Clinical, Histopathologic, Radiographic and Molecular Classification of Medulloblastoma

David R Raleigh¹, Vanja Varenika², Tarik Tihan³ and Daphne A Haas-Kogan¹*

¹Department of Radiation Oncology, University of California San Francisco, USA
²Department of Radiology and Biomedical Imaging, University of California San Francisco, USA
³Department of Pathology and Laboratory Medicine, University of California San Francisco, USA

INTRODUCTION

Central nervous system neoplasms are the second most prevalent cancer in children, and remain the leading cause of cancer-related death in pediatric patients [1]. Medulloblastoma, the most common malignant pediatric brain tumor, is an invasive, rapidly growing neoplasm that originates exclusively in the posterior fossa. In the United States, the overall incidence of medulloblastoma has remained stable at 1.5 per million people for the past 40 years, with a slight male to female preponderance of approximately 1.5:1 [2]. Despite its overall rarity, children age 1 through 9 are 10-fold more likely to be affected than older individuals, and medulloblastoma accounts for approximately 20 percent of all pediatric central nervous system tumors.

Keywords
• Medulloblastoma
• Sonic hedgehog
• Wnt
• Myc

Abstract

Medulloblastoma, a malignant neoplasm of the posterior fossa with a high propensity for metastatic spread, is one of the most common central nervous system tumors in children. Despite the long-term survival of many patients, definitive therapy is associated with significant morbidity. Adjuvant craniospinal irradiation and chemotherapy leaves many survivors debilitated from treatment-associated side effects including endocrine dysfunction, growth defects, and intellectual impairment. Although classically staged by clinical features, medulloblastoma has been the subject of extensive histopathologic, radiographic, and molecular studies aimed at defining prognosis and identifying novel therapeutic targets. These analyses have revealed specific tumor characteristics that are predictive of outcome independent of clinical stage, as well as biologic pathways that drive tumorigenesis. Despite these scientific discoveries that carry a potential for targeted therapy, there have been few recent innovations in the clinical management of medulloblastoma. Here we summarize the clinical, histopathologic, radiographic, and molecular characteristics of both pediatric and adult medulloblastoma. Although these features are employed in clinical practice to counsel patients and predict clinical course, they have yet to be translated into therapeutic advances. Ultimately, the precise classification of medulloblastoma is likely to facilitate modification of therapy with reduced toxicity in some cases, while improving clinical outcome with targeted therapies in others. However, additional data concerning the molecular pathways that facilitate medulloblastoma development and progression are necessary before therapeutic innovations are likely to be realized.

ABBREVIATIONS

APC: Adenomatous Polyposis Coli; CT: Computed Tomography; FAP: Familial Adenomatous Polyposis; FLAIR: Fluid Attenuated Inversion Recovery; GLI: Glioma-Associated Oncogene Transcription Factor; GAB1: Growth Factor Receptor-Bound Protein 2-Associated-Binding Protein 1; Hh: Hedgehog; WNT: Int/Wingless; MRI: Magnetic Resonance Imaging; MYC: Myelocytomatosis Viral Oncogene; NBCCS: Nevoid Basal Cell Carcinoma Syndrome; Ptc: Patched; PI3Kca: Phosphoinositide 3-Kinase Catalytic-A Subunit; PNET: Primitive Neuroectodermal Tumor; Smo: Smoothened; Shh: Sonic Hedgehog; Sufu: Suppressor of Fused; WHO: World Health Organization
Treatment consists of maximal safe surgical resection followed by adjuvant craniospinal irradiation and chemotherapy for most patients. Although this multi-modal approach results in long-term survival for approximately 75 percent of patients, most survivors suffer from an impaired quality of life due to treatment-associated side effects. Chief among these is cognitive dysfunction, including slowed processing speed, and deficits in working memory and attention [3]. Many patients also develop hearing deficits, endocrine dysfunction, and growth defects, and are at an increased risk for secondary malignancies following the presently non-specific, highly toxic treatment regimen [4-7]. Given the relative morbidity of definitive therapy, significant effort has been dedicated to stratifying medulloblastoma patients based on presentation, tumor characteristics, and survival with the overall goal of de-escalating therapy in appropriate cases and intensifying treatment in others. In this regard, clinical and histopathological features are being combined with radiographic findings and molecular studies in increasingly complicated risk stratification schemes. This review aims to summarize the utility and limitations of the clinical, histopathological, radiographic, and molecular characteristics of medulloblastoma that are used to classify patients and guide therapeutic decisions.

**CLINICAL RISK STRATIFICATION**

**Pediatric medulloblastoma**

Controversy exists concerning the most meaningful prognostic factors for clinical stratification of medulloblastoma patients. Based on primary tumor size and the extent of metastases, the Chang Criteria have served as the primary staging system for medulloblastoma since their inception in 1969 (Table 1) [8]. Spread to the leptomeninges is particularly critical, and 5-year progression-free survival in patients with microscopic or gross disease in the neuraxis or systemically is approximately half that of individuals with tumor confined to the posterior fossa [9]. With respect to surgical technique, five-year actuarial survival increases from approximately 40 percent following biopsy alone, to nearly 75 percent for those who undergo gross or even subtotal resection [10,11]. Despite the increase in overall survival following introduction of postoperative craniospinal radiation and chemotherapy in the 1970s, patients diagnosed before age 3 continue to have a markedly lower survival than older individuals [9,12]. Consequently, medulloblastoma is often viewed in terms of average- and high-risk features, which are defined by (i) age at presentation, (ii) extent of residual tumor on the most involved computed tomography (CT) axial image following resection, and (iii) the presence or absence of metastases (Table 2).

<table>
<thead>
<tr>
<th>Extent of Tumor</th>
<th>Average Risk</th>
<th>High Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 primary tumor &lt; 3 cm in diameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2 primary tumor &gt; 3 cm in diameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T3 primary tumor &gt; 3 cm in diameter with radiographic or operative extension beyond the posterior fossa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4 primary tumor &gt; 3 cm in diameter with extension past the aqueduct of Sylvius and/or the foramen magnum</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Following surgery, average risk medulloblastoma patients typically receive 2340 cGy of craniospinal irradiation in 180 cGy fractions, followed by a selective posterior fossa boost to a total dose of 5400 cGy. In contrast, high-risk patients are generally treated with 3600 cGy of craniospinal radiation, again followed by a posterior fossa boost to a total dose of 5580 cGy. The particular emphasis placed on posterior fossa control through maximal safe resection and radiotherapy is derived from the finding that 50-70 percent of recurrences occur in the tumor bed, and are often associated with leptomeningeal spread [13]. Following radiation, chemotherapy is employed to decrease the risk of recurrence, and minimize the dose of craniospinal radiation required for disease control in average risk patients post hoc. Patients younger than 3 to 5 years of age similarly benefit from chemotherapy as a temporizing measure to delay radiation and allow for brain and spinal cord development. Nevertheless, consensus is lacking concerning the appropriate timing of radiotherapy for pediatric medulloblastoma patients who have yet to complete neurocognitive development. Although radiation unequivocally improves local control and survival, intellectual impairment from ionizing radiation is well-known to be inversely related to patient age at the time of treatment [3,14]. More so than any other side effect of treatment, neurocognitive dysfunction following craniospinal irradiation has driven the histopathological, radiographic and biologic investigations aimed at re-defining the staging system of medulloblastoma with the overall goal of de-escalating definitive therapy in appropriate cases.

**Adult medulloblastoma**

Although approximately 20 percent of medulloblastomas occur in patients above the age of 16, there are no prospective randomized trials upon which to base treatment recommendations for adults [15]. Consensus is particularly vague with respect to role of chemotherapy in adult medulloblastoma patients, where toxicity is generally greater and there is less urgency to reduce the dose of craniospinal radiation. Irrespective of treatment, late relapses are more common in adults, as are systemic metastases [16]. Regardless, long-term survival for adults with medulloblastoma ranges from 58 to 84 percent, similar to pediatric patients [17]. Also similar to childhood medulloblastoma cases, adults who undergo gross total resection or who were diagnosed in the era of multi-modal therapy have a better prognosis than historic controls. Despite these similarities,
clinical prognostic factors for adult medulloblastoma are controversial, and contrasting studies have variably supported and invalidated the classic pediatric predictive elements in adult tumors [15]. Molecular profiling has similarly demonstrated both overlapping and disparate features between pediatric and adult medulloblastomas, although these characteristics will be discussed in further detail below.

**HISTOPATHOLOGY**

Medulloblastomas are primitive embryonal tumors primarily composed of small, blue round cells with hyperchromatic nuclei and scant cytoplasm. The tumors are often hypercellular, and mitotic figures and apoptotic cells are readily discernable even though their frequency may vary based on the histological subtype. Classic medulloblastomas are associated with rings of malignant cells arranged into primitive rosettes (Figure 1B). The Homer Wright, or neuroblastic rosette is defined by the presence of a fibrillar core typically composed of neuropil; Flexner-Wintersteiner rosettes are less common but have a true central lumen. The latter is also found in other central nervous system primitive neuroectodermal tumors (PNET), as well as pineoblastoma and retinoblastoma [18]. In addition to these classical structures, most medulloblastomas also demonstrate stacked, linear arrangement of tumor cells, and an angiocentric pattern reminiscent of perivascular pseudorosettes in ependymal tumors. Given this similarity, high-grade ependymomas in this age group and location may simulate medulloblastoma and can pose a diagnostic challenge.

As defined by the current World Health Organization (WHO) classification scheme, the two most biologically critical histopathologic subtypes of medulloblastomas are nodular/desmoplastic and large cell/anaplastic, both of which differ significantly from classical medulloblastoma (Figure 1A-D). A rich reticulin network separates nodular/desmoplastic medulloblastoma into paucicellular, neuropil-rich nodules (also referred to as “pale islands”), and hypercellular, mitotically active, reticulin-poor internodular areas (Figure 1A and 1D) [19]. A subgroup of this variant is medulloblastoma with extensive nodularity, which has been reported to have a unique radiological appearance and a better overall survival [20]. Although essentially two morphological subtypes the current classification system combines large cell and anaplastic features into a single stratum due to their mutually aggressive behavior. Large cell medulloblastoma is composed of discohesive populations of cells with prominent nucleoli reminiscent of a malignant lymphoma, while anaplastic variants harbor wildly pleomorphic cells with abundant mitoses, apoptotic markers, and engulfed remnants of previously adjacent tumor cells (Figure 1C). Often, these two histological patterns coexist in the same tumor, further giving credence to the suggestion of combining them into a single category.

Histological characteristics are important prognostic markers of medulloblastoma in children less than 5 years of age irrespective of disease burden [21]. Moderate and severe anaplasia are found in approximately one quarter of tumors in larger studies, and both the variant and the extent of anaplasia are strongly associated with worse clinical outcomes [19]. Numerous studies have further demonstrated that histopathological subtype correlates with molecular determinants of medulloblastoma pathogenesis, which will be discussed at greater length below. In brief, nodular/desmoplastic and large cell/anaplastic tumors seem to predominantly fall into Sonic Hedgehog and group 3 molecular categories, respectively. In contrast, the classical variant, which comprises the majority of the tumors, is common to all molecular subtypes.

![Figure 1](image-url)  **Figure 1** Histopathological Categories of Medulloblastoma. Hematoxylin and eosin staining of human surgical specimens demonstrating (A) extensively nodular/desmoplastic medulloblastoma, (B) classical medulloblastoma with abundant Homer Wright rosettes, (C) anaplastic medulloblastoma, and (D) medium power magnification of a nodule in a nodular/desmoplastic tumor. Also shown are immunohistochemical stains for (E) GAB1 in a case of nodular/desmoplastic medulloblastoma, and (F) β-catenin in the nuclei of a Wnt-medulloblastoma associated with CTNNB1 gene mutation.
Apart from the aforementioned strata, medulloblastoma can also display myogenic or melanotic differentiation. These patterns were recognized as distinct variants in the earlier editions of the WHO classification scheme, but are now considered histological patterns. Whether these extremely rare subtypes deserve recognition as distinct variants or are mere variations in the histological spectrum of classical medulloblastoma still needs to be determined. Immunohistochemical studies may be helpful in the recognition of medulloblastomas, but are currently better utilized to determine the molecular/genetic grouping of tumors (Figure 1E and 1F). All medulloblastomas demonstrate expression of neuronal proteins to some extent, although these are less common in more aggressive variants. Typical neuronal markers used in diagnostic neuropathology including synaptophysin, neurofilament protein, and rarely, nuclear antibody Neu-N [22-24]. Stains for the non-specific neuronal markers enolase and nestin are not practically beneficial in everyday practice.

**RADIOGRAPHIC FINDINGS**

Historically, imaging has not played a role in the definitive diagnosis or staging of medulloblastoma. Medulloblastomas classically appear as a hyperattenuating cerebellar mass on non-enhanced CT scans, and demonstrate homogeneous enhancement following contrast injection [25]. Although the majority of lesions are found at the posterior fossa midline, nodular/desmoplastic tumors are also known to localize to the cerebellar hemispheres. Irrespective of location, most medulloblastomas are further associated with significant peritumoral vasogenic edema, and approximately 20 percent have intratumoral calcifications [26]. CT scans have primarily served to identify the presence of a posterior fossa mass and to assess for residual tumor after resection. More recently, several studies have investigated the use of magnetic resonance imaging (MRI) as a means to differentiate histological subtypes. Even though several characteristic MRI findings have been attributed to the different pathological subgroups of medulloblastoma, biopsy remains the gold standard for diagnosis and in clinical practice imaging is not used in isolation.

On T1-weighted images, medulloblastoma is generally iso- or hypointense and avidly enhances after contrast administration (Figure 2A). The majority of tumors arise in the inferior vermis, but 10 to 15 percent occur within the cerebellar hemispheres, most commonly in older individuals and in patients and nodular/desmoplastic histology [26]. With respect to the distinct histologic variants of medulloblastoma, classic histological tumors can demonstrate more subtle, heterogeneous enhancement than other subtypes, as well as T2 signal hyperintensity from cysts and calcifications (Figure 2B). In contrast, nodular/desmoplastic lesions can appear isointense on T2-weighted sequences, presumably related to the highly cellular nature of the tumors (Figure 2C). Finally, relative to other histologic subgroups, large-cell/anaplastic tumors are most likely to have central necrosis on T1 imaging. Ring enhancement is also common, and T2 FLAIR (right) reveals extensive surrounding edema, consistent with the highly malignant nature of these lesions.

![Figure 2](https://example.com/figure2.png)
cell/anaplastic tumors can demonstrate ring enhancement as a result of tumor necrosis (Figure 2D). Consistent with clinical trends, leptomeningeal dissemination on MRI correlates with large-cell/anaplastic histology, and is inversely related to classic and nodular/desmoplastic subtypes [27].

Notably, retained blood products and surgical debris within the cerebrospinal fluid following primary tumor resection may obscure drop metastases in the lumbar spinal canal. It is therefore imperative that complete imaging of the brain and spinal canal be conducted prior to postoperative intervention. If not completed preoperatively, several weeks should be allowed to pass before staging scans of the spine can be undertaken. In contrast, it is imperative that post-operative restaging scans of the posterior fossa are completed within 72 hours of resection before granulation tissue obscures residual tumor.

**GENETIC AND MOLECULAR SUBTYPES OF MEDULLOBLASTOMA**

Classically, medulloblastoma was considered to be a member of the PNET family based on histopathological similarities, but modern genetic profiling suggest that these entities are molecularly distinct [28]. Biologic studies of pooled, multi-institution samples indicate that medulloblastoma can be divided into molecular subgroups with divergent cells of origin, clinical behaviors, and outcomes. Although a variety of molecular schemata have been proposed, the most widely accepted separates medulloblastoma into int/Wingless (Wnt), Shh, group 3, and group 4 tumors (Table 3). The majority of studies reported to date rely on high-throughput bioinformatic techniques such as genome-wide DNA copy number analysis, mRNA expression profiles, somatic copy number aberrations, whole genome sequencing, and whole exome sequencing. As such, the relatively rich understanding of medulloblastoma molecular signatures is offset by a lack of mechanistic insights. While unifying molecular characteristics are clear for Wnt- and Shh-tumors, specific driver mutations for group 3 and group 4 tumors remain largely elusive. Moreover, it is unclear to what extent group 3 and group 4 tumors are distinct variants, and future classification schemes may include Wnt, Shh, and non-Wnt/non-Shh categories. Despite these ambiguities, the prognostic significance of molecular strata in medulloblastoma are beginning to be elucidated [29].

**Inherited causes of medulloblastoma**

Although genetic syndromes account for less than 5 percent of all medulloblastomas, molecular profiling of tumors from these patients has been essential to understanding the distinct molecular subtypes of medulloblastomas that dominate sporadic cases [30,31]. Gorlin syndrome, also known as nevoid basal cell carcinoma syndrome (NBCCS), is an autosomal dominant condition that results from germline mutations to the patched-1 gene (*Ptch1*). *Ptch1* has an inhibitory effect on the Shh pathway, a partially conserved signaling network critical for regulating tissue patterning, cell cycle progression, and stem cell maintenance [32]. As such, Gorlin syndrome patients often demonstrate signs of dysfunctional tissue development including skeletal abnormalities, mandibular cysts, and skin pits. In the central nervous system, Shh is secreted by Purkinje neurons during cerebellar development to facilitate growth and migration of granule neuron precursor cells. However, in the absence of developmental *Ptch1* repression, inappropriate activation of the Shh pathway is associated with both medulloblastoma and basal cell carcinoma, the most common cancer in light-skinned adults [33]. Medulloblastoma formation is often the first indication of Gorlin syndrome, and given the well-known association between these two conditions, it is perhaps unsurprising that heterozygous *Ptch1* deletion was employed to create the first mouse model of medulloblastoma [34,35].

### Table 3: Molecular Subgroups of Sporadic Medulloblastoma.

<table>
<thead>
<tr>
<th>Pediatril prevalence</th>
<th>Wnt</th>
<th>Shh</th>
<th>group 3</th>
<th>group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>~10%</td>
<td>~30%</td>
<td>~25%</td>
<td>~35%</td>
<td></td>
</tr>
<tr>
<td>peak incidence</td>
<td>adolescents and young adults</td>
<td>infants and young adults</td>
<td>between infancy and adolescence</td>
<td>adolescence</td>
</tr>
<tr>
<td>5-year survival</td>
<td>~95%</td>
<td>~60-80%</td>
<td>~40-50%</td>
<td>~75%</td>
</tr>
<tr>
<td>cell of origin</td>
<td>lower rhombic lip progenitors</td>
<td>GNP s from EGL and cochlear nuclei; SVZ stem cells</td>
<td>GNP s from EGL</td>
<td>unknown</td>
</tr>
<tr>
<td>histology</td>
<td>classic</td>
<td>nodular/desmoplastic</td>
<td>classic and LC/A</td>
<td>classic</td>
</tr>
<tr>
<td>MRI characteristics</td>
<td>T2-FLAIR hyperintense</td>
<td>T2-FLAIR isointense</td>
<td>ring enhancement, central necrosis</td>
<td>T2-FLAIR hyperintense</td>
</tr>
<tr>
<td>TP53 correlate</td>
<td>Turcot syndrome (FAP)</td>
<td>Gorlin syndrome (NBCCS)</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>adult tumor</td>
<td>~15% rate of co-mutation, equivalent prognosis</td>
<td>~20% rate of co-mutation, worse overall survival (~40%)</td>
<td>uncommon</td>
<td>rare in conjunction with isochromosome 17, but common with MYCN amplification; prognostic significance unclear</td>
</tr>
<tr>
<td>characteristics</td>
<td>~10-20% incidence, worse prognosis</td>
<td>~50-75% incidence, comparable outcome</td>
<td>extremely rare, worse prognosis</td>
<td>~20-30%, prognostic significance unclear</td>
</tr>
</tbody>
</table>

*Most common histological association(s)

| Radiographic findings are not specific to molecular subtype, but are listed with respect to the most common histopathological association for each category.

**Abbreviations:** EGL: External Granule Layer; FAP: Familial Adenomatous Polyposis; FLAIR: Fluid Attenuated Inversion Recovery; GNP: Granule Neuron Precursor; LC/A: Large-Cell/Anaplastic; MRI: Magnetic Resonance Imaging; NBCCS: Nevoid Basal Cell Carcinoma Syndrome; Shh: Sonic Hedgehog; SVZ: Subventricular Zone; Wnt: Int/Wingless.
Turcot syndrome is defined by the simultaneously presence of brain tumors and inherited colonic polyposis. With respect to the latter, medulloblastoma is only found in familial adenomatous polyposis (FAP) but not microsatellite instability of DNA mismatches repair genes. FAP, an autosomal dominant condition, results from inactivating mutations in the adenomatous polyposis coli gene (APC). APC functions downstream of Wnt to assemble a protein complex that constitutively degrades β-catenin [36]. In the absence of APC activity, β-catenin is trafficked to the nucleus where it promotes expression of diverse genes to control cell proliferation. Although medulloblastoma develops in less than 1 percent of all patients with FAP, somatic driver mutations of the β-catenin gene are recognized in a specific subset of sporadic medulloblastoma [37-38].

**Wnt**

Wnt subgroup medulloblastomas account for approximately 10 percent of sporadic pediatric cases, are equally distributed between males and females, and typically occur in adolescents and young adults [39]. Wnt tumors are thought to arise from lower rhombic lip progenitors in the dorsal brainstem and generally exhibit classic histology (Figure 1B) [40]. With a very low rate of metastatic spread, it is perhaps unsurprising that 5-year overall survival for Wnt-medulloblastoma exceeds 95 percent, making it the most prognostically favorable molecular subtype of medulloblastoma [41].

Wnt peptides are a collection of highly conserved, lipid-modified glycoproteins which bind to the Frizzled family of transmembrane receptors to transduce intercellular signals upstream of APC and β-catenin. Although first identified as a proto-oncogene in a mouse model of breast cancer, autocrine and paracrine Wnt signaling is critical for embryonic development through regulation of cell migration and proliferation, body axis patterning, and cell fate determination [42]. Noncanonical Wnt pathways involved in planar cell polarity and calcium homeostasis have also been identified, but these networks operate independent of APC and β-catenin and are not involved in oncogenesis. In contrast, deregulation of the canonical Wnt pathway is associated with diverse malignancies, including skin, breast, lung, esophageal, colorectal, prostate, ovarian, and brain cancer [36]. With respect to the pathogenesis of medulloblastoma, alterations in Wnt signaling that promote stabilization and nuclear localization of β-catenin are common to both syndromic and sporadic tumors. In particular, somatic mutations of CTNNB1, the gene that encodes β-catenin, and loss of chromosome 6 are present in the majority of sporadic Wnt-medulloblastomas. As such, β-catenin immunohistochemistry has become a routine component of pathological analysis following resection of medulloblastoma (Figure 1F).

The first mouse model of Wnt-medulloblastoma was recently developed by selectively expressing a stabilized allele of CTNNB1 in progenitor cells from the lower rhombic lip [40]. Concordant with human Wnt-medulloblastoma, murine tumors from this system display classic histopathology. But unlike human cases, mouse Wnt-medulloblastomas have a low penetrance, long latency, and only develop in the setting of concurrent Tp53 mutation. Much like other molecular subgroups, Wnt-derived tumors are associated with mutations and copy number abnormalities to genes that are peripherally related to the driving oncogenic pathway. One such mutation identified in human tumors is phosphoinositide 3-kinase catalytic-α subunit E545K (PI3KcaE545K), a constitutively active, mitogenic enzyme that is also present in approximately 30 percent of breast cancers [43]. When incorporated into the aforementioned murine Wnt-medulloblastoma model, tumor formation is universal and complete within 3 months of birth [44]. Although neither Tp53 mutation nor PI3KcaE545K expression is sufficient for Medulloblastoma development in insolation of Wnt pathway misactivation, these data further support the hypothesis that peripheral regulators and downstream effectors are critical for medulloblastoma tumorigenesis.

**Sonic hedgehog**

More common in infants than adolescents, and slightly more prevalent in males than females, misactivation of the Shh pathway is found in approximately 30 percent of all sporadic pediatric medulloblastomas [41,45]. Shh-medulloblastoma, which is rarely metastatic upon diagnosis and frequently associated with nodular/desmoplastic histology, is considered to have an intermediate prognosis with approximate overall survival ranging from 60 to 80 percent (Figure 1D) [46,47]. Given the prevalence of this molecular signature, immunohistochemistry for growth factor receptor-bound protein 2-associated-binding protein 1 (GAB1), a transmembrane mediator of Shh signaling, is routinely performed for medulloblastomas (Figure 1E).

Hedgehog (Hh) proteins, such as Shh, are secreted glycolipoproteins that control cell fate and proliferation by binding to the transmembrane protein PtcH [32]. In the presence of Shh, PtcH-mediated repression of Smoothened (Smo) is released. In turn, Smo triggers a signal transduction pathway that culminates in Glioma-associated oncogene transcription factor (Gli) activation [48,49]. Gli2 is the primary downstream effector of developmental Hh signaling, but the mechanism and targets by which Gli2 facilitates Hh-dependent gene expression to drive medulloblastoma formation remain unclear [50].

Cells from the external granule cell layer, cochlear nuclei, and subventricular zone have been variably associated with Shh-medulloblastoma pathogenesis [51-55]. Mouse models of Shh-medulloblastoma in each of these progenitors have been available for many years, and numerous Shh pathway inhibitors have been developed with therapeutic purposes in mind [56]. However, despite promising preclinical and early clinical data, targeted therapies against the Shh pathway for medulloblastoma have been hindered by early relapses and acquired resistance [57,58]. The collection of Shh antagonists thus far reported work upstream of effector proteins, and given the preponderance of co-activated regulatory and accessory signaling networks in medulloblastoma, it is unlikely that targeted agents as monotherapy will significantly alter disease progression [39]. Moreover, it is unclear to what extent these agents will stunt normal development in pediatric patients.

Beyond germline inactivation of PtcH1 in Gorlin syndrome, somatic mutations of both PtcH1 and its homolog PtcH2 have been associated with medulloblastoma formation [59]. Misactivation of the Shh pathway in medulloblastoma patients has also been linked to Suppressor of fused (Sufu) mutation, which is critical
for shuttling Gli2 from the nucleus to the cytoplasm [60]. Finally, driver mutations have also been identified in Gli3, a member of the Gli family that has an antagonistic effect on the activity of Gli1 and Gli2 [61]. Chromosomal aberrations are common in Shh-mediated medulloblastoma, but the mechanistic impact of these changes has yet to be completely characterized [41].

Although not as well understood as their role in tissue patterning, the ways in which Gli transcription factors regulate cell proliferation has begun to be elucidated. In particular, the Hh cascade promotes cell cycle progression through expression of Cyclin D, Cyclin E, and N-myc in cultured granule neuron precursors, and also inhibits the ATR-Chk1 checkpoint in radiation-induced Ptch1−/− murine medulloblastoma [62-64].

Consistently, Hh also attenuates apoptosis through upregulation of HIP1 and Bcl-2 in human colon carcinoma cell lines, and down-regulates Fas in murine basal cell carcinoma [65,66]. In vitro experiments further suggest that Hh signaling overrides p53-, p21-, and RB-mediated tumor suppression, but these findings have yet to be validated in disease models [67-69]. Given the failures of targeted therapies which function upstream of Gli, further mechanistic data are needed before effective molecular agents are likely to be identified and translated into clinical use for medulloblastoma.

**Group 3**

Large cell anaplastic medulloblastoma, which has a high propensity for cerebrospinal fluid spread and an aggressive clinical course, is most commonly associated with the molecular subtype of medulloblastoma known as group 3 (Figure 1C) [70]. Rarely found in adults or infants and associated with a mere 40 to 50 percent overall survival, nearly 50 percent of these tumors present with metastatic spread at the time of diagnosis [39]. Notably, males are approximately 2-fold more likely to be effected with group 3 medulloblastoma than females [39].

Group 3 medulloblastoma is characterized by high-levels of myelocytomatosis virus oncogene (Myc) amplification, and genomic instability including gain of chromosomes 1q, 7, and isochromosome 17q, as well as loss of chromosomes 10q, 11, 16q, and 17p [41,7,17]. Myc is targeted by a plethora of mitogenic signals including the Wnt, Shh, and mitogen-activated protein kinase to regulate of cell proliferation, growth, differentiation, apoptosis, and stem cell self-renewal [73]. Irrespective of clinical stage or risk-stratification, MYC amplification in medulloblastoma is significantly associated with poor clinical outcome [29].

Twenty-five percent of all sporadic tumors fall into group 3, which are thought to arise from cerebellar granule neuron precursors in the external granule layer and multipotent progenitor cells from the subventricular zone [39,74,75]. Although TP53 alterations are not found in human group 3 medulloblastomas, both of the recently-reported animal models of MYC medulloblastoma require concurrent loss of endogenous TP53 activity for tumorigenesis [74-76]. Thus, the currently available transgenic reagents will likely serve as useful tools to test targeted agents against upstream drivers of group 3 medulloblastoma. However, better murine models are clearly needed to fully understand the impetus behind the most aggressive subgroup of medulloblastoma.

**Group 4**

Group 4 medulloblastomas are not unified by a single molecular signature, and are instead characterized by a collection of genomic alterations that variably occur in conjunction with one another. Amplification of MYCN and cyclin-dependent kinase 6, as well as cytogenetic abnormalities to isochromosome 17q and heterozygous loss of the X chromosome in females are all common to group 4 tumors [39]. Usually found in adolescent males and associated with classic histology, group 4 medulloblastomas have a 35 to 40 percent rate of metastasis at diagnosis. Approximately 35 percent of sporadic medulloblastomas meet molecular criteria for group 4, and prognosis is comparable to Shh-associated tumors with 75 percent 5-year overall survival. MYCN amplification and abnormalities of chromosome 17 are not negative prognostic factors in isolation, but the combination of MYCN overexpression with large-cell/anaplastic histopathology portends a particularly poor outcome [29]. Presently, there are no murine models of group 4 medulloblastoma that faithfully recreate the molecular signature of human tumors. Brain-specific over-expression of MYCN in mice facilitates tumorigenesis with a long latency, but the resulting tumors are molecularly dissimilar from human medulloblastoma [77,78].

**Contributions from altered p53 signaling**

Somatic mutations of the ubiquitous tumor suppressor p53 are present in less than 10 percent of cases, and are most often seen in adolescent medulloblastoma patients with average-risk disease. However, recurrent tumors are overwhelmingly likely to harbor TP53 mutation, and are frequently associated with large-cell/anaplastic histology [79]. p53 immunopositivity is both highly sensitive and specific for TP53 mutation, but controversy exists concerning the prognostic implication of p53 stabilization in medulloblastoma [79,80]. Individuals with Li-Fraumeni syndrome and germline TP53 mutation rarely develop medulloblastoma, suggesting that p53 may play an accessory rather than primary role in medulloblastoma. Consistent with this hypothesis, recent subgroup-specific analyses indicate that p53 status is only relevant to overall prognosis in the context of certain co-activated molecular networks.

Loss of p53 accelerates medulloblastoma formation in Ptch1−/− mouse models of medulloblastoma, and TP53 mutations have been identified in approximately 20 percent of Shh-associated human tumors [76,81]. However, unlike typical Shh-medulloblastomas, tumors bearing concurrent TP53 mutation occur almost exclusively in adolescents and are disproportionately associated with chromosomal instability [76,82]. Moreover, five-year overall survival for children with Shh-medulloblastoma is approximately 40 percent in the setting of TP53 mutation. p53 stabilization is similarly seen in approximately 15 percent of Wnt-medulloblastomas, and also enhances tumorigenesis in mouse models of Wnt-medulloblastoma [80]. However, in contrast to Shh-tumors, TP53 mutation does not alter the otherwise favorable prognosis characteristic to Wnt misactivation [76].

Consistently, anaplastic histopathology is not seen in Wnt/p53-medulloblastoma, whereas greater than 50 percent of Shh/p53 tumors are characterized by severe histopathologic anaplasia. Conflicting data exist concerning the association between TP53 mutation and MYC amplification, but the preponderance...
of evidence suggests that these alterations are not found concurrently in human patients [76,79]. In terms of group 4 medulloblastoma, TP53 mutation rarely occurs in the setting of alterations to chromosome 17, but is common in tumors carrying excessive MYCN amplification although the prognostic implication of this finding is unclear [76,80].

**Molecular profiling of adult medulloblastoma**

Whereas four distinct molecular variants exist in pediatric medulloblastoma patients, gene expression analyses demonstrated that adult medulloblastoma is dominated by three biologic signatures [71,83]. Approximately 30 percent of adult medulloblastomas are associated with expression of group 4 markers, and 21 percent of patients display misactivation of the Wnt pathway. Both Wnt and group 3 tumors in adult patients are associated with a worse prognosis than pediatric standards, although there is an exceptionally low prevalence of MYC amplification and other group 3 markers in adults [46,83].

Aberrant activation of the Shh pathway is the most common molecular variant in adult medulloblastomas and accounts approximately 50 to 75 percent of cases. When presenting with metastatic disease, adult Shh-medulloblastoma patients have an exceptionally poor prognosis relative to children, and do not have a favorable outcome if associated with nodular/desmoplastic histology [84]. One potential explanation for these findings is that adult and pediatric Shh-medulloblastomas are molecularly distinct entities, with unique downstream signal networks and disparate natural histories. Consistently, statistical modeling suggests that adult and pediatric Shh-medulloblastomas molecularly segregate into distinct, age-related cohorts [84]. Cytogenetic analyses further reveal that disparate chromosomal aberrations are found in pediatric and adult medulloblastomas. Despite these data, as well as the observation that leptomeningeal dissemination is approximately 4-fold more likely with pediatric Shh-medulloblastoma, progression-free and overall survival for Shh tumors is similar for both age groups.

**DISCUSSION AND CONCLUSION**

In conclusion, significant progress has been achieved in the classification and risk stratification of medulloblastoma. However, beyond craniospinal irradiation dose reduction for average-risk patients, the standard of care has not been influenced by the wealth of emerging prognostic data. Identification of medulloblastoma patients with low-risk disease through clinical, histopathologic, radiologic, and genomic studies provides an opportunity to reduce adjuvant therapy and perhaps attenuate long-term side effects. Conversely, precise stratification and treatment escalation for high-risk patients is likely to improve outcomes. Clinical, histopathologic, and radiographic analyses have established distinct favorable and unfavorable characteristics, but further understanding of the molecular subgroups of medulloblastoma is required before significant progress is likely to be achieved. In particular, a better understanding of the downstream mechanisms involved in cell fate determination following inappropriate activation of Wnt and Shh pathways in medulloblastoma may lead to targeted approaches that enhance treatment efficacy. Murine models that more accurately recapitulate human disease are central to this goal, not only to elucidate mechanistic data, but also to test novel therapeutic agents. Further studies are also needed to characterize potential synergistic connections between TP53 mutation and the molecular pathogenesis of medulloblastoma. Finally, adult patients have drastically different patterns of presentation and prognosis than children, and genetic profiling indicates that age-related differences exist in the molecular pathogenesis of medulloblastoma.

**REFERENCES**


42. Haas-Kogan et al. (2014)


