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Molecular Diagnostics and Pathology of Major Brain Tumors

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Abstract

Tumors of central nervous system (CNS) account for a small portion of tumors of human body, which include tumors occurring in the parenchyma of brain and spinal cord as well as their coverings. The following chapter covers some new development in some major brain tumors in both pediatric and adult populations, as well as some uncommon but diagnostic and management challenging tumors.

Keywords: gliomas, astrocytomas, oligodendrogliomas, mixed oligoastrocytomas, WHO (World Health Organization), WHO grades, medulloblastomas (MBs), midline diffuse astrocytoma, diffuse intrinsic pontine gliomas (DIPG), hemangioblastomas (HMBs), metastatic renal cell carcinoma (RCC), formalin-fixed, paraffin-embedded (FFPE), H3 K27M mutation, immunohistochemical (IHC) stain, fluorescence in situ hybridization (FISH)

1. Introduction

Tumors of central nervous system (CNS) include the tumors of the brain and spinal cord, as well as their covers. Those tumors are uncommon tumors, accounting for approximately 1% of all human body tumors. They can be divided into primary or secondary/metastatic tumors, benign or malignant tumors, based on the WHO classification; brain tumors are assigned into four grades, from Grade 1 very benign tumor to Grade IV highly malignant tumors (see below). By location, those tumors can be divided into extra-axial tumors (outside brain/spinal cord parenchyma), such as meningiomas, and intra-axial tumors (inside brain/spinal cord parenchyma), such as gliomas. Diagnosis of brain tumors is primarily based on the WHO Classification of Tumors of CNS; this expert consensus scheme was first completed in 1979 and then revised in 1993, 2000, and 2016. This scheme is currently the most widely utilized by neuropathologists worldwide for typing and grading the CNS tumors [1]. Neoplasms, especially those malignant ones, are biologically characterized by noncontrolled tumor cell proliferation; this uncontrolled growth is best explained by recently discovered EGFR (epidermal growth factor receptor) mutations, which mutations result in uncontrolled signal transduction downward to nuclei without ligand binding to the receptor and led to unlimited cell proliferation.

During the last two decades, a lot of gene mutations are identified, especially in the oncology field, which has been helpful for the development of new generation of antitumor medication focusing on the mutated gene products. As a matter of fact, those target treatments have already archived tremendous successes in the oncology field. For example, gefitinib, erlotinib, and afatinib are the current targeted medications against EGFR-mutated non-small-cell lung cancers, which already show great clinical success.

The following chapter is going to review some development in brain tumors, especially the recent understanding of adult gliomas and pediatric medulloblastomas, as well as some other uncommon tumors for their molecular diagnosis and genetic subgrouping.

2. Molecular diagnosis of adult gliomas

Glial tumors comprise approximately 25–30% of primary CNS tumors [1] and represent a spectrum ranging from low-grade, benign to the highly aggressive, malignant tumors. They are broadly classified by glial cell type of origin and determined by histology with or without the use of immunohistochemistry (IHC), which is then used to provide a WHO grade (see **Table 1**) [1]; however, histology has not been able to accurately predict response to treatment or clinical outcomes, and it is not uncommon for many of these tumors with nearly identical histologic features to have very different outcomes. As a result of these observations, and like many malignancies (lung and colorectal carcinoma for example), there has been increasing interest in attempting to further classify these tumors based on their molecular expression. With that interest there is an increase in available published data regarding these molecular alterations and a subsequent increase in the availability of myriad testing modalities; some of which are now considered well established, while others are not. In an era of test utilization awareness and rising healthcare costs, this phenomenon frequently leads to confusion regarding which tests should be utilized, how those tests should be interpreted, and how they should be reported, in order to best guide treatment and predict outcomes in this patient population [2].

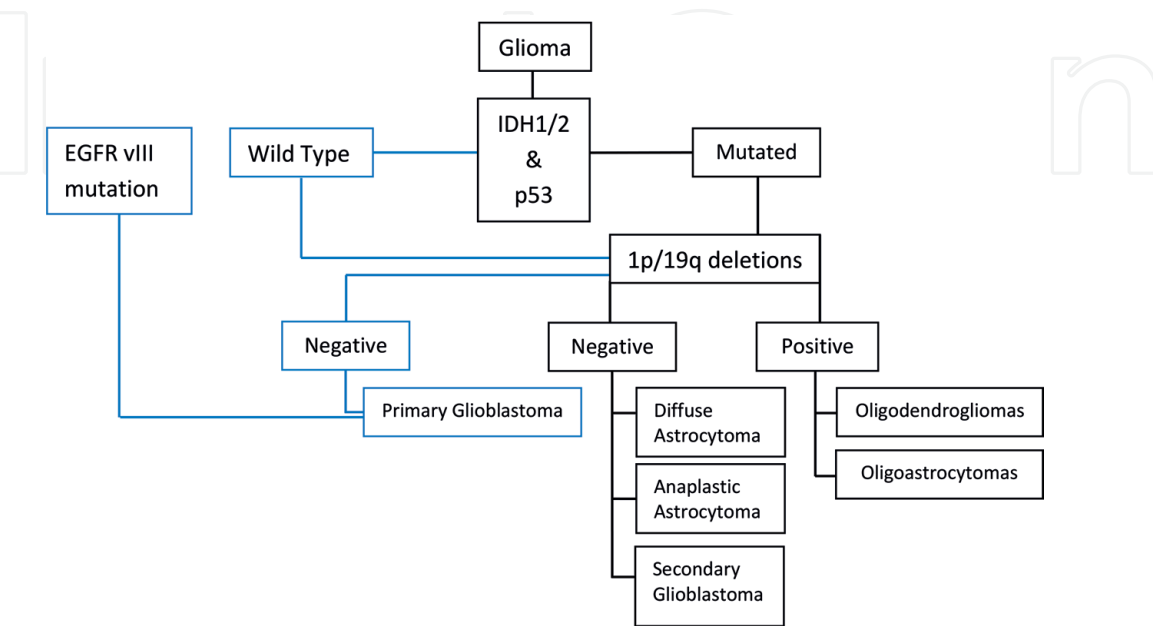


Table 1.
Molecular genetic map for the development of adult gliomas.

We will discuss here the well-established molecular concepts, touch briefly on the evolving molecular discoveries, and provide a testing algorithm (see **Table 1**).

Glioblastoma (GBM) is the most aggressive primary CNS malignancy, WHO Grade IV [1]. Despite decades of research and multiple new treatment modalities, little progress has been made in terms of substantial improvements of patients' outcomes, with the average long-term survival being measured in months rather than years [3]. However, this research has illuminated a complex series of molecular pathways and events that is improving our understanding of the pathophysiology of this aggressive entity.

2.1 LOH 10q

Loss of heterozygosity (LOH) at chromosome 10q occurs with high frequency in both primary and secondary GBMs, occurring in both in approximately 60–80% of cases [4]. Although this is an interesting primary event in the development of GBM of either type, because of its high frequency, it is not useful in distinguishing one from the other. Instead, GBM is currently subclassified by its molecular alterations into primary and secondary GBM, based on the presence or absence of IDH1/IDH2 and/or TP53 mutations [4]. EGFR status is also being increasingly used in this context.

2.2 IDH1/IDH2

Isocitrate dehydrogenase-1 (IDH1) is an NADP-dependent enzyme found in the cytoplasm responsible for the conversion of isocitrate to α -ketoglutarate and thereby producing NADPH, which reduces reactive oxygen species. IDH2 is similarly present in mitochondria. Their exact role in tumorigenesis is poorly understood; however, it is thought that mutations in this enzyme result in increased oxidative stress and subsequent carcinogenesis. It is therefore not surprising that this mutation is not found in primary GBMs, rather secondary GBMs that have progressed from a less aggressive tumor, and diffuse and anaplastic astrocytomas (WHO Grade II and III, respectively). IDH mutations are found with high frequency in the majority of astrocytomas, oligodendrogliomas, mixed gliomas, and secondary GBMs but not in pilocytic astrocytomas or primary GBMs. The most common mutation in IDH1 is a point mutation (arginine to histidine at codon 132), termed IDH1-R132H. IDH2 mutations (IDH2 172) represent only 3–5% of IDH mutations and are more commonly found in oligodendrogliomas. IDH mutations have also shown an association with hypoxia-inducible factor 1 alpha (HIF1), associated with upregulation of vascular endothelial growth factor (VEGF). Recent study indicates that low-grade astrocytomas with wild-type IDH1 behavior as glioblastoma clinically, suggesting again the importance of IDH1 mutation status in gliomas. IDH1 mutation can be detected by IHC, and commercially available mouse antihuman monoclonal antibody by Dianova with clone name H09 is a favored antibody to IDH1 R132H by most neuropathologists.

Secondary glioblastomas confer a significantly better prognosis than those arising de novo (primary GBM), and occur in a younger age group with a history of previous low grade gliomas [4]. Because primary and secondary GBMs cannot be distinguished morphologically, IDH1/2 mutation testing can be utilized for this task, allowing for better prognostication. IDH1/2 is commercially available as a reverse transcriptase PCR (RT-PCR) test. Although an IHC stain is available, it is not as sensitive to the less common variants of IDH1/2 mutation; however, it can be highly useful in the detection of single tumor cells in diffuse gliomas [5–7]. It is important to note that the role of IDH1/2 mutations in predicting response to therapy is still debated. It appears that

the mutation confers an increased response to radiation therapy, while others show an increased response with chemotherapy as well. Response appears to be multifactorial, dependent not only on the type of therapy, but also on the time of that therapy in relation to surgical resection [8]. Importantly, IDH mutations serve as a surrogate marker for secondary GBMs [9]. This testing should be performed in conjunction with TP53 and Ki67 on all GBMs, and considered standard of care.

2.3 P53

P53 is a cyclin-dependent kinase responsible for tumor suppression through prevention of cell replication. Mutations in p53 in malignant tumors are well established in the literature, with greater than 50% of cancers showing p53 loss of function mutations [10]. P53 is more commonly a missense mutation that results in accumulation of the protein in the cytoplasm, resulting in diffuse, strong nuclear staining by IHC; however, alternate mutations in p53 can show complete absence of staining or cytoplasmic staining only, whereas the wild type (unmutated) p53 will show weak to moderate, patchy positivity [11].

Most tumors that express p53 mutations typically have a more aggressive course than those that do not; however, this relationship has not been established in GBMs. Currently, no statistically significant difference has been established that GBMs with p53 mutations have a worse prognosis than those that do not [12]. The utility of p53 in GBMs, similar to IDH1/2 mutation status, is as additional evidence of a secondary GBM, rather than a primary GBM, as p53 mutations are far less frequent in primary GBMs, and, when present, likely represent secondary or late events associated with increasing genetic instability [4].

2.4 Epidermal growth factor receptor (EGFR)

EGFR is a member of the transmembrane tyrosine kinase receptor family that activates MAPK and PIK3 pathways resulting in cell proliferation. EGFR testing started to gain particular popularity due in part to the development of tyrosine kinase inhibitors (TKIs) and after a 2010 study by Verhaak et al. that attempted to further subclassify GBMs based on multiple molecular markers [15]. EGFR amplification confers more aggressive behavior and poorer outcomes, autophosphorylating the PIK3 pathway, leading to increased growth, angiogenesis, metastatic potential, and reduced apoptosis [16]. In contrast to secondary GBMs, epidermal growth factor receptor (EGFR) overexpression has been demonstrated in 36% of primary GBMs, with 70% of those showing amplification. It exists most commonly as the mutation EGFR variant 3 (EGFRvIII), which has deletion of exons 2 and 7 [17]. EGFRvIII mutation testing is performed with RT-PCR [17]. Unlike other malignancies where tyrosine kinase inhibitor (TKI) immunotherapy has shown wide successes, GBMs frequently do not respond, showed only a partial response, or develop rapid resistance to TKIs. This most often attributed to PTEN loss earlier in the EGFR pathway [4]. EGFR amplification or mutation in this context can be utilized, when present, as further support of a primary GBM over a secondary GBM.

In addition to the discovery of multiple molecular alterations in MET, PDGFRA, NF1, PTEN, PIK3 and CDKN2A/B, and several others, studies have discovered alterations of several microRNAs, which are also a field of current study. Importantly, none of these have been well established in terms of either their prognostic significance or their impact on treatment response, and several studies have shown contradictory results. This likely can be attributed to the marked heterogeneity of glial tumors, particularly GBMs. It is not currently recommended to add these markers to a broader profile until their clinical significance can be better established.

2.5 1p/19q co-deletion

Chromosome arms 1p and 19q deletions are the most characterized genetic aberrations of oligodendrogliomas, with up to 80% of classical oligodendrogliomas (WHO Grade II) and 60% of anaplastic oligodendrogliomas (WHO Grade III) [18, 19]. Although it seems unclear what impact these deletions have on cellular function, there are two identified roles for testing these deletions: the first is as a diagnostic marker for oligodendroglial tumors and the other as an indicator of response to treatment. One study demonstrated that the presence of complete or partial co-deletion of 1p and 19q conferred a significantly increased response to chemotherapy, and prolonged disease-free survival time, compared with those tumors with deletion of only one or the other chromosomal arm, regardless of histologic subtype [20], consistent with other studies, including mixed tumors. Due to the significant clinical implications for the presence of this gene, 1p/19q co-deletion testing should be performed on all glial tumors with or without oligodendroglial features since a small percentage (5%) of morphological astrocytomas are with 1p/19q co-deletion, which may confer a slightly better prognosis for the patients. Testing is available by both FISH and for LOH by real-time PCR. Evaluation of both chromosomal arms in their entirety is recommended due to molecular variability.

2.6 MGMT methylation

O⁶-methylguanine-DNA methyltransferase (MGMT) codes for MGMT repair protein, and methylation of this gene, which results in suppression and decreased expression of the MGMT protein, confers a significant survival benefit in patient treated with combined radiation and temozolomide therapy. MGMT methylation occurs in all types of gliomas and with frequency in primary and secondary GBMs and oligodendrogliomas (60–93%). In predicting a positive response to treatment, MGMT methylation also predicts an increased survival benefit. In lower-grade gliomas, MGMT methylation confers an increased response to radiation monotherapy, which is not well understood [9].

Methylation-specific PCR is the testing modality of choice and is widely available. An alternative is pyrosequencing, which shows high sensitivity, but is now less frequently used. Other testing modalities, such as western blot and IHC, have fallen into disuse due to issues with false-positive results.

Table 1 summarizes the current understanding of tumorigenesis for adult gliomas.

3. Molecular diagnosis of diffuse midline gliomas with H3 K27M mutation

It has been recognized for almost 20 years among pediatric neuro-oncologists that neuroimaging study defined diffuse intrinsic pontine gliomas (DIPG) had a very poor prognosis independent of histological grade (if biopsied). In that case, biopsy is most of the time considered unnecessary until recent identification of potential drug targets for individualized therapy has led to reevaluation of this approach [22]. Recent genomic analysis has demonstrated that specific genetic alterations drive distinct subsets of glial neoplasm of the central nervous system, dependent not only on tumor-type but also on the site of origin and patient age. Like this diffuse midline gliomas, with somatic mutations of the H3F3A and HIST1H3B gene encoding the histone H3 variants, H3.3 and H3.1, were recently identified in high-grade gliomas arising in the thalamus, pons, and spinal cord of

children and young adults; those tumors are named as diffuse midline gliomas with H3 K27M mutation [23].

Brainstem tumors affect primarily children and young adults. Each year, around 300–400 cases of brainstem tumors are diagnosed in the United States, and diffuse intrinsic pontine glioma (DIPG) accounts approximately 80% of these tumors [24]. DIPG has been recently categorized by WHO classification as high-grade (Grade IV) diffuse midline gliomas with H3 K27M mutation. It carries a poor prognosis, and only 1% of the patients live 5 years after diagnosis.

Clinically, diffuse midline gliomas result in brainstem dysfunction and the obstruction of cerebrospinal fluid (CSF) flow. The patients suffer from difficulty in ocular movements, weakness of facial muscles, sudden hearing problem, swallowing difficulty, muscle spasticity, clonus, and bladder dysfunction, along with multiple cranial neuropathies and ataxia.

Diagnosis of diffuse midline gliomas is initiated through imaging primarily by MRI scans indicating hypointense (T1) or hyperintense (T2) lesions, enhancing or non-enhancing after administration of contrast agents. Biopsy is a standard procedure for establishing the molecular and histopathological diagnoses. This tumor shows many histopathological features of glioblastoma such as pseudopalisading necrosis and microvascular proliferation, in addition to H3 K27M positive by immunohistochemical (IHC) stain (**Figure 1**).

Unlike many other adult gliomas, debulking surgery, with gross total resection (GTR) of the tumor, is not a treatment of choice for diffuse midline gliomas, mainly due to the location of the tumors. The brainstem regulates critical bodily functions, and therefore surgical resection without damaging the vital area of the brainstem is almost impossible. Surgery is indicated only for biopsy of the tumor and to relieve the hydrocephalus that may happen in a small fraction of cases. Currently, patients are treated primarily with radiation and adjuvant chemotherapy with temozolomide.

In diffuse midline gliomas with H3 K27 mutation, lysine in 27th position of tail of Histone 3.3 is replaced by methionine. Histones are the alkaline protein that provides a scaffold, around which DNA wraps. Solomon and colleague have observed that H3K37M mutation in pontine gliomas occurred at a much younger age (median 7 years of age) than gliomas of the thalamus (median age, 24 years) or spinal cord (median age, 25 years) [23]. The H3 K27M-positive gliomas have also been reported in adult in the brainstem [25]. High-grade gliomas with H3 K27M mutation may have additional mutations (WHO, 2016). These mutations are observed to occur in the critical genes, regulating cell divisions including cell cycle checkpoints and chromatin remodeling. The most frequent additional mutation noted is tumor suppressor p53, which is noted in an estimated half of the H3 K27M

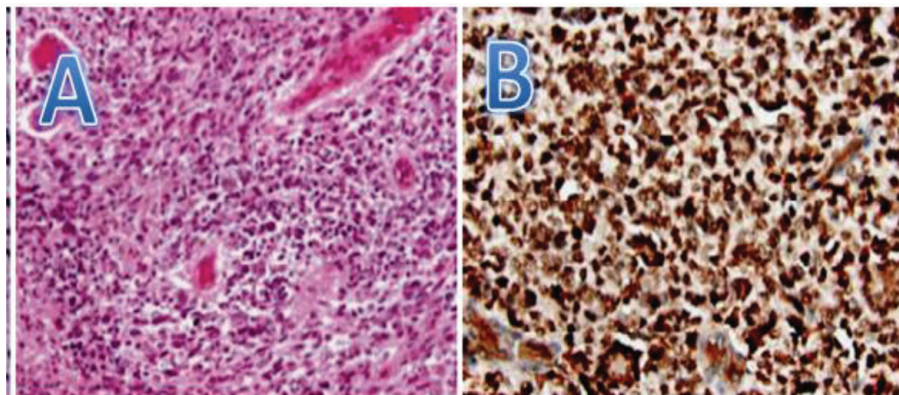


Figure 1.
(A) Grade IV midline glioblastoma and (B) IHC + for H3 K27M mutation.

midline gliomas. Amplification of platelet-derived growth factor alpha (PDGFRA), critical for cell survival and proliferation, is observed in about 30% of the H3 K27M tumors. Cyclin-dependent kinases 4 and 6 are reported to be amplified in 20% of the tumors, and homozygous deletion of cyclin-dependent kinase inhibitor 2A/2B is noted in 5% of the H3 K27M gliomas. Mutation of activin A receptor type-1 (AVCR1) is noted in 20% of H3 K27M gliomas. A mutation of protein phosphatase 1D (PPM1D) and amplification of MYC/PVT1 may present separately, in 15% of the H3 K27M gliomas [1]. In addition, histone H3 K27M mutation is found mutually exclusive with IDH1 mutation and EGFR amplification, rarely co-occurred with BRAF-V600E mutation, and was commonly associated with p53 overexpression, ATRX loss (except in pontine gliomas), and monosomy 10 [23].

These mutations could provide us with a better understanding of the disease process and could potentially lead to the development of a better treatment strategy for this deadly disease. As a matter of fact, at least two clinical trials are underway with small molecule inhibitor of the histone demethylase, which showed some promising result [23].

4. Molecular subgroups of medulloblastomas

Medulloblastoma is the most common malignant brain tumor of cerebellum in childhood, although it rarely happens in adult patients, too. It is an embryonal neuro-epithelial tumor arising in the cerebellum or dorsal brainstem, which is a major cause of morbidity and mortality in pediatric brain tumor patients [27, 28], and was designed as WHO Grade IV neoplasm [1]. Histologically, medulloblastoma is a prototypical embryonal tumor, consisting of densely packed small round undifferentiated blue tumor cells with mild to moderate nuclear pleomorphism and a high mitotic count, mostly with Homer-Wright rosettes, and shows different morphological variants, such as desmoplastic/nodular, large cell, and anaplastic, etc., with predominantly neuronal differentiation and tendency to metastasize via CSF pathways [1] (**Figures 2 and 3**).

4.1 Morphologic features of medulloblastomas

Several morphological variants of MBs are recognized, alongside the classic tumor: desmoplastic/nodular MB, MB with extensive nodularity, and large-cell/anaplastic MB. A dominant population of undifferentiated cells with a high nuclear-to-cytoplasmic ratio and active mitotic figures is a common feature [1] (**Figures 2 and 3**). Classic MB composed of sheets of undifferentiated small blue

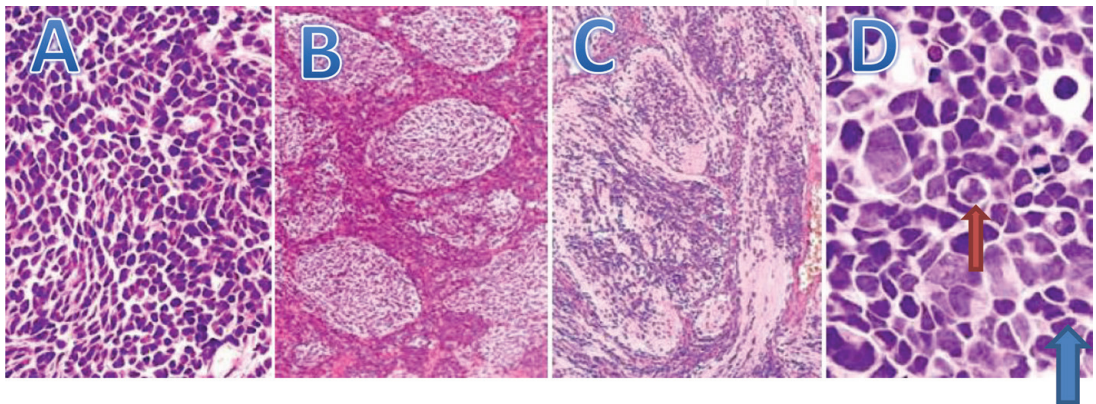


Figure 2.
Histopathology of MBs. (A) Classic type, 70%; (B) nodular, 10%; (C) extensive nodularity, 3%; and (D) large cell/anaplastic, 15% (arrow nuclear molding, blue; wrapping, red).

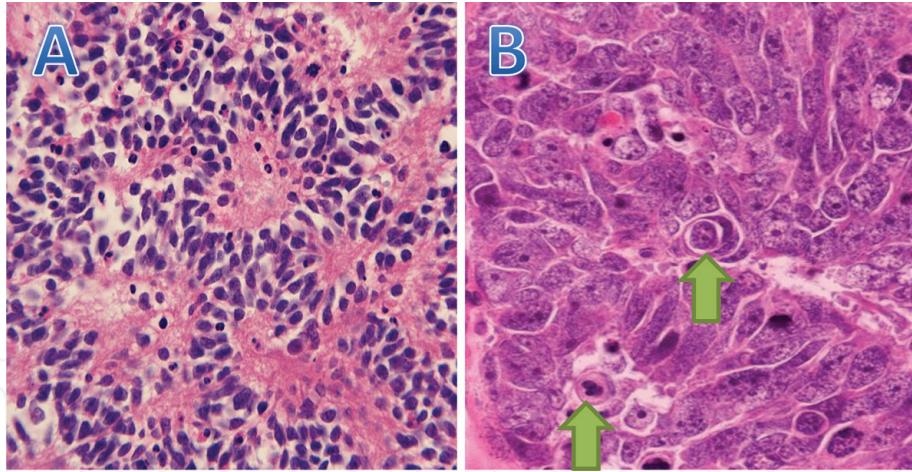


Figure 3. Histopathology of MBs. (A) Homer Wright rosettes and (B) nuclear wrapping (hugging), arrow, in large-cell/anaplastic MBs.

tumor cells with Homer-Wright rosette formations and/or palisading tumor cells forming a pseudoglandular feature, easily found mitoses and apoptosis. Other histological variants include desmoplastic MB, which contains abundant reticulin and collagen, characterized with nodular reticulin-free zones (pale islands). The nodules have reduced cellularity, a rarified fibrillar matrix and marked nuclear uniformity. A rare histologic type of MB is the so-called large-cell MB, which is composed completely or partially of cells with large, round nuclei and prominent nucleoli, commonly with large areas of necrosis. The large-cell MB sometimes resembles the rhabdoid/atypical teratoid (RT/AT) tumors of cerebellum, but its cytoplasm lacks globular hyaline inclusions and is diffusely reactive for synaptophysin, neurofilament protein, and vimentin and negative for epithelial membrane antigen, cytokeratins, and smooth-muscle actin by immunohistochemical (IHC) stains [1]. Most often associated with large-cell MB is anaplastic MB, which is characterized by angular, crowded, pleomorphic nuclei in large cells, sometimes with nuclear molding and wrapping (**Figure 3**), mitosis, and apoptosis, as well as prominent nucleoli. It has been noted for a long time that morphology of MBs was related to patient's prognosis and that those MBs with extensive nodularity are with better prognosis, while the large-cell/anaplastic MBs are usually associated with worse clinical outcomes (**Figure 1**).

Additional study indicates that poor survival outcome was significantly associated with chromosome 17p loss (loss of tumor suppressor) and high expression of oncogenes c-myc (MYCC) or N-myc (MYCN) [1].

Due to the highly heterogeneous nature of the MB, and complication caused by aggressive treatments, a more specific subgrouping of this tumor is becoming more and more important for clinical judgment.

Molecular studies from multiple groups around the world found that medulloblastoma is not a single disease but comprises a collection of clinically and molecularly diverse subgroups. Current consensus made in a 2010 meeting at Boston agrees that there are four principal subgroups of medulloblastomas [27, 28] termed as WNT, SHH, Group 3, and Group 4.

Two of these subgroups, characterized by either activated WNT or SHH signaling pathway, are thought to play prominent roles in the pathogenesis. Two other non-WNT/non-SHH groups are more closely related to each other and even produced additional different numbers of subgroups within these groups of MBs, pending additional evidence to further classify them [27, 28].

4.1.1 MB, WNT subgroup

The best known subgroup of the medulloblastoma is the WNT subgroup due to its very good long-term prognosis, compared to other subgroups. WNT indicates the wingless signaling pathway.

WNT medulloblastomas are characterized by upregulation of the canonical WNT signaling pathway, which results in translocation of β -catenin to the nucleus. About two-thirds harbor a CTNNB1 mutation. Mutations in other pathways, such as APC and AXIN1, have been reported in the absence of a CTNNB1 mutation but are much less frequent [27]. The extent of β -catenin nuclear immunoreactivity in these WNT pathway MBs always amounts to more than a third of the total tumor area and is clearly different from the situation where very few scattered β -catenin nucleopositive cells, representing less than 2% of tumor cells, are evident. Assays for CTNNB1 mutation and monosomy 6, which occurs in nearly all WNT pathway MBs, have helped to establish the status of tumors in these immunohistochemical categories [27]. There is a close association between a WNT pathway immunophenotype and CTNNB1 mutation or monosomy 6, predicting a good outcome for medulloblastomas with these genetic abnormalities [27].

More than 90% of WNT subgroup medulloblastoma patients achieved long-term survival, with those patients whose death is due to more complications of therapy or secondary tumors rather than due to recurrent WNT medulloblastomas. Germline mutations of the WNT pathway inhibit APC predispose to Turcot syndrome, which includes a proclivity to medulloblastoma; in addition, somatic mutations of CTNNB1 encoding β -catenin have been found in sporadic medulloblastomas [27]. These strong germline and somatic genetic data support an etiological role for canonical WNT signaling in the pathogenesis of this group of tumors and lead to the nomenclature of “WNT subgroup of medulloblastomas.”

Almost all WNT medulloblastomas have classic histology, which often described as having CTNNB1 mutations, with nuclear labeling for β -catenin by immunohistochemical stain, and monosomy 6 (deletion of one copy of chromosome 6 in tumor cells). Medulloblastomas with large-cell/anaplastic features have also been reported in the WNT subgroup, although they appear to maintain the excellent prognosis associated with the WNT subgroup. Which of monosomy 6, nuclear staining for β -catenin, mutation of CTNNB1, immunohistochemical staining for DKK1, or a transcriptional signature that clusters with other WNT tumors should be used as a gold standard for the diagnosis of WNT medulloblastomas awaits further validation on large cohorts of well-characterized medulloblastomas.

WNT-activated MBs account for approximately 10% of all MBs, most of them present in children aged between 7 and 14 years, but they can also occur in young adults. Genetically, besides CTNNB1, genes that are recurrently mutated in WNT-activated MBs include TP53, SMARCA4, and DDX3X [1].

4.1.2 MB, SHH subgroup with TP53 mutant

The SHH group of MBs are named after the sonic hedgehog signaling pathway. In large series of tumors, SHH-activated MBs tend to have similar transcriptome, methylome, and microRNA profiles. SHH pathway activation in TP53-mutant tumors is associated with amplification of GLI2, MYCN, or SHH. Mutations in PTCH1, SUFU, and SMO are genetically absent. Large-cell/anaplastic morphology and chromosome 17p loss are also common in SHH-activated and TP53-mutant tumors. Patterns of chromosome shattering known as chromothripsis are often present.

SHH-activated tumors account for approximately 30% of all MBs and originate from rhombic lip-derived cerebellar granule neuron precursors. SHH-activated and TP53-mutant MBs are rare and generally found in children aged 4–17 years. Clinical outcomes in patients with SHH-activated and TP53-mutant tumors are very poor [1].

4.1.3 MB, SHH subgroup, with TP53 wild type

SHH pathway activation in TP53 wild-type tumors can be associated with germline or somatic mutation in the negative regulations PTCH1 or SUFU, as well as activating somatic mutations in SMO or (rarely) amplification of GLI2. Desmoplastic/nodular MBs and MBs with extensive nodularity are always included in the SHH-activated group, but tumors with a SHH signaling pathway can also have a classic or large-cell/anaplastic morphology, particularly in older children. Patients with SHH-activated and TP53 wild-type MBs are generally children aged <4 years, adolescents, or young adults. In addition to genetic changes activating SHH signaling, mutations in DDX3X or KMT2D and amplification of MYCN or MYCL are sometimes seen, as are deletions of chromosomal arms 9q, 10q, and 14q. Clinical outcomes in patients with SHH-activated tumors are variable [1].

4.2 Epidemiology

Research data from 1973 to 2007 suggested MB incidence rates of 0.6 cases per 1 million children aged 1–9 years and 0.6 cases per 1 million adults aged >19 years. SHH-activated MBs in general show a bimodal age distribution, being most common in infants and young adults, with a male-female ratio of approximately 1.5:1. In contrast, SHH-activated and TP53-mutant tumors are generally found in children aged 4–17 years. In one study including 133 SHH-activated MBs, 28 patients (21%) had a TP53 mutation, and the median age of these patients was approximately 15 years [1].

4.2.1 Groups 3 and 4/non-WNT and non-SHH groups

Groups 3 and 4 MBs are usually called “the non-WNT/non-SHH groups.” They share some of the similarities in both clinical presentation and molecular profiling. Most tumors in these groups display classical histology. The large-cell/anaplastic and desmoplastic histologies are present but at a lower frequency. The age of onset is distributed in both groups with most patients are children; they are relatively uncommon in infants and adults. Although both groups have similar frequency of metastasis, Group 3 shows poor prognosis, while Group 4 shows intermediate prognosis. Non-WNT and non-SHH tumors account for approximately 60% of all MBs and typically have classic histopathological features [1].

One characteristic similarity between Groups 3 and 4 is both subgroups are enriched for expression of genes involved in photoreceptor differentiation, and they express high level of OTX2 and FOXP1B, well-known oncogenes of MB. However, Group 3 is distinguished by its enriched gene signatures functioning in cell cycle, protein biosynthesis, glutamate receptor signaling, and p38 mitogen-activated protein kinase (MAPK) pathway, while Group 4 is overrepresented by genes involved in neuronal differentiation, development, cytoskeleton organization, etc.

In addition, isochromosome 17q (I17q) represents the most common structural abnormality in Groups 3 and 4. Other chromosomal alteration identified in these subgroups includes gain of 7 and 18q and loss of 8 and 11q. The major difference

between these two groups is the enrichment of MYC amplification in Group 3, which is very rarely observed in Group 4, as well as in WNT and SHH. Another difference is the enrichment of chromosome X loss in Group 4, found in 80% female MBs in Group 4.

The signaling pathway or biological programs driving the tumorigenesis of Groups 3 and 4 still remain largely unknown, although some reports suggest that disruption of chromatin genes associated with histone methylation may be a critical event driving Groups 3 and 4 tumor developments.

4.3 Medulloblastoma molecular subgroups: immunophenotypes and histopathological associations

After multigroup extensive researches, the development and validation of immunohistochemical stains to define molecular subgroups of MBs finally archived, with 4 immunohistochemical staining marker identified in order to MBs subgrouping, which can be used in FFPE tissue and greatly improve the routine pathological diagnosis process for these types of tumors. Four immunostaining markers were selected for pathological subgrouping of MBs: they are β -catenin, GAB1, filamin A, and YAP1 [27].

4.3.1 SHH pathway MBs

Combined immunoreactivities for GAB1, filamin A, and YAP1, indicating a SHH profile, were found in 31% of MBs, including all desmoplastic tumors. Desmoplastic MBs constituted 54% of SHH pathway tumors, and classic and large-cell/anaplastic tumors contributed 29 and 17%, respectively. While non-desmoplastic SHH tumor generally showed widespread and strong immunoreactivities for GAB1, YAP1, and filamin A, all three types of desmoplastic tumors displayed stronger staining for these proteins within internodular regions. Immunoreactivities for filamin A and YAP1 in SHH tumors were always strong and generally widespread. This was not always the situation for GAB1 immunoreactivity; no more than weak cytoplasmic staining for GAB1 was seen in a few non-desmoplastic SHH tumors ($n = 6$). These tumors were all strongly immunopositive for filamin A and YAP1, which acted to confirm the SHH phenotype [27].

4.3.2 WNT pathway MBs

Antibodies to β -catenin for identifying WNT tumors effective on formalin-fixed and paraffin-embedded (FFPE) tissue are well established in the diagnostic laboratory [27]. Widespread intermediate or strong cytoplasmic β -catenin immunoreactivity was a feature of nearly all MBs in the series; very few showed only patchy weak cytoplasmic staining for this antigen. WNT pathway MBs were identified by nuclear, as well as cytoplasmic, immunoreactivity for β -catenin (**Figure 4**). WNT pathway MBs defined by these types of nuclear β -catenin immunoreactivity also express filamin A. Typically, this was patchy staining and less intense than that seen in SHH tumors. Strong and widespread nuclear immunoreactivity for YAP1 was also a feature of WNT pathway tumors. This distinctive combination of β -catenin, filamin A, and YAP1 immunoreactivities robustly confirmed the status of MBs in this molecular subgroup. WNT tumors contributed 14% of all MBs in this study. Nearly all WNT pathway MBs were classic tumors. Large-cell/anaplastic tumor ($n = 2$, 6%) was exceptional among WNT tumors, while desmoplastic MBs were not represented [27].

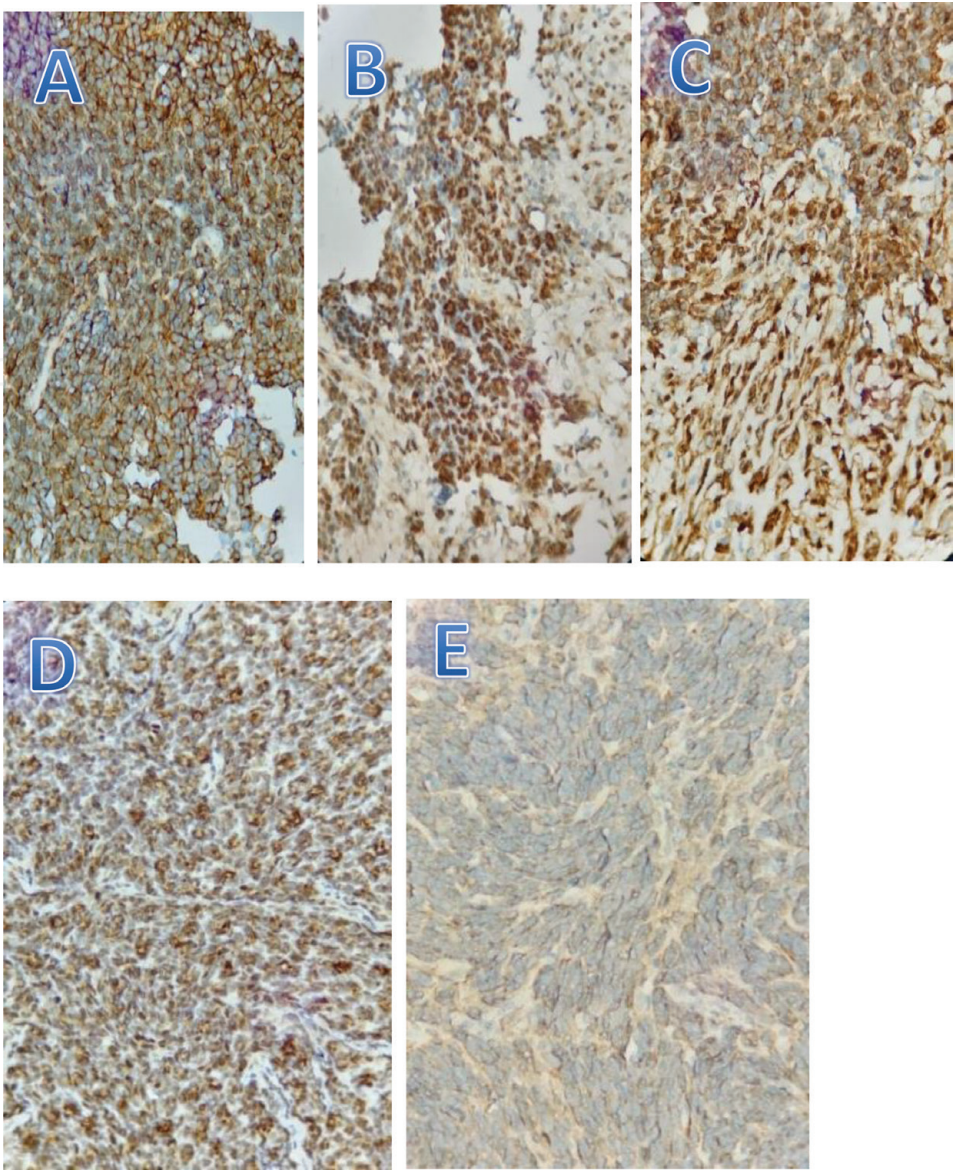


Figure 4.
β-Catenin IHC stain with both nuclear and cytoplasmic positive (A and C), GAB1 stain positive (B), YAP1 (D), and filamin A (E).

4.3.3 Non-SHH/WNT MBs

MBs (N = 130, 55%) falling outside the SHH and WNT categories displayed cytoplasmic, but not nuclear, immunoreactivity for β-catenin. Tumor cells were negative for GAB1 and YAP1. In general, tumors in this category were also immunonegative for filamin A, though very weak and patch immunoreactivity for this antigen was evident in rare non-SHH/WNT MBs (n = 9), which were classified as such on the basis of the panel of immunoreactivities. Intrinsic vascular elements were immunopositive for YAP1 and filamin A, providing an internal control. This subgroup of MBs was dominated by classic tumors (92%), including all non-desmoplastic nodular tumors and all but one MB that contained small clusters of densely packed neurocytic cells, the exception being a WNT tumor. Large-cell/anaplastic tumors made up the remainder (n = 11) [27].

4.3.4 Metastatic MBs

Despite four subgroups, metastatic MBs exist among all subgroups although the incidence of metastatic dissemination is higher in Group 3 and 4 than WNT and

IHC marker	IHC stain for MBs		
	WNT	SHH	Non-WNT/non-SHH
β -Catenin	N+, C+, ¼ focal	C+	C+
GAB1	Neg	C+	Neg
Filamin A	C+	C+	Neg
YAP1	N+, C+	N+, C+	Neg

N+, nuclear staining positive; C+, cytoplasmic staining positive; SHH, Sonic Hedgehog.

Table 2.
Subgrouping MBs by immunohistochemical stains.

SHH [33]. Metastatic MBs occur in approximately 40% of all MBs at diagnosis and are associated with poorer prognosis [34]. In 2001, McDonald et al. [35] identified potential therapeutic targets, e.g., PDGFR α PDGFR for metastatic MBs using expression array analysis. However, Gilbertson and Clifford [36] found that the probe McDonald used for PDGFR α was PDGFR β . They further demonstrated that PDGFR β is overexpressed in metastatic MB. Then, Kohane and his co-workers did an interesting experiment and found that genomically, human MBs were closest to mouse P (postnatal) 1-P10 cerebella, and normal human cerebella were closest to mouse P30-P60. Metastatic human MBs were highly associated with mouse P5 cerebella (non-metastatic human MB with mouse P7 cerebella). PDGFR α is highly expressed in P5; PDGFR β in P7 [37]. However, which isoform of PDGFRs plays a role in metastatic MBs kept controversial. Ten years later, we demonstrated that PDGFR α inhibits while PDGFR β promotes MB cell proliferation and cell survival as well as cell invasion [38], highlighting that PDGFR β may serve as a potential therapeutic target for metastatic MBs and warrants further investigation, including clinical studies.

Table 2 summarizes the IHC staining for subgroups of MBs.

5. Hemangioblastoma, metastatic renal cell carcinoma, and von Hippel-Lindau disease

Hemangioblastoma (HMB) is a benign, slow-growing, WHO grade I tumor, most likely occurs in cerebellum, brainstem, and spinal cord. Most hemangioblastomas are cystic on neuroimaging with intramural nodule. Histologically, the tumor has two major components, one is tightly packed capillary small vessels, and another is so-called stromal cells with low-grade nuclei, foamy cytoplasm, and no prominent nucleoli. Mitosis and necrosis are absent. But some degenerative features are often present [1].

On the other hand, cerebellum is a favorite location for metastatic renal cell carcinoma (RCC). Histologically, most RCC has clear cytoplasm with rich vascular supply, but slightly higher-grade nuclei mostly have small nucleoli.

Due to the similarity in histology and the same preference location, 70% of HMBs occur in sporadic forms, while approximately 30% of HMBs are associated with the inherited von Hippel-Lindau disease. The VHL tumor suppressor gene is inactivated both in VHL-associated cases and in most sporadic cases [1].

Von Hippel-Lindau disease (VHL) is a familial disorder predisposing patients to cysts and hypervascular neoplasm of multiple organs, including the CNS, eye, kidneys, adrenal medulla, pancreas, inner ear/temporal bone, and epididymis.

VHL is an autosomal dominant disorder, with roughly 20% of patients presenting as sporadic cases with no family history. The VHL tumor suppressor gene maps to chromosome 3p25 and includes three highly conserved exons [31].

VHL-associated disease includes [31] the following:

- Retinal hemangioblastomas (40–60%)
- CNS hemangioblastomas (60–80%)
- Endolymphatic sac tumor (2–11%)
- Pheochromocytomas (10–25%)
- Pancreatic cysts or islet tumors (60–80%)
- Renal cysts and RCCs (30–60%)
- Papillary cystadenomas of the epididymis (20–60%)

Solitary and especially multiple HMBs are diagnostic hallmarks of VHL. Roughly 75% are infratentorial, mainly involving the cerebellum. The rest of them are found

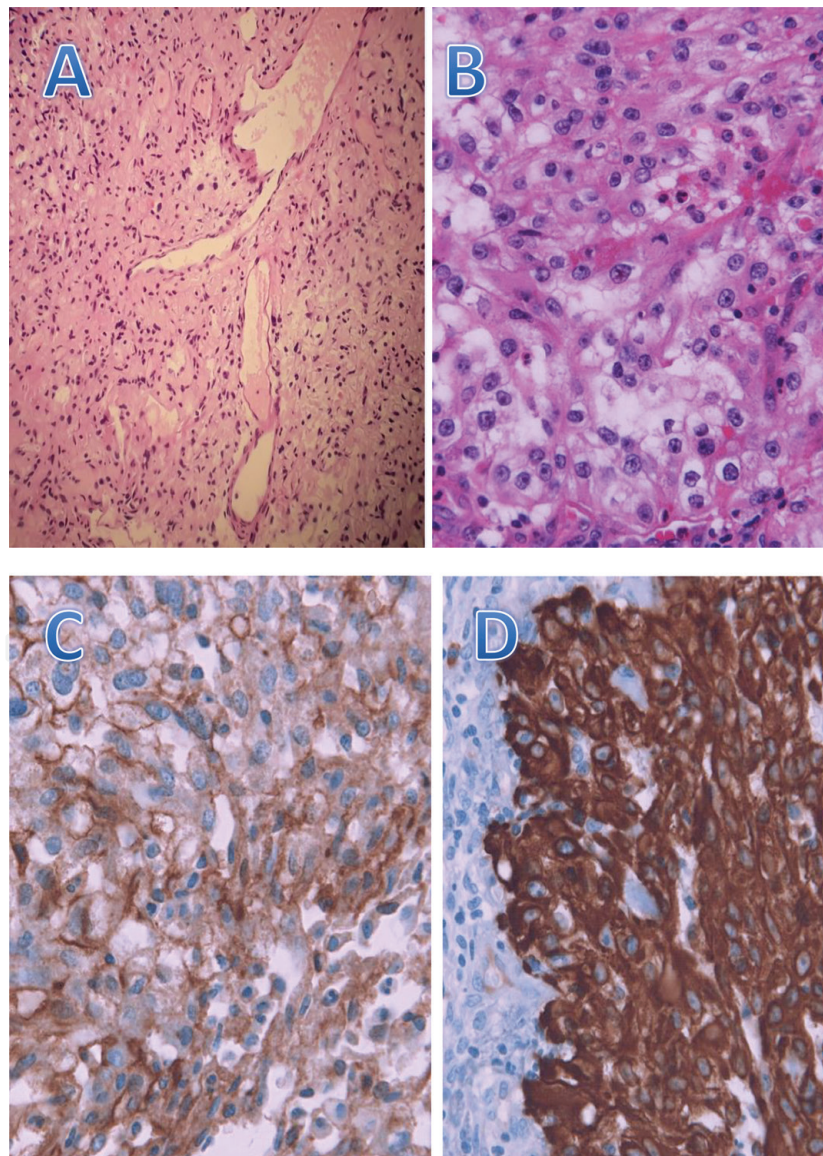


Figure 5. Hemangioblastoma, H&E stain $\times 200$, with low-grade nuclei and foamy stromal cells (A); metastatic RCC with clear cytoplasm, larger nuclei, and prominent nucleoli H&E stain $\times 400$ (B); and RCC is immunoreactive for CD10 (C) and cytokeratin (D).

in the spinal cord, brainstem, and lumbosacral nerve roots. Supratentorial HMBs are extremely rare. Of interest, only about 25–30% of cerebellar HMBs are seen in VHL patients, whereas this fraction rises to 80% in the spinal cord [1].

It may be those two tumors share the same chromosome locus of 3p25; they have some histological overlapping as well as the same preference of anatomic location (cerebellum); HMB and metastatic RCC are two tumors almost always request differentiation diagnosis, since one is benign and another is malignant, both carry different prognoses, and this two tumors become “forever differential diagnosis” for most diagnostic neuropathologists. Luckily, a simple small panel of immunohistochemical (IHC) stain would easily resolve this puzzle. HMB is negative for cytokeratin but positive for inhibin and 2D40, while RCC will be positive for cytokeratin, CD10, and PAX-8 and negative for inhibin [31] (**Figure 5**).

6. Summary

Research work in the last two decades discovered lots of genetic alterations in human brain tumors. More work will be done to further facilitate the diagnosis and classification. A recent proposal is suggested by using the epigenomics, like methylation status, to enhance brain tumor classification [32]. A new clinical trial with medication focusing on the IDH1 mutation is underway now; as more and more research data collected, we believe more effective treatment options will be developed in the near future. For a more detailed review on the molecular neuropathology of brain tumors, please refer to Ref. [39].

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