Molecular markers in pediatric neuro-oncology

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Pediatric molecular neuro-oncology is a fast developing field. A multitude of molecular profiling studies in recent years has unveiled a number of genetic abnormalities unique to pediatric brain tumors. It has now become clear that brain tumors that arise in children have distinct pathogenesis and biology, compared with their adult counterparts, even for those with indistinguishable histopathology. Some of the molecular features are so specific to a particular type of tumors, such as the presence of the KIAA1549-BRAF fusion gene for pilocytic astrocytomas or SMARCB1 mutations for atypical teratoid/rhabdoid tumors, that they could practically serve as a diagnostic marker on their own. Expression profiling has resolved the existence of 4 molecular subgroups in medulloblastomas, which positively translated into improved prognostication for the patients. The currently available molecular markers, however, do not cover all tumors even within a single tumor entity. The molecular pathogenesis of a large number of pediatric brain tumors is still unaccounted for, and the hierarchy of tumors is likely to be more complex and intricate than currently acknowledged. One of the main tasks of future molecular analyses in pediatric neuro-oncology, including the ongoing genome sequencing efforts, is to elucidate the biological basis of those orphan tumors. The ultimate goal of molecular diagnostics is to accurately predict the clinical and biological behavior of any tumor by means of their molecular characteristics, which is hoped to eventually pave the way for individualized treatment.

Keywords: astrocytomas, intracranial germ cell tumors, medulloblastomas.

Introduction

Brain tumors are the most common solid malignancy in children. Over the past few years, we have witnessed a dramatic development in the field of pediatric molecular neuro-oncology, largely because of the emergence of new technologies that allow unbiased, high-throughput, genome-wide analysis, most notably, next-generation sequencing. We now know that some pediatric brain tumors have molecular features so unique compared with their adult counterparts that they perhaps should belong to a separate entity. These features may serve as diagnostic or prognostic marker or even as potential targets for novel therapy. This review attempts to summarize current knowledge, rather than to provide a comprehensive list, of the molecular markers that may be used for diagnosis and/or predicting therapy response or prognosis in the brain tumors that commonly occur in children. Typical genetic/chromosomal/expression markers are discussed for a selected set of major pediatric brain tumors divided into the following 3 categories: gliomas, embryonal tumors, and germ cell tumors. Commonly found abnormalities in each tumor type (i.e., molecular markers), as discussed in the text, are listed in Table 1.

Gliomas

Pilocytic Astrocytomas

Pilocytic astrocytoma (PA) is the most common central nervous system tumor in children (17.7% among patients <14 years of age). PAs typically arise in the cerebellum (67%) and in the optic nerve and the hypothalamic/chaismatic region, but they may also occur in the cerebral hemispheres, brain stem, or even the spinal cord. They are generally slow-growing and macroscopically well-circumscribed, although they may microscopically permeate into surrounding tissues and almost never show malignant progression.
Unlike other gliomas, PAs may be surgically curable when completely removed. The prognosis of PA is good, with a 10-year overall survival of around 90%. As such, PA is classified as World Health Organization (WHO) grade I. Despite the distinct clinical features, the histopathological diagnosis of PA can sometimes be challenging to make. This is because PAs show a wide variety of morphology and may share some histopathological features with other gliomas of higher malignancy grades, including glioblastoma, such as mitosis, endothelial proliferation, and necrosis. It is crucial to distinguish PA from astrocytomas of higher malignancy, because the latter may require more aggressive adjuvant therapy. Thus, a specific molecular marker for PA would be clinically highly valuable.

Table 1. Molecular markers of pediatric brain tumors

<table>
<thead>
<tr>
<th>Tumor types</th>
<th>Histopathological Diagnosis</th>
<th>Subgroup</th>
<th>WHO Grades</th>
<th>Molecular Markers</th>
</tr>
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<tbody>
<tr>
<td>Astrocytic tumor</td>
<td>Pilocytic astrocytoma</td>
<td>cerebellar/optic/brain stem</td>
<td>I</td>
<td>KIAA1549-BRAF fusion</td>
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<tr>
<td></td>
<td></td>
<td>cerebral/diencephalic</td>
<td>I</td>
<td>BRAF mut</td>
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<tr>
<td>Diffuse astrocytoma</td>
<td>Adult*</td>
<td></td>
<td>II</td>
<td>IDH1/IDH2 mut, TP53 mut, RB1 mut, PTEN mut, EGFR amp</td>
</tr>
<tr>
<td></td>
<td>Pediatric</td>
<td></td>
<td>II</td>
<td>BRAF mut</td>
</tr>
<tr>
<td>Pleomorphic</td>
<td>Xanthoastrocytoma</td>
<td></td>
<td>II</td>
<td>BRAF mut</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>Adult*</td>
<td></td>
<td>IV</td>
<td>CDKN2A HD, TP53 mut, RB1 mut, PTEN mut, EGFR amp, 9p loss</td>
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<td></td>
<td>Pediatric, DIPG</td>
<td></td>
<td>IV</td>
<td>H3F3A mut, ATRX mut, DAXX mut, ADAM3A HD, PDGFRA amp/mut</td>
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<tr>
<td>Neuronal tumor</td>
<td>Ganglioglioma</td>
<td></td>
<td>I</td>
<td>BRAF mut</td>
</tr>
<tr>
<td>Epithelial tumor</td>
<td>Ependymoma</td>
<td>Posterior fossa Group A</td>
<td>II-III</td>
<td>1q gain</td>
</tr>
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<td></td>
<td>Ependymoma</td>
<td>Posterior fossa Group B</td>
<td>II</td>
<td>6q loss, 22q loss, 9q gain, 15q gain</td>
</tr>
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<td>Embryonal tumor</td>
<td>Medulloblastoma</td>
<td>Wnt</td>
<td>IV</td>
<td>CTNNB1 mut, MLL2/MLL3 mut, SMARCA4 mut, DDX3X mut</td>
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<tr>
<td></td>
<td></td>
<td>Shh</td>
<td>IV</td>
<td>PTCH1 mut, SUFU mut, GLI amp, MYCN amp</td>
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<tr>
<td></td>
<td></td>
<td>Group 3</td>
<td>IV</td>
<td>SMARCA4 mut, MYC amp i17q, 5q loss, 10q loss, 1q gain</td>
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<tr>
<td></td>
<td></td>
<td>Group 4</td>
<td>IV</td>
<td>KDM6A mut i17q</td>
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<td>PNET</td>
<td></td>
<td></td>
<td>IV</td>
<td>IDH1 mut, CDKN2A HD, PDGFRA amp</td>
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<tr>
<td>ETANTR</td>
<td></td>
<td></td>
<td>IV</td>
<td>miRNA372-373 amp</td>
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<td>AT/RT</td>
<td></td>
<td></td>
<td>IV</td>
<td>SMARCB1 mut, SMARCA4 mut</td>
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<tr>
<td>Germinal cell tumor</td>
<td>Germinoma</td>
<td>KIT mut</td>
<td>I2p</td>
<td>Trisomy X KIT</td>
</tr>
</tbody>
</table>

The table is meant to provide an overview and for this purpose only the most typical findings for each tumor type are listed. For gene nomenclature, see text. PNET, Primitive neuroectodermal tumor; ETANTR, Embryonal tumor with abundant neuropil and true rosettes; AT/RT, Atypical teratoid/rhabdoid tumor; DIPG, Diffuse intrinsic pontine glioma. *Although adult tumors are not described in the text, they are included in the table for comparison to pediatric tumors. For more details on molecular markers in adult tumors, see Riemenschneider et al. Acta Ne neuropathol 120:567–584, 2010. Abbreviations: mut, mutation; HD, homozygous deletion; amp, amplification; i17q, isochromosome 17q; i12p, isochromosome 12p;
One such specific marker for PA is the KIAA1549-BRAF (v-raf murine sarcoma viral oncogene homolog B1) fusion gene (BRAF fusion). This fusion gene is generated by the tandem duplication of a region approximately 2 Mb in size at 7q34 that spans from KIAA1459 to BRAF. The tandem duplication transposes the 5' half of KIAA1549 with the 3' half of BRAF in frame, resulting in a fusion gene expressed under the KIAA1549 promoter.4,5 The BRAF fusion gene is highly specific to PA.4–6 It is found in 60%–80% of PA, whereas only very infrequently, if at all, in any other type of intracranial tumors. Its presence therefore constitutes a very useful diagnostic marker for PA that predicts good prognosis. The fusion event can directly be evidenced by amplifying the fusion transcripts using reverse-transcriptase polymerase chain reaction (PCR);4 however, in the clinical setting, it would likely be more practical to instead demonstrate the presence of a tandem duplication by using interphase FISH.5

Although the tandemly duplicated regions at 7q34 are almost identical at the chromosomal level in all tumors with the fusion gene, the nucleotide positions of the break points greatly vary. As a result, the exonic composition of the fusion gene shows some diversity. The 3 most common types of the fusion gene consist of KIAA1549 exons 1–16 and BRAF exons 9–18 (K:B16.9), which comprises approximately 60% of all KIAA1549-BRAF genes, followed by K:B15.9 accounting for about 30%, and K:B16.11 with about 10%.7 Other rare variants include K:B18.10 and K:B19.5.5 All of them represent in frame fusions of KIAA1549 to BRAF, retaining the entire kinase domain of BRAF intact, whereas its regulatory region is replaced by the N-terminal end of KIAA1549.4,5 The new chimeric gene functions as a constitutively active BRAF kinase and transforms NIH3T3 cells when overexpressed,4 thus acting as a classical activated oncogene.

On rare occasions, BRAF may be fused to the family with sequence similarity 131, member B gene (FAM131B), a gene located approximately 2.5 Mb downstream of BRAF, as a result of an interstitial deletion that removes the N-terminal half of BRAF and the majority of the C-terminal half of FAM131B.8 A subset of PAs without BRAF fusion may have the BRAF V600E mutation or in some cases a 3-bp insertion encoding an extra threonine (T599_V600insT, or BRAFins3987T).9–11 RAF1 (v-raf-1 murine leukemia viral oncogene homolog 1), which encodes another member of the RAF kinase family, is fused to SLIT-ROBO Rho GTPase activating protein 3 (SRGAP3) by tandem duplication, a mechanism similar to the KIAA1549-BRAF fusion, in a few cases.4,5 Furthermore, up to 10% of PAs are associated with neurofibromatosis type 1 (NF1). NF1 is an autosomal-dominant hereditary tumor-bearing disease. Most NF1 patients inherit germ-line mutations of the NF1 gene, which is a member of the Ras GTPase-activating protein family (RasGAP).7 These alterations, which occur in a mutually exclusive manner, lead to activation of the mitogen-activated protein kinase (MAPK) signal transduction pathway. Overall, up to 90% of PAs have genetic abnormalities that activate the MAPK pathway.

The frequency of the BRAF fusion is strongly associated with the clinicopathological features of the tumors. It is predominantly seen in pediatric PAs with either cerebellar, optic nerve, hypothalamus, or brain stem location, but is less frequent among adult PAs and very rare in hemispheric locations.12,13 On the other hand, the BRAF V600E mutation is more common in supratentorial tumors than in infratentorial ones.11 Horbinski et al. reported that PAs with BRAF V600E mutations showed an increased risk of progression, compared with BRAF fusion cases.14

**Diffuse Astrocytomas Grade II**

Diffuse astrocytomas WHO grade II (DA) occur in children and young adults. The most common and characteristic genetic changes in DA are mutations of isocitrate dehydrogenase 1 (IDH1) or IDH2, which almost always coincide with tumor protein p53 (TP53) mutations. Mutations of IDH1/IDH2 or TP53 will be discussed elsewhere in this issue. These alterations are however limited to adult tumors, whereas very few pediatric diffuse astrocytomas have IDH1/IDH2 mutations.6,15 The molecular pathogenesis of the pediatric DA is currently unknown. Concurrent BRAF V600E mutations and CDKN2A homozygous deletions (HD) have been found in a subset of DAs without IDH1 mutations.5,16 This combination of abnormalities has also been observed in pediatric astrocytic tumors of higher malignancy grades.16 Whether BRAF mutations account for all pediatric DAs that develop without IDH1 mutations, or they define yet another, perhaps more malignant, subset in this category, remains to be investigated.

**Pleomorphic Xantheastrocytomas and Gangliogliomas**

Pleomorphic xanthoastrocytoma (PXA) is a rare subtype of astrocytic tumors classified as WHO grade II that often occur in children and young adults. Although they have distinct histopathology characterized by dense cellularity and high pleomorphism, it differentially diagnose them from more malignant astrocytic tumors, including glioblastomas, may sometimes be troublesome. Ganglioglioma (GG) is a well differentiated slow-growing tumor composed of neoplastic ganglion cells. They may arise at any age but most frequently among children and young adults.2 The histopathology can be very heterogeneous and may contain cell types resembling DA, oligodendrogliomas, or PA. Overlapping histological features may sometimes make it difficult to render the differential diagnosis.17 BRAF mutations (V600E or T599_V600insT) are found in >60% of pleomorphic xanthoastrocytomas with or without anaplasia and slightly less frequently in gangliogliomas (18%–45%).5,11,17,18 Their much higher incidence of BRAF mutation than in PA is intriguing, because these tumors may show overlapping morphology.17 BRAF
mutations are found in both adult and pediatric PXA, although pediatric PXA with anaplasia is more strongly associated with BRAF mutations. Correlation with localization is less evident, compared with PA. PXA or GG very rarely has IDH1 mutations. None of these tumors have BRAF fusion. Of note, CDKN2A deletions have been observed in PXA, reminiscent of the concurrent genetic alterations of BRAF and CDKN2A reported in a subset of DA without IDH1 mutation (see above), and they may play a role in the pathogenesis of PXA.

The occurrence of IDH1 mutations and BRAF fusion/mutation is mutually exclusive, regardless of the tumor type. To date, it has been suggested that the combination of IDH1 and BRAF status may serve as a diagnostic marker for low-grade gliomas. It has to be noted, however, that a significant proportion of those tumors have neither mutation, particularly in pediatric diffuse astrocytomas.

Pediatric High-Grade Gliomas

Although glioblastoma (GBM) is by far the most common primary malignant brain tumor in adults, it is relatively uncommon among children. Nonetheless, they do arise in children with similar dismal outcome to the adult tumors. Pediatric GBM not only develop in the cerebral hemispheres like their adult counterparts but also arise in the brain stem (BS), in the form of diffuse intrinsic pontine gliomas (DIPG), which is the subtype almost exclusively found in children. Although pediatric GBM are histopathologically indistinguishable from the adult tumors, they have distinct molecular signatures, strongly suggesting that they develop through a different mechanism.

It has been shown recently that pediatric GBM, whether they arise in the brain stem or cerebral hemisphere, frequently have mutations in genes involved in the H3.3-ATRX-DAXX chromatin remodeling pathway. The H3 histone, family 3A (H3F3A) gene, which encodes the replication-independent histone 3 variant H3.3, was mutated in about 60% of DIPG and 30% of non-brainstem pediatric gliomas. All mutations were heterozygous missense changes affecting only 1 of 2 codons, encoding lysine 27 (K27) or glycine 34 (G34), at or around amino acid residues on which critical repressive or activating modifications take place. Taken together, almost 80% of DIPG and &gt;30% of non-BS pediatric GBM had histone H3 mutations. Furthermore, in a series of pediatric GBM with predominantly non-BS location, mutations of α-thalassaemia/mental retardation syndrome X-linked (ATRX) or death-domain associated protein (DAXX) were found in 31% of tumors, some of them concurrently with H3F3A mutations. Overall, 44% of pediatric GBM in this series had a mutation(s) in ≥1 of these 3 genes. Whether the presence of these mutations has a prognostic value awaits further investigation, although a preliminary study suggests otherwise. ATRX/DAXX mutations, but not H3F3A mutations, have also been reported in pancreatic neuroendocrine tumors. IDH1 mutations are found mutually exclusively to H3F3A mutations, whereas a few pediatric GBM with ATRX mutations also had IDH1 mutations.

Most strikingly, mutations of H3F3A/ATRX/DAXX are almost exclusively found among GBM that arise in children, whether they are located in the brain stem or elsewhere, but very rarely in adults. They were neither found in any other type of brain tumors, glial or non-glial, regardless of patient age, nor in any other non-central nervous system pediatric tumors, indicating that alteration of this pathway is very specific to pediatric GBM. ATRX dimerizes with DAXX and forms a transcription/chromatin remodeling complex required for the incorporation of H3.3 at telomeres and pericentric heterochromatin. Tumors with these mutations are associated with alternative lengthening of telomeres. The observation that a large proportion of pediatric GBM develops through alterations of the H3F3A/ATRX/DAXX chromatin-remodeling pathway, which distinguishes pediatric tumor from their adult counterparts, confirms that these tumors may have a very different biology and should be considered as separate entities. Furthermore, the most recent study has shown that the different mutations, IDH1, H3F3A K27 or G34, are linked with clearly distinct patterns of methylation, and the presence of IDH1 mutation is associated with longer overall survival (OS), whereas H3F3A K27 mutations show a trend toward shorter survival. K27-mutated tumors predominantly occur in the midline (thalamus, pons, spinal cord), whereas all the other subgroups almost exclusively arise in the cerebral hemispheres, which may be reflecting different cellular origins and/or time of tumor initiation.

Other notable genetic abnormalities frequently observed in pediatric GBM include the homozygous deletion of ADAM3A (8p11) and amplification/mutation of PDGFRα (4q12), although the latter may also occur in adult GBM. Schindler et al. found BRAF mutations exclusively among pediatric GBMs. However BRAF mutations have never been identified among DIPG. Genome-wide copy number and gene expression analysis suggest that pediatric GBM have a distinct genomic profile, compared with adult tumors, and that DIPG may be further divided into 2 subtypes. The prognostic value of these genetic changes in pediatric GBM is currently still controversial.

Ependymomas

Ependymomas may develop in all age groups but predominantly in children and young adults. They arise literally throughout the neural axis from the cerebral hemispheres to the filum terminale of the spinal cord. Ependymomas in pediatric patients typically arise in the posterior fossa, in contrast to adult tumors, which are most often found in the supratentorial or spinal region. Patients with pediatric ependymoma
tend to have a worse prognosis than adults.\textsuperscript{34} It is now well-established that pediatric posterior fossa ependymomas comprise 2 distinct subtypes, each of them harboring vastly different clinical and molecular features.

Analysis of gene expression profiles in 177 ependymomas identified 2 histopathologically indistinguishable yet molecularly distinct subgroups (termed Group A and B) among posterior fossa ependymomas.\textsuperscript{35} Group A tumors arise in very young patients (median age, 2.5 years; more commonly male), are more often classified as WHO grade III, show invasive growth into the cerebellum, and have higher incidence of metastasis/recurrence and a shorter PFS and OS, compared with group B tumors.\textsuperscript{35}

Genetically, group A ependymomas exhibited a largely balanced genomic profile with the exception of a relatively frequent 1q gain, compared with group B tumors. In contrast, group B ependymomas have numerous cytogenetic abnormalities involving whole chromosomes or chromosomal arms, including losses of 6q and 22q and gains of 9q, 15q, and 18q, among others.\textsuperscript{35} This is in line with the previously suggested molecular cytogenetic subgroups based on a study of 292 intracranial ependymomas using array-CGH and FISH, in which the presence of 1q gain and CDKN2A HD independently predicts a worse prognosis, whereas gains of chromosomes 9, 15q, and 18 combined with loss of chromosome 6 were associated with longer survival.\textsuperscript{34} The findings have further been validated in a large series of ependymomas using immunohistochemistry for laminin alpha-2 (LAMA2) and neural epidermal growth factor like-2 (NELL2), representing expression markers for group A or B, respectively, and FISH for 1q gain in group A and for 6q loss, 9q gain, 15q gain, 18q gain, and 22q loss in group B.\textsuperscript{35} Taken together, these molecular markers appear to be able to identify 2 groups of pediatric posterior fossa ependymomas that have distinct clinical course and perhaps underlying pathogenesis.

**Embryonal Tumors**

**Medulloblastoma**

Medulloblastoma research is the area of neuro-oncology currently experiencing the biggest breakthrough in terms of translational research, with tremendous progress achieved over a short period. Medulloblastomas have traditionally been classified, based solely on the histopathology of the tumors, into several variants, including desmoplastic/nodular, medulloblastomas with extensive nodularity, classic, large cell, and anaplastic.\textsuperscript{2} The prognostic significance of these histopathological variants is however not clear. In the past few years, a number of independent studies have found that medulloblastomas may comprise several subgroups with distinct clinical features and molecular pathogenesis (reviewed in).\textsuperscript{36} The current consensus for the molecular subgroups of medulloblastomas recognizes 4 distinct major groups, named as Wnt, Shh, group 3, and group 4.\textsuperscript{36,37} The existence of these 4 groups has been confirmed with an international meta-analysis followed by a large-scale independent validation.\textsuperscript{38} It has been shown that medulloblastomas from each group may have distinct cellular origins.\textsuperscript{39} As described below, these molecular subgroups predict patients' outcome better than any clinical markers such as patient age, histopathological subtype, or metastatic stage at diagnosis, which have traditionally been used for prognostication.

The Wnt group is the best defined and analyzed of all groups. It is characterized by the upregulation of genes involved in the WNT pathway, which plays an important role during the embryonal development of the central nervous system. Mutations of *catenin (cadherin-associated protein) beta 1, 88kDa (CTNNB1)* are found in the majority of Wnt medulloblastomas.\textsuperscript{40,41} Monosomy 6 is also a common finding.\textsuperscript{31} *CTNNB1* mutations are associated with nuclear immunohistochemical staining for a–catenin. Dickkopf 1 homolog (Xenopus laevis) (Dkk1) positive immunostaining is also observed.\textsuperscript{42} Histopathologically, the classic variant dominates this group, although the large cell variant may also be found. The patients with Wnt medulloblastoma may be adults or children but rarely infants. They have the best prognosis of all groups, with metastases being rare.

The Shh group is characterized by upregulation of genes involved in the Sonic Hedgehog signaling pathway, which also plays a role in embryonal central nervous system development and the maintenance and proliferation of stem cells in adults. Frequent mutations of patched 1 (*PTCH1*) and, less commonly, suppressor of fused homolog (Drosophila; SuFu) are reported.\textsuperscript{41,42} Amplifications of *GLI family zinc finger 1 or 2 (GLI1/ GLI2)* may also be found.\textsuperscript{43} Deletion of 9q is a frequent event, which is associated with *PTCH1* (9q22) mutations. Isochromosome 17q (i17q, see below) is typically absent from Wnt and Shh tumors.\textsuperscript{44} Of note, Shh medulloblastomas with TP53 mutations, particularly those associated with Li-Fraumeni syndrome (with germ-line *TP53* mutations), may display a chromothripsis phenotype, which is characterized by numerous intra- and inter-chromosomal rearrangements that presumably took place in a single catastrophic genetic event.\textsuperscript{45} Positive immunostaining of secreted frizzled-related protein 1 (SFRP1) or GRB2-associated binding protein 1 (GAB1) has been used to identify Shh medulloblastomas.\textsuperscript{46,47} Almost all of the nodular/desmoplastic variants are Shh tumors, although other histopathological variants may also belong to this group. Patients in this group are either infants or adults but rarely children (3–16 years).\textsuperscript{44} The prognosis for this group of patients is intermediate between Wnt and group 3 (see below).

Groups 3 and 4 are less well defined. The current standard for diagnosis of a group 3 tumor is rather succinct, namely “a transcriptional profile that clusters with other Group 3 tumors.”\textsuperscript{36} Currently, the pathways in which the relevant genes are involved are not as well characterized as for the Wnt and Shh groups. Positive immunostaining of natriuretic peptide receptor C/guanylate cyclase C (atriotriuretic peptide receptor C;
NPR3) is considered to be a marker for group 3. Amplification of v-myc myelocytomatosis viral oncogene homolog (avian; MYC) is almost exclusively found in this group, but not v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian; MYCN) amplification.\textsuperscript{40,44} Recurrent translocations that result in a fusion gene between the PVT1 oncogene (non-coding gene hosting for 4 micro RNA) and MYC, which arise through chromothripsis, are restricted to group 3.\textsuperscript{46} MYC is overexpressed, and MYCN expression levels remain low. Isochromosome 17q may be observed (26%), although less frequently than in group 4. A number of chromosomal aberrations, including losses of 5q or 10q and/or gains of 1q or 17q, may also be found. Group 3 medulloblastomas are mostly classic variants, but many of the large cell or anaplastic variants also belong to this group. Group 3 tumors are more common in males than in females, occur both in infants and children, but almost never in adults. The metastasis rate is very high, and the prognosis is the poorest of all groups. It has been suggested that group 3 may also have further subsets, for example, based on MYC status. Tumors with MYC amplification have the highest risk of recurrence and the shortest survival. Those without MYC amplification have an intermediate prognosis, which is comparable to group 4 patients.

Group 4 medulloblastomas have been defined by their unique transcriptional profile in which genes involved in neuronal differentiation and development are upregulated; however, the underlying common pathogenesis has yet to be discovered.\textsuperscript{56} They have the highest incidence of i17q (up to 70%).\textsuperscript{44} Loss of chromosome X occurs in 80% of females with group 4 tumors. Positive potassium voltage-gated channel, shaker-related subfamily, member 1 (episodic ataxia with myokymia; KCNA1) immunostaining has been proposed as a marker for group 4 tumors. The majority of tumors have classical histology. Patients are in all age groups, but predominantly male, and have an intermediate prognosis.

There are also other genetic changes that are observed in >1 group. TP53 mutations are found at a low frequency (overall up to 7%) in Wnt, Shh, and group 4 but not among group 3.\textsuperscript{47} The status of TP53 is not associated with clinical outcome for medulloblastomas. MYCN amplification is detected either in the Shh group or in group 4.\textsuperscript{47,48} Isochromosome 17q occurs as the result of a complex chromosomal rearrangement involving loss of 17p and gain of the whole 17q arm, with break points generally located within 17p11 (isodicentric 17q) rather than at the centromere.\textsuperscript{49} There are multiple break point clusters involving regions of low copy repeats.\textsuperscript{49} Neither the molecular targets nor the consequences of the rearrangement are currently known. Isochromosome 17q is a predominant feature in group 4 and can also be seen in group 3 but very rarely in the WNT or SHH groups.

Very recently, a series of whole genome/exome mutation analyses have provided further insight into the biology of medulloblastoma subgroups.\textsuperscript{50–53} Myeloid/lymphoid or mixed-lineage leukemia 2 or 3 (MLL2/MLL3), genes encoding histone-lysine N-methyltransferases involved in histone H3K4 methylation, are mutated mainly in WNT or SHH subgroup medulloblastomas.\textsuperscript{50–53} Lysine (K)-specific demethylase 6A (KDM6A, also known as UTX), which functions as a H3K27 demethylase, is mutated mostly in group 4 but not in WNT or SHH medulloblastomas. These indicate that alterations of genes involved in histone modification are common events across the subgroups. Genes encoding subunits of the SWI/SNF-like chromatin-remodeling complex, most typically the SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 (SMARCA4) gene, are mutated in WNT and group 3 tumors but not in others. Apart from these, DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, X-linked (DDX3X), encoding an ATP-dependent RNA helicase, is mutated predominantly in WNT tumors.\textsuperscript{50,52,53}

Thus, comprehensive genome sequencing has further helped delineation, as well as adding complexity, of medulloblastomas. It is likely that each group potentially consists of additional levels of hierarchy, however, and refining the definition of existing molecular groups to more accurately predict outcome should be a priority for future research. For example, it has been suggested that adult medulloblastomas may develop through a molecular pathogenesis that differs from their pediatric counterpart, which may give rise to distinctive subclasses within each group.\textsuperscript{54–56}

It is likely that these molecular groups will become an integral part of the routine diagnosis for medulloblastomas, because the current histopathological subclassification bears several limitations. The classic medulloblastomas, for example, contain the 2 clinically most distinct groups (i.e., the WNT group and group 3) that have best and worst prognosis, respectively. On the other hand, it is worth noting that large cell/anaplastic histology may be a prognostic factor independent of molecular grouping.\textsuperscript{54} Histopathological and molecular classification may thus complement each other to help further delineate the proposed prognostic groups.

**PNET and ETANTR**

Central nervous system primitive neuroectodermal tumors (PNETs) are a heterogeneous group of tumors classified as malignant embryonal neoplasms of WHO grade IV.\textsuperscript{2} They are highly aggressive, located supratentorially, and histopathologically similar to medulloblastomas. Expression profiling demonstrated that they clustered separately from medulloblastomas or other embryonal tumors, with PNETs lacking expression of external granular cell and proneuronal genes, suggesting that they form a separate entity.\textsuperscript{57} Unlike medulloblastomas, IDH1 mutations may be found in PNETs at a low frequency.\textsuperscript{58} This finding is limited to adult patients. CDKN2A HD or amplification of PDGFRA, v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (KIT) or v-myc myelocytomatosis viral oncogene homolog 1, lung carcinoma derived (avian) (MYCL) may be detected in a subset of tumors.\textsuperscript{59,60} Gains of 1q
and 19p are also common findings.\textsuperscript{60,61} On the basis of gene expression signatures, Picard et al. recently proposed 3 molecular subgroups of CNS PNET with differential expression of cell-lineage markers LIN28 and OLIG2 that could predict outcome.\textsuperscript{62} To establish a molecular classification comparable to that of medulloblastomas, a genetic signature unique to PNET needs to be identified.

Recently, it has been shown that a subset of embryonal brain tumors have amplification of 19q13.42, a unique genetic change not shared by any other embryonal tumors, such as medulloblastomas or AT/RT.\textsuperscript{63–65} They are called ependymoblastoma or embryonal tumor with abundant neuropil and true rosettes (ETANTR) and may actually belong to a single entity of embryonal tumor with multilayered rosettes (ETMR).\textsuperscript{66} These rare tumors arise almost exclusively in very young children where they adopt a very aggressive clinical course. Within the 19q13.42 amplicons, which involve a number of microRNA genes, overexpression of miR372 and 373 is highly associated with the genetic amplification.\textsuperscript{63}

**AT/RT**

As with ETANTR, atypical teratoid/rhabdoid tumor (AT/RT) is a highly malignant neoplasm that occurs in very young children. They may be found throughout the CNS, although about half of them develop in the posterior fossa. AT/RT are resistant to therapy and take a more dismal clinical course than do other embryonal tumors, such as medulloblastomas or PNET; however, histopathologically distinguishing them may prove to be challenging, because they share many morphological features.

The great majority of AT/RT has complete inactivation of the SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1 gene (SMARCB1), also known as INI1 or bSNF5, by mutations combined with hemizygous deletion, or homozygous deletion of the gene.\textsuperscript{66,67} SMARCB1 is a member of the ATP-dependent SWI/SNF chromatin-remodeling complex and is involved in the transcriptional regulation of a variety of genes that control cellular proliferation or differentiation.\textsuperscript{68} SMARCB1 inactivation is so highly specific to AT/RT that it is now considered as a hallmark of the disease. The absence of SMARCB1 protein expression can be immunohistochemically established in the routine histopathological laboratory and used to identify AT/RT from other embryonal pediatric brain tumors. Germ-line mutations of SMARCB1 predispose the patients to develop AT/RT.\textsuperscript{59,70} Although the diagnosis of AT/RT may be made on the basis of the lack of SMARCB1 protein, screening for germ-line mutations may help identify unaffected carriers within the family. On rare occasions, AT/RT may develop through mutations of SMARCA4/BRG1, another member of the chromatin remodeling complex.\textsuperscript{71} AT/RT may also progress from existing GG or PXA, presumably acquiring SMARCB1 mutations, in addition to BRAF V600E mutations in their preceding tumors.\textsuperscript{17}

**Germ Cell Tumors**

The WHO classification recognizes several major subtypes of intracranial germ cell tumors (IGCT; i.e., germinoma, teratoma [mature, immature or teratoma with malignant transformation], yolk sac tumor, embryonal carcinoma, and choriocarcinoma) and mixed germ cell tumor. In contrast to most other major pediatric brain tumors discussed above, the molecular pathogenesis of intracranial germ cell tumors is still largely unknown. This is partly attributable to the fact that the incidence of these tumors is very low in the western countries, accounting for only 0.3%–0.5% of all primary intracranial neoplasms.\textsuperscript{2} The prevalence of IGCT is however significantly higher in Far East Asian countries, comprising >15% of all primary brain tumors in children <14 years of age in Japan, IGCT thus being the second most common pediatric brain tumors after astrocytomas.\textsuperscript{72} Among IGCT, pure germinomas generally respond well to chemo/radiotherapy and have a good prognosis. However, non-germinoma IGCT and approximately 10% of pure germinomas are resistant to therapy. Elucidating the molecular pathogenesis of germ cell tumors is therefore essential to predict therapy response/prognosis and crucially also to develop better treatments.

One of few known genetic abnormalities is mutations of KIT, a gene frequently altered in a variety of tumors including gastrointestinal stromal tumors (GIST) and testicular germ cell tumors (seminomas).\textsuperscript{73} KIT mutations are found in approximately 25% of pure germinomas.\textsuperscript{74,75} The position of the KIT mutation within the gene is of therapeutic significance, as GIST with mutations in KIT exon 11 respond well to imatinib.\textsuperscript{76} In IGCT, the mutations can be found in a range of exons including exons 1, 10, 11, 13, and 17. No correlation between the presence of KIT mutations and prognosis has been observed thus far. All germinomas and some mixed IGCT show strong membranous staining for the KIT protein by IHC regardless of the mutation status. This discrepancy between protein expression and mutation of KIT has also been observed in testicular germ cell tumors.\textsuperscript{77}

A high incidence of CDKN2A HD has been reported in IGCT.\textsuperscript{78} However, because a metaphase CGH analysis did not identify frequent 9p loss,\textsuperscript{79} the true impact of CDKN2A alterations needs to be further validated. Isochromosome 12p (i12p) has been described in up to 25% of IGCT.\textsuperscript{80,81} No molecular target for i12p has yet been identified. Gains of 1q and 8q are also found.\textsuperscript{79} The presence of an extra copy of chromosome X is particularly interesting and possibly of pathogenic significance, because extragonalad germ cell tumors are often associated with Klinefelter syndrome, which is characterized by a 47,XXX karyotype.\textsuperscript{79,80}

IGCT clearly needs to be the focus of more research to uncover the mechanism of disease and to overcome
therapy resistance. A multicenter study has been initiated in Japan aimed at facilitating a comprehensive genome-wide molecular analysis in a large cohort of patients.

**Future perspective**

It has become clear that pediatric brain tumors have distinct biology and pathogenesis, compared with adult tumors, even if they share the same histopathological features. We already have several highly specific molecular markers that could perhaps practically determine the diagnosis, such as the BRAF fusion gene for PA or SMARCB1 mutation for AT/RT.

It has also become evident that malignant pediatric brain tumors of any type display alterations of epigenetic regulation as a common feature, although mutations in components of the chromatin remodeling complexes or in histone modifying enzymes (sometimes even histones themselves). In the latter target, in particular, the alterations could lead to altered H3K27 or H3K36 methylation or equivalent, which could in turn alter transcription of lineage-specific genes and may keep the cells in an undifferentiated (or stem-like) state. This may be essential for the development of pediatric brain tumors and makes them distinct from adult tumors.

One of the current limitations is that not all tumors are positive for these markers. For example, approximately one-third of PAs are negative for the BRAF fusion. Some of them may harbor BRAF mutations; however, the presence of the BRAF V600E mutation alone does not technically distinguish a PA from some other gliomas, such as PXA. There are also a number of molecular targets for which no specific markers are available. Ongoing whole-genome analysis projects may provide new evidence to decipher the pathogenesis of these orphan tumor types. Conventional morphology-based diagnosis is currently the gold standard, with its overall consistency, cost-effectiveness, and vast amount of accumulated knowledge and technical expertise. We are now, however, progressively moving into a new era of molecular diagnosis as an aid to predicting a particular biological behavior, which would subsequently be linked to the conventional histopathological diagnosis. Just as the introduction of immunohistochemistry has greatly helped to advance histopathological diagnosis, molecular testing is now also becoming an integral part of disease diagnosis. Integration of all information sources, from clinical and histopathological to molecular, will transform diagnosis into the true state of the art.

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**References**


