drug resistance and limited efficacy of current treatments. For example, the identification and assessment of novel Gli inhibitors for SHH-mediated cancers should be evaluated in the context of medulloblastoma. In addition, the effects of the crosstalk of intersecting pathways on medulloblastoma tumorigenesis should be further studied.

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