Review Article

Signaling pathway and molecular subgroups of medulloblastoma

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Abstract: Medulloblastoma (MB) is the most common malignant brain tumor in children. Although multimodality treatment regimens including surgery, radiotherapy and chemotherapy have greatly improved disease outcome, about one-third of MB patient remains incurable, and many long-term survivors are suffered from deleterious effects due to aggressive treatment. Understanding the signaling pathways and the genetic mechanisms contributed to MB development would be the key to develop novel therapeutic treatment strategies for improving survival and outcome of MB. In this review, we discuss the biological signaling pathways involved in MB pathogenesis. We also go through the current international consensus of four core MB subgroups namely, SHH, WNT, Group 3, and Group 4. This is adopted based on the knowledge of genomic complexity of MB as analyzed by recent high-throughput genomic technology. We talk about immunohistochemistry assays established to determine molecular subgroup affiliation. In the last part of review, we discuss how identification of molecular subgroups is going to change our routine disease diagnosis and clinical management.

Keywords: Medulloblastoma, signaling pathway, molecular subgroups

Introduction

Medulloblastoma (MB) is the most common malignant brain tumor of childhood and accounts for about 20% of all central nervous system (CNS) tumors in children [1-3]. It was first described by neurosurgeon Harvey Cushing and his associate Percival Bailey in 1925 as a tumor of primitive origin arising in the posterior fossa of young children. The current World Health organization (WHO) classification of MB, which was updated in 2007, recognizes five variants: classic MB, desmoplasic/nodular MB, MB with extensive nodularity (MBEN), large cell (LC) MB, and anaplastic (A) MB [4]. Majority of MB arise in the vermis of cerebellum and some occupy the fourth ventricle and brainstem. MB demonstrates a slight male predominance over female, with a gender ratio about 1.5:1. For a long time, MB has been stratified into high-risk and average-risk groups according to age, metastatic stage at diagnosis and extent of surgical resection [5]. The average-risk patients are those diagnosed at the age greater than three years, have non-metastases at presentation, and have manageable residual tumor (<1.5 cm²) post-operation. High-risk patients are those do not fulfill these criteria. Current multimodal treatment of MB has led to a five year overall survival about 90% with average risk and 70% for high risk [6]. However, five year disease-free survival remains low (36%) for patients with dissemination, and prognosis remains poor for patients with recurrent MB [7]. In addition, majority of survivors exhibit long-term neurocognitive and neuroendocrine complication as a result of therapy [8, 9].

During the past decades, research studies have greatly improved our understanding of MB oncogenesis through elucidation of several developmental signaling pathways, namely, sonic hedgehog (SHH), Wingless (WNT) and NOTCH. These pathways involve cell signaling receptors, intracellular second messengers, transcription factors, and gene regulation. Tight regulation of these signaling cascades is essential to normal cerebellum development. Dysregulation of these pathways has linked to MB tumorigenesis.
The surge of advancement in genome analysis technologies has accelerated our understanding of molecular basis of MB. MB is no longer considered as a single disease. Rather, it is comprised of at least four molecularly distinct subgroups. Each of them is characterized by discrete clinical presentation, demographic features, prognosis, expression profilings and genomic abnormalities. The identification of molecular subgroups has a great impact on clinical management, including patient stratification, treatment strategy, and design and implementation of targeted therapy. In this review, we will focus on the signaling pathways contributed to MB tumorigenesis and the updated molecular features identified in MB subgroups. In the last part, we will discuss future perspective of molecular subgroups and application of molecular subgroups in disease diagnosis and patient management.

Signaling pathway

SHH

Normal development of the cerebellum is regulated by a number of complex, hierarchical signal transduction pathways. The SHH pathway is a major of these [10]. Aberrant SHH pathway causes severe cerebellar development and MB [11]. The link between MB and SHH pathway was initiated from observation that patients with rare, hereditary condition known as Gorlin’s syndrome or nevoid basal cell carcinoma (NBCCS), are predisposed to multiple tumors including MB [12-15]. Genetic analysis has demonstrated that the gene PTCH of SHH pathway is mutated in these patients [15, 16]. Mutation of other players of SHH players including SMO (5%) and SUFU (9%) has also been identified MB patients [17, 18].

PTCH is a transmembrane receptor with 12 membrane-spanning domains and 2 extracellular loops [15, 19, 20]. In the absence of hedgehog (Hh), a secreted protein, PTCH represses the SHH pathway by inhibition of activity of SMO, a seven transmembrane G coupled protein [21]. Upon Hh binding, PTCH relieves the suppression of SMO on the membrane. SMO is then translocated into the cytoplasm where transcription factor GLI family zinc finger 1 (GLI1) activity is repressed by the antagonist suppressor of fused (SUFU) through an unidentified mechanism [22]. In return, GLI1 is modified and translocated from cytoplasm to nucleus [23]. The presence of GLI1 in nucleus would activate transcription of SHH targets, including oncogene MYCN and cyclin D1 (CCND1), and cyclin D2 (CCND2) [24-26].

A number of mouse models have revealed that activation of SHH pathway through manipulation of main effectors including Ptc, Smo, and Sufu would give rise to MB formation. First, mice homozygous for Ptc(−/−) died at embryonic stage, and 19% of hemizygous mice developed tumors closely resembled human MB within the first 25 weeks after birth [27]. Second, 58% of Sufu(+/−) and p53(−/−) mice developed MB [28]. Moreover, homozygous mice bearing the constitutive active form of Smo through point mutation in the transmembrane domain resulted in 94% incidence of MB formation by 2 months of age [29], and hemizygous mice developed MB at 48% incidence at a median age of 26 weeks [30]. Taken together, these models highlight the importance of dysregulation of SHH pathway in the contribution of MB tumorigenesis.

WNT

The first evidence demonstrating the involvement of WNT signaling pathway in MB came from genetic study of patients affected by Turcot syndrome, who have a 92-fold higher relative risk of developing MB than the general population. These patients carried a germ-line mutation of the adenomatous polyposis coli (APC) gene in the WNT pathway [31]. Subsequently, a small subset of sporadic MB was showed to harbor mutation of genes essential in WNT pathway. These included APC, β-catenin (CTNNB1), and axin 1 (AXIN1) [32-36].

Activation of WNT requires the interaction of Wingless (WNT) ligand. In the absence of ligand Wingless, the key downstream effector, CTNNB1, is undergone ubiquitination and degradation. This pathway is activated when the ligand binds to a receptor complex composed of a seven transmembrane Frizzled (FZ), serpentine receptor and low density lipoprotein receptor-related protein (LRP). This leads to phosphorylation of dishevelled (DVL), association with AXIN, and prevention of CTNNB1 phosphorylation by glycogen synthase kinase-3β (GSK-3β) [2]. The stabilized CTNNB1 is then
translocated to the nucleus where it interacts with transcription factors T-cell factor (TCF) and lymphoid enhancer factor (LEF) to activate transcription of targets genes such as MYC, JUN, FRA, AXIN2, and CCND1 [37-43].

Very recently, the mouse models of WNT MB have been established. Gibson and his colleagues demonstrated that 15% of Ctnnb1 and Tp53 double mutant mouse developed tumors resembled MB [44]. Furthermore, Rogers et al. showed that Myc immortalized cerebellar progenitor cells with activation of WNT pathway through stably expressed Wnt1 became tumorigenic and form tumors resembled classical MB in vivo [45].

**NOTCH**

At least 4 Notch receptors (NOTCH-1, -2, -3, and -4) have been identified in mammalian [46-50]. Notch is a single transmembrane protein which exists as a heterodimeric receptor [51]. The extracellular domain contains epidermal growth factor-like repeats, participates in ligand binding and prevents signaling in the absence of ligand binding. The cytoplasmic domain contains a RAM domain, six ankyrin (also known as CDC10) repeats, two nuclear-localization signals, a transcription transactivation domain (TAD) and a PEST sequence. Upon binding to ligands Jagged (JAG-1, JAG-2) and Delta-like (DLL-1, DLL-2, DLL-3) family members, a cascade of proteolytic cleavage is initiated, and the soluble Notch intracellular domain (NCI) is released and translocated to the nucleus. The NCI interacts with DNA binding proteins (CBF1) to activate transcription of downstream target genes such as HES1 and HES5 [52].

In MB, NOTCH1 and NOTCH2 have opposite biological effects. NOTCH1 inhibits proliferation of MB whereas NOTCH2 promotes cell growth of MB [53]. Moreover, expression of NOTCH1 is not detectable whereas NOTCH2 is overexpressed in MB [53, 54].

**Our understanding of MB heterogeneity**

Prior to the early 1990’s, MB was regarded as a single disease. Then, Giangaspero and associates recognized that “large cell” MB as a distinct, aggressive group of MB [55]. These tumors display large vesicular nuclei with prominent nucleoli appearance, show frequent amplification of oncogene c-myc and isochromosome 17q, and are associated with adverse clinical outcome due to cerebrospinal fluid dissemination [55]. This “subgroup” and “stratification” concepts were confirmed and expanded by several groups of investigators. In year 2000, Brown and his colleagues analyzed a large cohort of study comprised of 495 MB tumors, and showed that large cell/anaplastic MB are featured with distinct histological and cytogenetic characters [56]. Further, Lamont et al. demonstrated combined histopathological and molecular abnormalities would stratify MB patients. The group of patients with large cell/anaplastic histology and chromosome 17p loss had a shorter overall survival than patients without these characteristics [57]. Later, chromosome 17 alteration was demonstrated as a biological marker to stratify clinical outcome [58]. Further investigation illustrated that a “subgroup” of MB harbored p53 mutation displayed poor overall survival [59]. In contrast, a “subgroup” of MB with chromosome 6 loss showed favorable clinical outcome [60]. These accumulative data showing the diversity of clinical presentation and genetic abnormalities among MB patients prompt for a hypothesis that MB is a heterogeneous disease comprised of various subgroups with distinct histological features, molecular profiles, and clinical behaviors.

However, it is only recently that high-throughput, robust, integrative studies using gene expression profiling, array comparative genomic hybridization (aCGH), and single nucleotide polymorphism (SNP) array allow researchers to reinforce this idea. The researchers working in this field have come to a consensus that MB can be classified into four core subgroups: WNT, SHH, Group 3 and Group 4 [61]. Each of these subtypes has distinct molecular profile and genomic defects. The clinical parameters and patient outcomes are vary among subgroups. In addition, the introduction of whole genome sequencing has expanded our knowledge of subgroups. The followings will summarize the current known characteristics of each subgroup.

**SHH**

SHH subgroup comprises approximately 25-30% of MB [62-64]. SHH subgroup is characterized by the high frequency of desmoplastic histology (40%) although other variants are
Medulloblastoma, signaling pathway, molecular subgroups

found in this subgroup [62-65]. The age distribution of SHH subgroups displays a bimodal shape, with majority of SHH are found in infant under the age of 3 or adult above age 16 [65]. SHH subgroup comprises of half of the adult MB [66]. The prognosis of SHH tumors is good in infant and intermediate in adult [67].

Regulators and target genes of SHH signaling pathway are overexpressed in SHH subgroup as revealed by global gene expression profilings [68-70]. At the chromosomal level, loss of 9q is the most frequent abnormality in SHH, accounting for 21-47% [63, 65, 68, 69]. Other chromosomal aberrations found in this group of tumors include gain of chromosome 3q and 9p and loss of chromosome 10q, 20p and 21p [63, 71].

Very recently, Northcott and his colleagues describe multiple focal somatic copy number aberration (SCNA) in SHH tumors. They have showed genes of SHH signaling pathway are genomically altered. In addition, they report that SHH tumors harbor amplification and loss of genes associated with p53 and PI3K signaling pathways. These genomic aberrations are only restricted in SHH tumors [72]. The results point out that TP53 signaling and PI3K signaling may cooperate with SHH pathway to contribute the MB formation or sub-groups of SHH tumors are present in SHH tumors.

The hypothesis of the presence of sub-groups within SHH tumors is also supported by the observation that infant and adult SHH can be distinguished by different clinical and transcription profile [71]. Infant SHH is characterized by the upregulation of genes functioning in neuronal development. In contrast, adult SHH shows elevation of members of the homeobox (HOX) family. Nearly 80% of infant SHH tumor is located in vermis, whereas majority of adult SHH tumor is found in cerebellar hemispheres. The ongoing genomic studies will soon provide further evidence to illustrate the existence of SHH-subgroup heterogeneity.

WNT

WNT subgroup accounts for about 10-15% MB [62, 64, 65, 69]. This subgroup is characterized by classic histology, age above 3, good prognosis, and infrequent metastasis at presentation [65, 68, 70, 73]. More than 90% of WNT tumors display classic histology, and few show LC/A histology [62, 65, 66, 74]. Almost all WNT tumors are found in children and adult and rarely do these tumors find in infant [68, 69, 75].

Early studies by global gene expression array, aCGH, and mutational analysis have demonstrated the enrichment of genes of WNT signaling pathway, CTNNB1 mutation, and loss of chromosome 6 in WNT subgroup [68, 70, 75]. Few studies have showed that all WNT tumors harbor CTNNB1 mutation [6, 62]. Other studies demonstrate CTNNB1 mutation in 70-90% WNT tumors [64, 66, 72]. Complete or partial loss of chromosome 6 is accounted for approximately 90% of WNT tumors [62, 64, 65]. Interestingly, other than chromosome 6 loss, WNT tumors display only few, recurrent chromosomal aberration as compared with other subgroups [64, 72].

Whole genome sequencing has indicated that WNT tumors have a relatively high mutation rate compared to other subgroups, and gene mutation is appeared in a WNT-enriched manner [62, 64, 72, 74]. For instance, mutation for DDX3X is identified in greater than 50% of WNT. Such mutation is not found in Groups 3 and 4, and less than 10% SHH tumors harbor DDX3X mutation [62, 64]. DDX3X encodes a DEAD-box RNA helicase which displays RNA-dependent ATPase and ATP-dependent RNA helicase activities [76]. DDX3X is participated in mRNA splicing and processing, translational control, chromosome segregation, cell cycle regulation and cancer progression [76-78]. Mutations of SMARCA4, CREBBP, TRRAP, and MED13 have also been described in WNT tumors [64]. Interestingly, all of them participate in regulation of gene expression by remodeling the chromatin structure. These novel findings suggest that development of WNT MB may require additional factors in addition to the activation of WNT signaling pathway.

Groups 3 and 4

Groups 3 and 4 were originally so called the non-WNT/non-SHH groups. They share some of the similarities in both clinical presentation and molecular profiling. Most tumors in these group display classical histology. LC/A and desmoplastic histologies are present but at a lower frequency [62, 64, 65, 69]. The age of onset is distributed in both groups with most cases are
Medulloblastoma, signaling pathway, molecular subgroups

Early transcriptome profiling study indicates that these two subgroups are more similar to each other, and some characteristics of Group 3 are also observed in Group 4 [69]. For instance, both subgroups are enriched for expression of genes involved in photoreceptor differentiation [68, 75]. Furthermore, they express high level of OTX2 and FOXG1B, well-known oncogenes of MB [69]. Nevertheless, Group 3 is distinguished by its enriched gene signatures functioned in cell cycle, protein biosynthesis, glutamate receptor signaling, and p38 mitogen-activated protein kinase (MAPK) pathway. In contrast, Group 4 is overrepresented by genes involved in neuronal differentiation, neuronal development, cytoskeleton organization and biogenesis or vesicle mediated transport [68, 69].

Isochromosome 17q (I17q) represents the most common structural abnormality in Groups 3 and Group 4, with higher incidence observed in Group 4 than in Group 3 (66% vs 26%) [62, 69, 75]. Other chromosomal aberration events identified in these subgroups include gain of 7 and 18q and loss of 8 and 11p [65, 68, 69, 79]. A main difference between Groups 3 and 4 is the enrichment of MYC amplification in Group 3, a feature very rarely observed in Group 4, as well as WNT and SHH [62, 64, 69]. MYC amplification is observed in approximately 15% of Group 3 [62, 64, 65, 68, 69, 72, 75]. Another difference is the enrichment of chromosome X loss in Group 4, which is observed at a frequency of 80% in female with Group 4 [61, 68].

Signaling pathways or biological programs driving the pathogenesis of Groups 3 and 4 still remain largely unknown. Two latest studies illustrate that genes regulated H3K27me3 (histone H3 lysine K27 trimethylation) are recurrently dysregulated in Groups 3 and 4, but not in SHH and WNT [62, 64]. Methylation of H3K27 is associated with gene repression in many developmental processes [80]. The methyltransferase EZH2, which is a component of Polycomb complex PRC2, plays a role in maintenance of H3K27me3 mark [81]. The demethylases, lysine (K)-specific demethylase 6A (KDM6A) and lysine (K)-specific demethylase 6B (KDM6B), act on H3K27 to derepress gene silencing [81]. Through whole genome sequencing, mutation of KDM6A in Groups 3 and 4 has been identified [62]. Mutations of other KDM family members (KDM1A, 3A, 4C, 5A, 5B, AND 7A) and proteins participated in H3K27me3 epigenetic mark (CDH7 and ZMYM3) have also been described and they are observed exclusively in Groups 3 and 4 [64]. Furthermore, EZH2 overexpression and gain of 7q, where EZH2 is located, were significantly enriched in these subgroups [64]. These results suggest that disruption of chromatin genes associated with histone methylation may be a critical event driving Group 3 as well as Group 4 tumor development.

Development of subgroup affiliation assays

With the establishment of molecular subgroups of MB, our next major challenge is the development of accurate subgroup affiliation assay. An optimal assay needs to be rapid and robust. In addition, it can be applied on common diagnostic materials, such as paraffin-embedded tissue. At present, two research groups have established immunohistochemical method for identification of molecular subgroups.

The first proposed subgroup affiliation assay involves immunohistochemical staining of a panel of antibodies. These include CTNNB1 or DKK1 for WNT, SFRP1 for SHH, NPR3 for Group 3, and KCNA1 for Group 4 [66, 69, 82]. These antibodies are introduced based on subgroup-specific signature genes found in global gene

### Table 1. Immunostaining patterns of four antibodies for subgroup affiliation

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>WNT</th>
<th>SHH</th>
<th>Groups 3 and 4</th>
</tr>
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<tbody>
<tr>
<td>CTNNB1</td>
<td>nuclear and cytoplasmic</td>
<td>cytoplasmic</td>
<td>cytoplasmic</td>
</tr>
<tr>
<td>FilaminA</td>
<td>cytoplasmic</td>
<td>cytoplasmic</td>
<td>negative</td>
</tr>
<tr>
<td>GAB1</td>
<td>negative</td>
<td>cytoplasmic</td>
<td>negative</td>
</tr>
<tr>
<td>YAP1</td>
<td>nuclear and cytoplasmic</td>
<td>nuclear and cytoplasmic</td>
<td>negative</td>
</tr>
</tbody>
</table>

Medulloblastoma, signaling pathway, molecular subgroups

The WHO classification of MB have been undergone several editions (year 1993, 2000, and 2007), and the changes are reflection of our understanding and perception of MB pathogenesis. For instance, MB was separated from PNET entity in year 2000 partly because of the recognition of the differences in morphology and cytology features between these two diseases. Given the facts that MB is now recognized as a heterogeneity disease with multiple subgroups featuring with unique genetic abnormalities and clinical presentation, and advance techniques and platforms are made available to expand our knowledge of molecular subgroups of MB, it is not difficult to anticipate that disease diagnosis of MB is going to change, from heavily dependent on microscopic morphology to a combination of morphology supplementary with genetic identity. The expand-

expression microarray [69]. Northcott and his colleagues have demonstrated that 98% of samples stained positive for one antibody, suggesting a high specificity.

Ellison et al. have also described another set of immunohistochemistry markers, namely, GAB1, CTNNB1, filamin A, and YAP1, for identification of WNT, SHH, and non-WNT/non-SHH [73]. Table 1 summarizes the immunoreactivity of the panel of antibodies recommended by Ellison and his colleagues. WNT tumors show strong nuclear and cytoplasmic CTNNB1 staining (Figure 1A). These tumors are immunoreacted to filaminA and YAP1, but not GAB1. SHH tumors display cytoplasmic staining of CTNNB1, and exhibit positive immunostaining for filaminA (Figure 1B), GAB1, and YAP1 (Figure 1C). Non-WNT/non-SHH tumors are only present with cytoplasmic CTNNB1 staining, and they are immunonegative for filaminA, GAB1, and YAP1.

Perspectives of MB subgrouping and clinical management

The WHO classification of MB have been undergone several editions (year 1993, 2000, and 2007), and the changes are reflection of our understanding and perception of MB pathogenesis. For instance, MB was separated from PNET entity in year 2000 partly because of the recognition of the differences in morphology and cytology features between these two diseases. Given the facts that MB is now recognized as a heterogeneity disease with multiple subgroups featuring with unique genetic abnormalities and clinical presentation, and advance techniques and platforms are made available to expand our knowledge of molecular subgroups of MB, it is not difficult to anticipate that disease diagnosis of MB is going to change, from heavily dependent on microscopic morphology to a combination of morphology supplementary with genetic identity. The expand-
Medulloblastoma, signaling pathway, molecular subgroups

of SHH, WNT, and NOTCH signaling pathways are now known to contribute to MB development. The advancement in high-throughput technology has led to an understanding that MB is a heterogeneous disease comprised of multiple molecular subgroups with differential gene signatures, distinct genetic abnormalities, and various clinical outcomes. Our knowledge potentiates change in MB classification, development of diagnostic test for subgroup assignment, and improvement in treatment strategy for MB patients. We hope that in the near future MB patients will be evaluated for molecular subgroups, and tailored-made treatment will be given based on the results of these molecular testing.

Disclosure of conflict of interest

All authors have no conflict of interest.

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medulloblastoma, signaling pathway, molecular subgroups


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Medulloblastoma, signaling pathway, molecular subgroups


Medulloblastoma, signaling pathway, molecular subgroups


