
New Molecular Targets and Treatments for Pediatric Brain Tumors

Claudia C. Faria, Christian A. Smith and
James T. Rutka

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/53300>

1. Introduction

The outstanding progress in advanced molecular technologies has provided a tremendous amount of data that has altered the way in which we classify and categorize pediatric brain tumors. From the initial identification of chromosomal aberrations by karyotyping and comparative genomic hybridization, we have rapidly moved to expression array studies and to integrative genomic approaches which allowed the stratification of several pediatric brain tumors into molecular subgroups. These data have not only increased our understanding of the molecular pathogenesis of pediatric brain tumors, but have also identified prognostic markers and opened new avenues for targeted therapies.

One of the most important discoveries in pediatric astrocytomas was the duplication and the mutation of the v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*) gene, found in pilocytic astrocytomas and malignant astrocytomas, respectively. Clinical trials using *BRAF* signaling pathway inhibitors are currently ongoing.

Until recently, the biology of diffuse intrinsic pontine glioma (DIPG) was poorly understood. Overexpression of epidermal growth factor receptor (*EGFR*) and platelet-derived growth factor receptor alpha (*PDGFR α*) amplification have now been reported in multiple studies in DIPG and clinical trials with the respective inhibitors (nimotuzumab for *EGFR* and dasatinib for *PDGFR α*), alone or in combination with other drugs, are in progress.

Integrated genomics has shown that medulloblastoma, the most common malignant pediatric brain tumor, comprises four distinct molecular and clinical variants. The stratification of patients into the Wnt subgroup, the sonic hedgehog (SHH) subgroup, Group 3 and Group 4 may lead to the identification of those patients that will most likely benefit from targeted

therapies, like SMO inhibitors. Moreover, this molecular classification may help identify patients predicted to have a poor prognosis, who may benefit from intensified therapies, and patients with a favorable prognosis that potentially benefit from reduced radiation and chemotherapy regimens. It is already known from four independent studies that the Wnt subgroup patients have a very good prognosis while patients in Group 3 have a high incidence of metastasis and a dismal prognosis.

The transcriptional profiling of two large independent cohorts of posterior fossa ependymomas also identified two groups with distinct molecular and clinical features. Group A ependymomas have a balanced genome, occur in younger patients and tumors are located laterally. These patients have a worse clinical outcome and higher incidence of metastasis and recurrence. In contrast, Group B ependymomas occur in the midline and have a better prognosis. Since ependymomas are usually refractory to current chemotherapeutic agents, these discoveries may shed some light into the pathways involved in tumor initiation and progression and also identify new targets for therapy.

We are now facing the next-generation (Next-Gen) sequencing era which will provide further insights into the dysregulated signaling pathways in each tumor type. The identification of driver mutations will allow a better understanding of tumorigenesis and lead to the development of more accurate preclinical models. Moreover, the profiling of transcriptomes, genetic and epigenetic events of large cohorts of tumors will eventually shed some light into the cells of origin of specific subgroups and also the important driver events for tumor initiation, maintenance and progression. On the clinical side, this knowledge will allow the stratification of patients into appropriate risk groups and the tailoring of treatments according to each tumor's genomic landscape. In this chapter we describe the latest advances in molecular genomics of the most common pediatric brain tumors as well as its prognostic significance and relevance to the clinic. We also highlight the most recent clinical trials using molecular targeted therapies and discuss further possible avenues in the treatment of pediatric brain cancer.

2. Astrocytomas

Astrocytomas are a common brain tumor in children. According to the 2007 classification of the World Health Organization (WHO) they can be classified into four grades (WHO grade I-IV), which reflect their biological and clinical behavior. Pilocytic astrocytomas (WHO grade I) represent the most frequent brain tumor in the pediatric population and have excellent survival rates over 95% [1]. On the other hand, glioblastomas (WHO grade IV) are aggressive tumors, often non-responsive to treatments and with survival rates ranging from 10% to 30% [1]. We will focus on the molecular biology of these two types of pediatric astrocytomas.

2.1. Pilocytic astrocytoma

The first-line treatment for pilocytic astrocytoma (PA) is surgical resection. Although most children with these tumors survive for a long time, some will experience tumor recurrence, especially if the tumor resection is incomplete. Recurrent and progressive tumors are treated

with radiation and/or chemotherapy but, until recently, the mechanisms of recurrence and malignant transformation were poorly understood.

Early studies on chromosomal aberrations in PAs showed normal karyotypes in most cases. Trisomy of chromosomes 5 and 7 and 7q gains were some of the few cytogenetic abnormalities found [2]. Using an array-based comparative genomic hybridization (array-CGH), Pfister *et al.* studied a large series of pediatric low-grade astrocytomas and uncovered mechanisms of mitogen-activated protein kinase (MAPK) activation in these tumors. The authors identified a tandem duplication of the *BRAF* gene locus (7q34) in more than 50% of the PAs and, in a smaller percentage (~6%) of tumors, a *BRAF* activating mutation V600E (a valine to glutamate change at hotspot codon 600) [3]. Later, it was demonstrated that the constitutive activation of BRAF was due to a fusion between a novel gene (*KIAA1549*) and the *BRAF* oncogene and also that this fusion was related to a better clinical outcome in incompletely resected low-grade astrocytomas [4]. Other, less frequent, reported gene fusions in PAs include the fusion of *BRAF* with the *FAM131B* gene and the fusion between a Raf kinase family member (*RAF1*) and SLIT-ROBO Rho GTPase-activating protein 3 (*SRGAP3*) gene [5]. The combined analysis of *BRAF* and isocitrate dehydrogenase 1 (*IDH1*) was shown to be both sensitive and specific to separate PAs from diffuse astrocytomas (WHO grade II). PAs contained the *KIAA1549:BRAF* fusion in 70% of cases but no *IDH2* or *IDH1* mutations while diffuse astrocytomas exhibited 76% of cases with *IDH1* mutations but no *BRAF* fusion [6]. There is also an interesting correlation between tumor location and the type of MAPK alteration. Posterior fossa tumors usually have increased incidence of *KIAA1549:BRAF* fusion while supratentorial tumors have high frequency of *BRAF*^{V600E} mutation. Approximately 80-90% of PA cases reported in the literature have at least one alteration in the MAPK pathway. Interestingly, all the alterations described in the MAPK pathway seem to be mutually exclusive suggesting that a single hit may be sufficient to induce transformation [5].

With the discovery of constitutive activation of MAPK pathway and BRAF alterations in most PAs, there has been an increasing interest in using these targets for novel therapeutic approaches. A number of phase I and phase II clinical trials are ongoing to test small molecule inhibitors of MAPK and related pathways (Table 1).

Drug		Clinical Trial	Reference (http://clinicaltrials.gov)
Group	Name		
MEK inhibitor	AZD6244	Phase I	NCT01386450, NCT01089101
RAF inhibitor (multikinase inhibitor)	Sorafenib	Phase II	NCT01338857 (suspended)
mTOR inhibitor	Everolimus	Phase II	NCT01158651, NCT00782626

Table 1. Pediatric clinical trials for pilocytic astrocytomas using targeted therapies

2.2. Glioblastoma multiforme

Glioblastoma multiforme (GBM) is the most common brain tumor in adults and is less frequent in the pediatric population. Despite improvements in neurosurgery and neuro-oncology, children diagnosed with GBM still have a dismal outcome with low survival rates even with aggressive therapeutic regimens. Although the histology of pediatric and adult GBMs appears identical, the molecular biology of these tumors is different. Therefore, the development of effective therapies for GBM in children should probably not rely on advances made in adult tumors.

Mutations in phosphatase and tensin homolog (*PTEN*) and amplifications of *EGFR* are common in adult malignant gliomas but less frequent in the pediatric setting. However, when compared to pediatric low-grade gliomas, pediatric GBMs have a significant higher expression of *EGFR*, although with no reported mutations and few deletions (~17%) in *EGFRvIII* [7]. While in adult GBMs the activation of the AKT pathway is a result of *PTEN* mutations, in pediatric tumors these are infrequent, although overexpression of AKT and its association with poor survival has been reported [8,9]. In a study with a large cohort of pediatric high-grade gliomas (HGGs) (37 GBMs out of 63 tumor samples), Bax *et al.* identified *PDGFR α* amplification and cyclin-dependent kinase inhibitor 2A/B (*CDKN2A/B*) deletion as the most frequent focal events. Interestingly, the patients with high-grade tumors and a stable genomic profile had a better survival, independent of the histological grade or type [10]. Several studies showed that *IDH1* mutations, common in adult secondary GBM (98%), are rare in pediatric GBM [7]. *TP53* (tumor protein 53) mutations were reported in pediatric high-grade tumors with an increased frequency in older children (40%) when compared to those less than 3 years (12%) [11]. Overexpression of p53, but not *TP53* mutations, was also associated with a significant reduction in the 5 year progression-free survival [12]. A recent study used high resolution single nucleotide polymorphism (SNP) arrays to identify novel chromosomal alterations in pediatric GBM, including amplifications of 7q21-22 and 1q43-44 and loss of heterozygosity (LOH) in 15q15.1-15q23 and 17p13-17p11.2. Genes involved in cell-cycle regulation and cell death pathways were the predominant targets of LOHs [13].

It has been shown that overexpression of O⁶-methylguanine-DNA methyltransferase (MGMT) is rare in HGGs but it has also been reported that it is associated with a poor overall survival in children. The *MGMT* gene encodes a DNA-repair enzyme that can reduce the efficacy of alkylating agents. When the *MGMT* gene is silenced by promoter methylation, DNA repair in tumor tissue is compromised and patients have a better survival [7].

Finally, a very recent study uncovered an interesting interplay between genetic and epigenetic events in pediatric GBM. The authors performed whole-exome sequencing in 48 pediatric GBMs and identified 44% of tumors harbouring somatic mutations in genes involved in the chromatin remodeling pathway, including the histone H3 gene (*H3F3A*), the α -thalassaemia/mental retardation syndrome X-linked (*ATR*X) gene and the death-domain associated protein (*DAXX*) gene [14]. Interestingly, the *H3F3A* mutations were found to be specific of GBMs and more prevalent in the pediatric population. Moreover, 54% of all cases and 86% of patients with *H3F3A* and/or *ATR*X mutations, also showed *TP53* somatic mutations [14].

The novel targeted therapies that are currently under clinical investigation for high-grade gliomas, including GBM, are reviewed in reference [15].

3. Diffuse intrinsic pontine glioma

Despite decades of clinical research, the prognosis of diffuse intrinsic pontine glioma (DIPG) remains dismal with more than 90% of affected children dying within two years of diagnosis [16]. The only known effective treatment is radiation therapy although in most patients it has a transient effect. Different combinations of chemotherapy, radiation and radiosensitizers were attempted but failed to improve long-term survival [17]. The indication for biopsy in DIPG has been reserved for atypical tumors (prominent enhancement in magnetic resonance imaging (MRI), low T2/FLAIR signal and/or high signal on diffuse imaging). However, the recognition that it is crucial to understand the biology of DIPG in order to design more rational drug treatments, led to a plea for routine biopsies in these patients. Some groups are already performing regular image-guided stereotactic biopsies in patients with DIPGs [18,19] and, furthermore, biopsies have been included in a clinical trial to decide which patients with DIPG would benefit from targeted therapy with erlotinib, an *EGFR* inhibitor [20]. Autopsies became another important source of DIPG tissue collection. Recent studies showed that up to 50% of parents agree with the autopsy and, if done within a short period of time after death, it is possible to obtain good quality DNA and RNA and even culture primary DIPG cell lines [21-23].

The paradigm shift to obtain tissue from patients with DIPG not only increased our knowledge in the molecular biology of the disease but also raised important clinical considerations. Paugh *et al.* described differences at the copy number and expression level in both adult and pediatric HGGs, including DIPGs. They showed that gain of chromosome 1q and *PDGFR α* amplification were frequent in children while chromosome 7 gain, 10q loss and *EGFR* amplification were characteristic of adult HGGs [24]. The identification of these two distinct biological entities has important clinical implications since pediatric clinical trials over the past decades have been designed with drugs known to have some activity in adult HGGs. It is now clear that DIPG has to be considered and treated as a separate disease. The same group compared the copy number alterations (CNAs) in DIPGs and nonbrainstem pediatric glioblastomas concluding that they are also genomically distinct. They identified receptor tyrosine kinase and retinoblastoma protein (*RB*) amplifications in 47% and 30% of DIPG cases, respectively, and found high frequency of focal amplifications of *PDGFR α* , *MET* and insulin-like growth factor receptor 1 (*IGF1R*) [25]. Furthermore, Zarghooni *et al.*, using SNP arrays, showed that DIPGs have distinct CNAs from pediatric supratentorial high-grade astrocytomas, with gains in *PDGFR α* and poly (ADP-ribose) polymerase-1 (*PARP-1*) amongst the most frequent [26]. Another group showed other differences between these tumors including frequent losses of 17p and 14q in DIPGs [27]. A very recent study used DNA from tumor tissue obtained at diagnosis by stereotactic biopsies and identified oncogenic mutations in *TP53* (40%), *PI3KCA* (15%) and *ATM/MPL* (5%). In fact, *PI3KCA* represents the first mutated oncogene described in DIPG [28].

Another level of gene expression regulation in DIPG occurs through epigenetic mechanisms, including histone modifications. Very recent studies used whole-genome sequencing in large cohorts of DIPGs to identify somatic mutations in the *H3F3A* gene. Khuong-Quang *et al.* reported mutations in H3.3 that resulted in the substitution of lysine by methionine at amino acid 27 (K27M-H3.3) in 71% of cases and the presence of these mutations were associated with a worse survival independent of patient age and histological grade [29]. Furthermore, gains or amplifications in *PDGFR α* and *MYC/PVT1* were exclusively seen in K27M-H3.3 mutants. The authors also described *TP53* mutations in 77% and *ATRX* mutations in 9% of DIPGs [29]. Interestingly, both the mutated and wild-type H3.3 subgroups showed high frequency of *TP53* mutation. In another study, Wu *et al.* identified mutations in *H3F3A* (encodes histone H3.3) and *HIST1H3B* (encodes histone H3.1) in 78% of DIPGs and 22% of nonbrainstem pediatric glioblastomas [30]. The different H3 mutations were mutually exclusive and seemed to be a feature of pediatric HGGs.

The discoveries from the molecular biology of DIPGs identified several drugable targets allowing the development of molecular target-based trials that are summarized in Table 2. Some of these trials have shown a subset of patients with survival longer than expected and, therefore, several other trials are ongoing, some of them including combined therapies [17,31].

Finally, DIPG treatment involves another challenge which is drug distribution. Probably due to an intact blood-brain barrier, penetration of drugs in the pons seems to be poor, a fact demonstrated by the lack of gadolinium enhancement in the MRI in most DIPGs. Therefore, it is crucial to improve drug delivery either by disrupting the blood-brain barrier (e.g. focused ultrasound or drugs that increase permeability such as mannitol), by local delivery (tumor injection or convection enhanced delivery) or even nanoparticles [17]. These techniques may promote high drug concentrations in the pons, including agents that normally don't cross the blood-brain barrier.

Drug	Clinical Trial	1 year Overall Survival (OS)	Reference
Imatinib	Phase I	45.5%	2007 [32]
Tipifarnib	Phase I	36.4%	2008 [33]
Gefitinib	Phase I	48%	2010 [34]
Vandetanib	Phase I	37%	2010 [35]
Erlotinib	Phase I	50%	2011 [20]
Gefitinib	Phase II	56%	2011 [36]
Nimotuzumab	Phase II	Median OS 9.6 months	2011 [37]

Table 2. Clinical trials for diffuse intrinsic pontine glioma (DIPG) using targeted therapies

4. Medulloblastoma

Medulloblastoma, the commonest malignant brain tumor in the pediatric population, is no longer considered a single disease. Recent efforts of multiple independent groups have reached to a consensus that medulloblastoma comprises four distinct molecular variants named WNT, SHH, Group 3 and Group 4 [38]. The subgroups have different demographics, genetic profiles and prognosis [39]. This may explain why patients with the same histological disease have different clinical outcomes and a variable response to current treatments including surgery, whole-brain radiation and intensive chemotherapy. Figure 1 summarizes the main features of the four medulloblastoma subgroups.

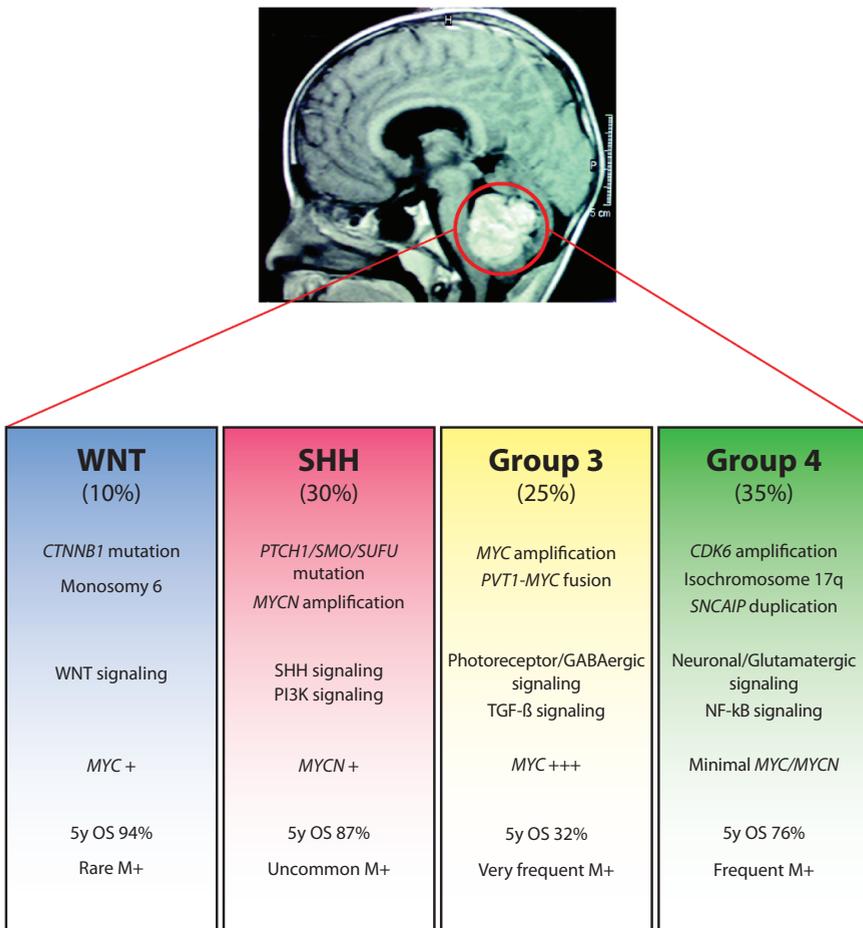


Figure 1. Features of the four medulloblastoma subgroups, including molecular genetics and clinical outcome

4.1. WNT medulloblastomas

WNT medulloblastomas are frequent in older children and teenagers and are rarely seen in infants. Patients within this subgroup have usually an excellent outcome with survival rates over 90%. However, Remke *et al.* showed that this is only true for pediatric cases as adult WNT patients exhibit survival rates of approximately 80% [40]. Histologically, WNT tumors are almost always of the classic variant and they rarely disseminate.

This subgroup is enriched in genes of the WNT pathway. Although few gains and losses were reported in the WNT genome, mutations in *CTNNB1* are frequent and usually occur with deletion of one copy of chromosome 6 (monosomy 6). Patients with *CTNNB1* mutation have accumulation of β -catenin in the nucleus and better survival rates. Positive nuclear immunostaining for β -catenin is now currently accepted as a marker of WNT medulloblastomas [41,42], although *DKK1* and *DKK2* were also proposed as markers for this subgroup [42,43]. The overall good outcome of WNT tumors suggests that this subgroup may be a good candidate for de-escalation therapy in future clinical trials.

4.2. SHH medulloblastomas

SHH medulloblastomas are frequently found in infants and adults (approximately 60% of cases in each age group) but are rare in childhood. In the SHH subgroup, prognostic factors such as M-stage and desmoplasia are age-dependent. Metastasis at presentation represents a negative prognostic factor only in adults while desmoplasia is associated with worse outcome only in pediatric cases [44]. From a histological point of view this subgroup is unique since it includes tumors of the four main variants (classic, nodular desmoplastic, large-cell anaplastic and medulloblastoma with extensive nodularity).

SHH medulloblastomas are characterized by aberrant expression of SHH pathway genes including *SMO*, *PTCH1*, *SUFU* and *GLI2*. Amplifications of *MYCN* and *YAP1* are also seen in this subgroup. Additionally, deletion of chromosome 9q is a common and highly restricted event in SHH tumors, most likely secondary to *PTCH1* mutation on chromosome 9q22 [45]. Of notice is the fact that the transcriptomes of pediatric and adult SHH tumors have different expression profiles with increased levels of genes related to extracellular matrix function in the first group and elevated levels of *HOX* family genes and genes involved in tissue development in the second group [44,46]. In an attempt to simplify the molecular subgrouping of medulloblastomas, different laboratories used formalin-fixed paraffin-embedded tissues (FFPE) to test a variety of markers for SHH medulloblastomas including *SFRP1*, *GLI1* and *GAB1* [42,43,47].

The clinical and molecular distinction of infant and adult SHH medulloblastomas suggests a disparate underlying biology and raises the question of possible different responses to current targeted therapies.

4.3. Group 3 medulloblastomas

Group 3 medulloblastomas are restricted to pediatric patients. Indeed, two recent studies concluded that Group 3 tumors are extremely rare in adults [40,48]. Another feature of this

subgroup is its aggressive behavior with high incidence of metastasis, frequent large-cell anaplastic histology and an invariable dismal prognosis (approximately 20 to 30% overall survival). It has been shown that Group 3 medulloblastomas consist of two distinct subtypes one of which harbors frequent amplifications of the *MYC* gene and has the worse outcome [49]. Although Group 3 and 4 have some common genetic features, including gain of chromosome 7, losses of chromosomes 5q and 10q and gain of chromosome 1q are more frequent in Group 3 tumors. *NPR3* and *KCNA1* were proposed as biomarkers for Group 3 and Group 4, respectively [42].

Until recently, there were no known targetable pathways in Group 3 medulloblastomas and the suggested intensification of treatment for these patients would necessarily result in increased toxicity and morbidity (see below).

4.4. Group 4 medulloblastomas

Group 4 tumors represent simultaneously the most common and the less well-understood subgroup of medulloblastomas. They are found across all age groups and have an intermediate prognosis although the adult patients show a reduced survival when compared to their pediatric counterparts. Group 4 medulloblastomas are usually of the classic variant and they rarely present with metastasis. The most frequent genetic aberration in Group 4 tumors, present in up to 80% of cases, is isochromosome 17q (i17q) although *MYCN* amplifications were also reported. The expression of follistatin-related protein 5 (*FSTL5*) was identified as a marker of high-risk Group 4 patients [50].

4.5. From genomic revolution to clinical trials in medulloblastoma

Despite important advances in medulloblastoma treatment, approximately 40% of children will have recurrence and 30% will die from the disease. Moreover, the survivors are often left with significant disabilities due to cytotoxic side effects of chemotherapy and radiation to the developing central nervous system (CNS). Identifying the genetic events that drive medulloblastoma is, therefore, critical to develop more effective and less toxic therapies.

Except for *SHH* inhibitors that have shown some promise in *SHH* patients [51], there were no other targetable genes or pathways for *WNT*, Group 3 and Group 4 medulloblastomas. However, very recently published studies from four independent groups dissected the genomic landscape of medulloblastoma using large cohorts of patients and the latest high-throughput technology.

Using SNP arrays in a large cohort of over 1,000 medulloblastoma samples, Northcott *et al.* reported that somatic CNAs are a common event in medulloblastoma and are subgroup-specific [52]. In Group 3 tumors the authors identified recurrent *PVT1* gene fusions with *MYC* and *NDRG1* through chromothripsis, a process of erroneous DNA repair after chromosome shattering. This process of catastrophic DNA rearrangement has been previously shown in *SHH* medulloblastomas with *TP53* mutations [53]. The most frequent somatic CNA was a duplication of *SNCAIP*, a gene on chromosome 5q23.2 involved in Parkinson's disease. Interestingly, *SNCAIP* duplication is restricted to Group 4 α , a subtype of Group 4

with a relatively balanced genome when compared to Group 4 β [52]. The authors also reported novel targetable pathways that could be the basis for future clinical trials, including PI3K pathway in SHH, TGF- β pathway in Group 3 and NF- κ B pathway in Group 4 [52].

Another interesting observation, consistent with the first published study using genome sequencing in medulloblastoma [54], is the low number of somatic mutations found in these tumors when compared to adult solid tumors and the increased mutation frequency with age [52,55-57]. Jones *et al.* also identified tetraploidy as an early event in Group 3 and Group 4 medulloblastomas, concomitant with *TP53* mutations in some tumors [55].

The genome sequencing of independent cohorts of medulloblastoma samples and matched blood, identified previously known mutated genes (*CTNNB1*, *PTCH1*, *MLL2*, *SMARCA4*, *TP53*) but also allowed the discovery of new recurrent somatic mutations (*DDX3X*, *CTDNEP1*, *KDM6A*, *TBR1*, *GPS2*, *BCOR*, *LDB1*, *EZH2*, *CHD7*, *ZMYM3*), often subgroup-specific [52,55-57]. Notably, these studies identified genes involved in histone modification and chromatin remodeling complexes across all subgroups, which may explain the complexity and heterogeneity seen in medulloblastoma.

The ongoing genomic revolution in medulloblastoma is moving the next generation of clinical trials towards targeted treatments according to the molecular subgroup. SHH inhibitors, including GDC-0449 and NVP-LDE225, already proved its efficacy in tumor growth reduction but the reported acquired resistance suggests that a combination of targeted therapies may be a key approach to improve response [58]. Phase II clinical trials using GDC-0449 are now recruiting pediatric and adult patients with recurrent or refractory medulloblastoma and phase I trials with NVP-LDE225 are recruiting patients with advanced solid tumors including medulloblastoma. Under debate is the de-escalation of therapy in WNT patients and the intensification of treatment and/or targeted therapy for Group 3 patients [59,60]. Finally, the discovery of a significant number of chromatin modifier genes across medulloblastoma subgroups suggests that histone deacetylase inhibitors may constitute a good therapeutic option in the future.

5. Ependymoma

Ependymoma, the third most common brain tumor in childhood, is still incurable in up to 45% of patients [61]. The gold standard of treatment is maximal safe surgical resection followed by radiation since chemotherapy is usually ineffective. Ependymomas can arise in different regions of the CNS including the cerebral hemispheres, the posterior fossa and the spinal cord. This diversity is extended to its demographic, genetic, clinical and prognostic characteristics. Both children and adults can be affected although posterior fossa tumors are more common in children and supratentorial and spinal tumors occur more frequently in adults. The clinical behavior of ependymoma is variable with some patients experiencing a fatal clinical course while others have a long recurrence-free survival. The lack of novel targeted treatments for ependymoma can be explained by the paucity of cell lines and animal models of the disease.

The genetic heterogeneity of ependymoma has been highlighted by different studies with some cohorts of tumors showing frequent chromosomal alterations and others displaying a balanced genome. Korshunov *et al.* identified gain of chromosome 1q, *CDKN2A* homozygous deletion and age at diagnosis as independent factors of worse prognosis in ependymoma [62]. Johnson *et al.* described subgroups of ependymoma clustered by their CNAs, messenger RNA (mRNA) and microRNA (miRNA) profiles and, interestingly, tumors were segregated by their CNS location [63]. Furthermore, the authors were able to generate a mouse model of supratentorial ependymoma presenting strong evidence that the radial glial cells are likely the cells of origin of this tumor [63]. More recently, Witt *et al.* transcriptionally profiled two large independent cohorts of posterior fossa tumors identifying two distinct subgroups, Group A and Group B ependymomas [64]. Group A tumors comprise only posterior fossa ependymomas while Group B tumors include posterior fossa tumors that clustered with spinal ependymomas. Patients with Group A ependymomas are younger (median age 2.5 years), with the majority of tumors located laterally and with a balanced genome. These patients have higher incidence of recurrence and metastasis and a worse prognosis (5 year overall survival of 69%). Patients with Group B ependymomas are older (median age 20 years), with tumors in the midline (95%) and a high degree of genomic instability (Figure 2). Although several cytogenetic abnormalities were found in this group, including loss of chromosomes 1, 2, 3, 6, 8, 10, 14q, 17q, 22q, and gain of chromosomes 4, 5q, 7, 9, 11, 12, 15q, 18, 20, and 21q, patients have a good prognosis (5 year overall survival of 95%). The genes that characterize Group B ependymomas are involved in microtubule assembly and oxidative metabolism while Group A tumors include several pathways associated with cancer. The authors identified *LAMA2* and *NELL2* as markers of Group A and Group B ependymomas, respectively [64]. Using unsupervised cluster analysis of gene expression signatures, others also described two groups of infratentorial ependymomas (Group 1 and Group 2) that overlap with Group A and Group B of Witt *et al.*, respectively [65]. The distinct genetic profiles of posterior fossa ependymomas suggest that novel targeted therapies against subgroup-specific pathways maybe the key strategy to improve survival, particularly in Group A patients.

The interesting observation that up to 50% of pediatric ependymomas have a balanced genome [62] raises the possibility that epigenetic mechanisms may play a role in ependymoma pathogenesis. Most studies have focused on promoter hypermethylation of candidate genes known to be tumor suppressor genes in ependymoma or frequently methylated in other cancers. *HIC-1* and *RASSF1A* promoter hypermethylation were described in 83% and 86% of ependymomas, respectively. They are known to be tumor suppressor genes, silenced by hypermethylation in many human cancers [66]. Promoter hypermethylation of other genes, including *CDKN2A* (21%), *CDKN2B* (32%), *p14ARF* (21%), and *MGMT* (27%) were also reported in studies with large cohorts of tumor samples [66]. More recently, Rogers *et al.* used an array-based analysis to determine the methylation profile of 98 ependymomas [67]. The authors found that supratentorial and spinal ependymomas have a hypermethylated phenotype and that the genes identified are involved in cell growth and apoptosis. The increase in promoter methylation of CpG islands across a large number of genes has been described in other cancers as CpG island methylator phenotype (CIMP). Although it has been

correlated to a worse outcome in other cancers, the authors could not find an association between methylation and prognosis in ependymomas [67].

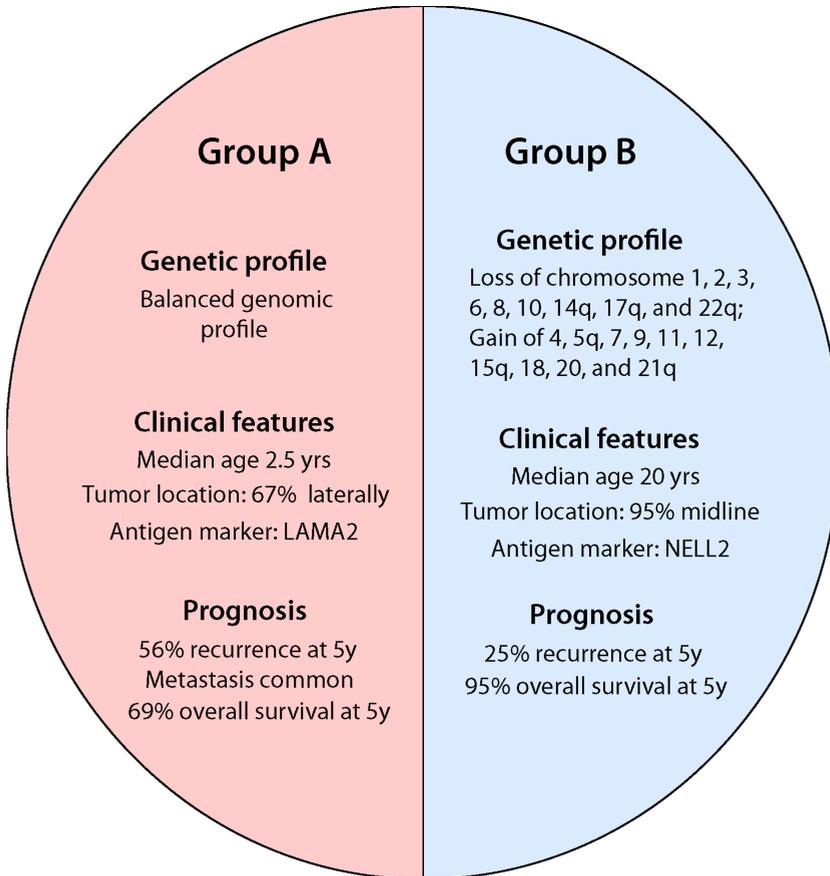


Figure 2. Features of the posterior fossa ependymoma subgroups, including genetic profile, clinical features and prognosis.

Another less frequent epigenetic event in cancer genomes is hypomethylation. This loss of DNA methylation occurs mainly in repetitive elements (Alu repeats). In a recent study, Xie *et al.* used a genome-wide approach to study the methylation profiles of Alu repeat sequences in pediatric intracranial ependymomas [68]. Notably, they identified a global loss of methylation in the regions flanking, rather than within, Alu sequences, and this was corre-

lated with a more aggressive tumor phenotype. The biological significance of this finding is yet to be unraveled.

The increased knowledge of the genetic and epigenetic events that drive ependymoma may lead to more effective targeted therapies aimed to repair molecular functions and dysregulated pathways. However, there are currently no clinical trials evaluating specific molecular therapies in ependymoma. Despite the recent achievements in ependymoma research, greater progress is needed to decipher the molecular and biological mechanisms of this disease and, ultimately, to improve patient's clinical outcome.

6. Conclusion

Major steps have been made to a better understanding of the molecular genetics underlying the most common pediatric brain tumors. An important advance was to recognize that adult and pediatric brain tumors are distinct and, therefore, need different therapeutic approaches. This knowledge opened new avenues for targeted therapies and clinical trials based on tumor-specific molecular subgrouping are currently ongoing. When compared to standard chemotherapy and radiation, the use of biological agents has several advantages. They can target cancer cells and spare normal cells in the developing CNS of children and also be used to delay radiotherapy, which is responsible for long-term side effects of treatment. Many of the newer agents are small molecules, with low molecular weight, which facilitates blood-brain barrier penetration. However, despite the enthusiasm with the phase I and phase II clinical trials using biological agents as monotherapy, mainly for progressive and recurrent brain tumors, efficacy has not yet been proven. In the future, combination therapies will likely be needed to target multiple pathways involved in tumorigenesis and to overcome the cytostatic effect of several biological agents. As the amount of data generated by high-throughput studies increases the drugable targets for each pediatric brain tumor, the number of clinical trials will continue to expand aiming a better control of the disease with less morbidity and extended survival.

Acknowledgments

Claudia Faria was supported by a fellowship from The Hospital for Sick Children Research Training Centre and the Garron Family Cancer Centre. She is a PhD candidate from The Programme for Advanced Medical Education, supported by Fundação Gulbenkian, Fundação Champalimaud, Ministério da Saúde e Fundação para a Ciência e Tecnologia, Portugal. This work was also supported by grants from the Canadian Cancer Society Research Institute (grant no. 019073), the Wiley fund, the Laurie Berman Fund for Brain Tumour Research, Pediatric Brain Tumour Foundation of the United States, and B.r.a.i.n.child.

Author details

Claudia C. Faria¹, Christian A. Smith² and James T. Rutka¹

1 Division of Neurosurgery and Labatt Brain Tumour Research Centre, The Hospital for Sick Children, University of Toronto, Canada

2 Labatt Brain Tumour Research Centre, The Hospital for Sick Children, University of Toronto, Canada

References

- [1] Ohgaki H, Kleihues P. Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas. *J Neuropathol Exp Neurol.* 2005 Jun;64(6):479-89.
- [2] Dubuc AM, Northcott PA, Mack S, Witt H, Pfister S, Taylor MD. The genetics of pediatric brain tumors. *Curr Neurol Neurosci Rep.* 2010 May;10(3):215-23.
- [3] Pfister S, Janzarik WG, Remke M, Ernst A, Werft W, Becker N, et al. BRAF gene duplication constitutes a mechanism of MAPK pathway activation in low-grade astrocytomas. *J Clin Invest.* 2008 May;118(5):1739-49.
- [4] Hawkins C, Walker E, Mohamed N, Zhang C, Jacob K, Shirinian M, et al. BRAF-KIAA1549 fusion predicts better clinical outcome in pediatric low-grade astrocytoma. *Clin Cancer Res.* 2011 Jul 15;17(14):4790-8.
- [5] Jones DT, Gronych J, Lichter P, Witt O, Pfister SM. MAPK pathway activation in pilocytic astrocytoma. *Cell Mol Life Sci.* 2012 Jun;69(11):1799-811.
- [6] Korshunov A, Meyer J, Capper D, Christians A, Remke M, Witt H, et al. Combined molecular analysis of BRAF and IDH1 distinguishes pilocytic astrocytoma from diffuse astrocytoma. *Acta Neuropathol.* 2009 Sep;118(3):401-5.
- [7] MacDonald TJ, Aguilera D, Kramm CM. Treatment of high-grade glioma in children and adolescents. *Neuro Oncol.* 2011 Oct;13(10):1049-58.
- [8] Faury D, Nantel A, Dunn SE, Guiot MC, Haque T, Hauser P, et al. Molecular profiling identifies prognostic subgroups of pediatric glioblastoma and shows increased YB-1 expression in tumors. *J Clin Oncol.* 2007 Apr 1;25(10):1196-208.
- [9] Pollack IF, Hamilton RL, Burger PC, Brat DJ, Rosenblum MK, Murdoch GH, et al. Akt activation is a common event in pediatric malignant gliomas and a potential adverse prognostic marker: a report from the Children's Oncology Group. *J Neurooncol.* 2010 Sep;99(2):155-63.

- [10] Bax DA, Mackay A, Little SE, Carvalho D, Viana-Pereira M, Tamber N, et al. A distinct spectrum of copy number aberrations in pediatric high-grade gliomas. *Clin Cancer Res*. 2010 Jul 1;16(13):3368-77.
- [11] Pollack IF, Finkelstein SD, Burnham J, Holmes EJ, Hamilton RL, Yates AJ, et al. Age and TP53 mutation frequency in childhood malignant gliomas: results in a multi-institutional cohort. *Cancer Res*. 2001 Oct 15;61(20):7404-7.
- [12] Pollack IF, Finkelstein SD, Woods J, Burnham J, Holmes EJ, Hamilton RL, et al. Expression of p53 and prognosis in children with malignant gliomas. *N Engl J Med*. 2002 Feb 7;346(6):420-7.
- [13] Qu HQ, Jacob K, Fatet S, Ge B, Barnett D, Delattre O, et al. Genome-wide profiling using single-nucleotide polymorphism arrays identifies novel chromosomal imbalances in pediatric glioblastomas. *Neuro Oncol*. 2010 Feb;12(2):153-63.
- [14] Schwartzenuber J, Korshunov A, Liu XY, Jones DT, Pfaff E, Jacob K, et al. Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. *Nature*. 2012 Feb 9;482(7384):226-31.
- [15] Nageswara Rao AA, Scafidi J, Wells EM, Packer RJ. Biologically targeted therapeutics in pediatric brain tumors. *Pediatr Neurol*. 2012 Apr;46(4):203-11.
- [16] Hargrave D, Bartels U, Bouffet E. Diffuse brainstem glioma in children: critical review of clinical trials. *Lancet Oncol*. 2006 Mar;7(3):241-8.
- [17] Jansen MH, van Vuurden DG, Vandertop WP, Kaspers GJ. Diffuse intrinsic pontine gliomas: a systematic update on clinical trials and biology. *Cancer Treat Rev*. 2012 Feb;38(1):27-35.
- [18] Roujeau T, Machado G, Garnett MR, Miquel C, Puget S, Geoerger B, et al. Stereotactic biopsy of diffuse pontine lesions in children. *J Neurosurg*. 2007 Jul;107(1 Suppl):1-4.
- [19] Pirotte BJ, Lubansu A, Massager N, Wikler D, Goldman S, Levivier M. Results of positron emission tomography guidance and reassessment of the utility of and indications for stereotactic biopsy in children with infiltrative brainstem tumors. *J Neurosurg*. 2007 Nov;107(5 Suppl):392-9.
- [20] Geoerger B, Hargrave D, Thomas F, Ndiaye A, Frappaz D, Andreiuolo F, et al. Innovative Therapies for Children with Cancer pediatric phase I study of erlotinib in brainstem glioma and relapsing/refractory brain tumors. *Neuro Oncol*. 2011 Jan;13(1):109-18.
- [21] Angelini P, Hawkins C, Laperriere N, Bouffet E, Bartels U. Post mortem examinations in diffuse intrinsic pontine glioma: challenges and chances. *J Neurooncol*. 2011 Jan;101(1):75-81.
- [22] Broniscer A, Baker JN, Baker SJ, Chi SN, Geyer JR, Morris EB, et al. Prospective collection of tissue samples at autopsy in children with diffuse intrinsic pontine glioma. *Cancer*. 2010 Oct 1;116(19):4632-7.

- [23] Caretti V, Jansen MH, van Vuurden DG, Lagerweij T, Bugiani M, Horsman I, et al. Implementation of a Multi-Institutional Diffuse Intrinsic Pontine Glioma Autopsy Protocol and Characterization of a Primary Cell Culture. *Neuropathol Appl Neurobiol.* 2012 Jul 27.
- [24] Paugh BS, Qu C, Jones C, Liu Z, Adamowicz-Brice M, Zhang J, et al. Integrated molecular genetic profiling of pediatric high-grade gliomas reveals key differences with the adult disease. *J Clin Oncol.* 2010 Jun 20;28(18):3061-8.
- [25] Paugh BS, Broniscer A, Qu C, Miller CP, Zhang J, Tatevossian RG, et al. Genome-wide analyses identify recurrent amplifications of receptor tyrosine kinases and cell-cycle regulatory genes in diffuse intrinsic pontine glioma. *J Clin Oncol.* 2011 Oct 20;29(30):3999-4006.
- [26] Zarghooni M, Bartels U, Lee E, Buczkowicz P, Morrison A, Huang A, et al. Whole-genome profiling of pediatric diffuse intrinsic pontine gliomas highlights platelet-derived growth factor receptor alpha and poly (ADP-ribose) polymerase as potential therapeutic targets. *J Clin Oncol.* 2010 Mar 10;28(8):1337-44.
- [27] Barrow J, Adamowicz-Brice M, Cartmill M, MacArthur D, Lowe J, Robson K, et al. Homozygous loss of ADAM3A revealed by genome-wide analysis of pediatric high-grade glioma and diffuse intrinsic pontine gliomas. *Neuro Oncol.* 2011 Feb;13(2):212-22.
- [28] Grill J, Puget S, Andreiuolo F, Philippe C, MacConaill L, Kieran MW. Critical oncogenic mutations in newly diagnosed pediatric diffuse intrinsic pontine glioma. *Pediatr Blood Cancer.* 2012 Apr;58(4):489-91.
- [29] Khuong-Quang DA, Buczkowicz P, Rakopoulos P, Liu XY, Fontebasso AM, Bouffet E, et al. K27M mutation in histone H3.3 defines clinically and biologically distinct subgroups of pediatric diffuse intrinsic pontine gliomas. *Acta Neuropathol.* 2012 Sep;124(3):439-47.
- [30] Wu G, Broniscer A, McEachron TA, Lu C, Paugh BS, Becksfors J, et al. Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. *Nat Genet.* 2012 Mar;44(3):251-3.
- [31] Bartels U, Hawkins C, Vezina G, Kun L, Souweidane M, Bouffet E. Proceedings of the diffuse intrinsic pontine glioma (DIPG) Toronto Think Tank: advancing basic and translational research and cooperation in DIPG. *J Neurooncol.* 2011 Oct;105(1):119-25.
- [32] Pollack IF, Jakacki RI, Blaney SM, Hancock ML, Kieran MW, Phillips P, et al. Phase I trial of imatinib in children with newly diagnosed brainstem and recurrent malignant gliomas: a Pediatric Brain Tumor Consortium report. *Neuro Oncol.* 2007 Apr;9(2):145-60.
- [33] Haas-Kogan DA, Banerjee A, Kocak M, Prados MD, Geyer JR, Fouladi M, et al. Phase I trial of tipifarnib in children with newly diagnosed intrinsic diffuse brainstem glioma. *Neuro Oncol.* 2008 Jun;10(3):341-7.

- [34] Geyer JR, Stewart CF, Kocak M, Broniscer A, Phillips P, Douglas JG, et al. A phase I and biology study of gefitinib and radiation in children with newly diagnosed brain stem gliomas or supratentorial malignant gliomas. *Eur J Cancer*. 2010 Dec;46(18):3287-93.
- [35] Broniscer A, Baker JN, Tagen M, Onar-Thomas A, Gilbertson RJ, Davidoff AM, et al. Phase I study of vandetanib during and after radiotherapy in children with diffuse intrinsic pontine glioma. *J Clin Oncol*. 2010 Nov 1;28(31):4762-8.
- [36] Pollack IF, Stewart CF, Kocak M, Poussaint TY, Broniscer A, Banerjee A, et al. A phase II study of gefitinib and irradiation in children with newly diagnosed brain-stem gliomas: a report from the Pediatric Brain Tumor Consortium. *Neuro Oncol*. 2011 Mar;13(3):290-7.
- [37] Massimino M, Bode U, Biassoni V, Fleischhack G. Nimotuzumab for pediatric diffuse intrinsic pontine gliomas. *Expert Opin Biol Ther*. 2011 Feb;11(2):247-56.
- [38] Taylor MD, Northcott PA, Korshunov A, Remke M, Cho YJ, Clifford SC, et al. Molecular subgroups of medulloblastoma: the current consensus. *Acta Neuropathol*. 2012 Apr;123(4):465-72.
- [39] Northcott PA, Korshunov A, Pfister SM, Taylor MD. The clinical implications of medulloblastoma subgroups. *Nat Rev Neurol*. 2012 Jun;8(6):340-51.
- [40] Remke M, Hielscher T, Northcott PA, Witt H, Ryzhova M, Wittmann A, et al. Adult medulloblastoma comprises three major molecular variants. *J Clin Oncol*. 2011 Jul 1;29(19):2717-23.
- [41] Fattet S, Haberler C, Legoix P, Varlet P, Lellouch-Tubiana A, Lair S, et al. Beta-catenin status in paediatric medulloblastomas: correlation of immunohistochemical expression with mutational status, genetic profiles, and clinical characteristics. *J Pathol*. 2009 May;218(1):86-94.
- [42] Northcott PA, Korshunov A, Witt H, Hielscher T, Eberhart CG, Mack S, et al. Medulloblastoma comprises four distinct molecular variants. *J Clin Oncol*. 2011 Apr 10;29(11):1408-14.
- [43] Thompson MC, Fuller C, Hogg TL, Dalton J, Finkelstein D, Lau CC, et al. Genomics identifies medulloblastoma subgroups that are enriched for specific genetic alterations. *J Clin Oncol*. 2006 Apr 20;24(12):1924-31.
- [44] Northcott PA, Hielscher T, Dubuc A, Mack S, Shih D, Remke M, et al. Pediatric and adult sonic hedgehog medulloblastomas are clinically and molecularly distinct. *Acta Neuropathol*. 2011 Aug;122(2):231-40.
- [45] Northcott PA, Dubuc AM, Pfister S, Taylor MD. Molecular subgroups of medulloblastoma. *Expert Rev Neurother*. 2012 Jul;12(7):871-84.

- [46] Al-Halabi H, Nantel A, Klekner A, Guiot MC, Albrecht S, Hauser P, et al. Preponderance of sonic hedgehog pathway activation characterizes adult medulloblastoma. *Acta Neuropathol.* 2011 Feb;121(2):229-39.
- [47] Ellison DW, Dalton J, Kocak M, Nicholson SL, Fraga C, Neale G, et al. Medulloblastoma: clinicopathological correlates of SHH, WNT, and non-SHH/WNT molecular subgroups. *Acta Neuropathol.* 2011 Mar;121(3):381-96.
- [48] Kool M, Korshunov A, Remke M, Jones DT, Schlanstein M, Northcott PA, et al. Molecular subgroups of medulloblastoma: an international meta-analysis of transcriptome, genetic aberrations, and clinical data of WNT, SHH, Group 3, and Group 4 medulloblastomas. *Acta Neuropathol.* 2012 Apr;123(4):473-84.
- [49] Cho YJ, Tsherniak A, Tamayo P, Santagata S, Ligon A, Greulich H, et al. Integrative genomic analysis of medulloblastoma identifies a molecular subgroup that drives poor clinical outcome. *J Clin Oncol.* 2011 Apr 10;29(11):1424-30.
- [50] Remke M, Hielscher T, Korshunov A, Northcott PA, Bender S, Kool M, et al. FSTL5 is a marker of poor prognosis in non-WNT/non-SHH medulloblastoma. *J Clin Oncol.* 2011 Oct 10;29(29):3852-61.
- [51] Rudin CM, Hann CL, Laterra J, Yauch RL, Callahan CA, Fu L, et al. Treatment of medulloblastoma with hedgehog pathway inhibitor GDC-0449. *N Engl J Med.* 2009 Sep 17;361(12):1173-8.
- [52] Northcott PA, Shih DJ, Peacock J, Garzia L, Morrissy AS, Zichner T, et al. Subgroup-specific structural variation across 1,000 medulloblastoma genomes. *Nature.* 2012 Aug 2;488(7409):49-56.
- [53] Rausch T, Jones DT, Zapatka M, Stutz AM, Zichner T, Weischenfeldt J, et al. Genome sequencing of pediatric medulloblastoma links catastrophic DNA rearrangements with TP53 mutations. *Cell.* 2012 Jan 20;148(1-2):59-71.
- [54] Parsons DW, Li M, Zhang X, Jones S, Leary RJ, Lin JC, et al. The genetic landscape of the childhood cancer medulloblastoma. *Science.* 2011 Jan 28;331(6016):435-9.
- [55] Jones DT, Jager N, Kool M, Zichner T, Hutter B, Sultan M, et al. Dissecting the genomic complexity underlying medulloblastoma. *Nature.* 2012 Aug 2;488(7409):100-5.
- [56] Pugh TJ, Weeraratne SD, Archer TC, Pomeranz Krummel DA, Auclair D, Bochicchio J, et al. Medulloblastoma exome sequencing uncovers subtype-specific somatic mutations. *Nature.* 2012 Aug 2;488(7409):106-10.
- [57] Robinson G, Parker M, Kranenburg TA, Lu C, Chen X, Ding L, et al. Novel mutations target distinct subgroups of medulloblastoma. *Nature.* 2012 Aug 2;488(7409):43-8.
- [58] Metcalfe C, de Sauvage FJ. Hedgehog fights back: mechanisms of acquired resistance against Smoothed antagonists. *Cancer Res.* 2011 Aug 1;71(15):5057-61.

- [59] Ramaswamy V, Northcott PA, Taylor MD. FISH and chips: the recipe for improved prognostication and outcomes for children with medulloblastoma. *Cancer Genet.* 2011 Nov;204(11):577-88.
- [60] Leary SE, Olson JM. The molecular classification of medulloblastoma: driving the next generation clinical trials. *Curr Opin Pediatr.* 2012 Feb;24(1):33-9.
- [61] Merchant TE, Li C, Xiong X, Kun LE, Boop FA, Sanford RA. Conformal radiotherapy after surgery for paediatric ependymoma: a prospective study. *Lancet Oncol.* 2009 Mar;10(3):258-66.
- [62] Korshunov A, Witt H, Hielscher T, Benner A, Remke M, Ryzhova M, et al. Molecular staging of intracranial ependymoma in children and adults. *J Clin Oncol.* 2010 Jul 1;28(19):3182-90.
- [63] Johnson RA, Wright KD, Poppleton H, Mohankumar KM, Finkelstein D, Pounds SB, et al. Cross-species genomics matches driver mutations and cell compartments to model ependymoma. *Nature.* 2010 Jul 29;466(7306):632-6.
- [64] Witt H, Mack SC, Ryzhova M, Bender S, Sill M, Isserlin R, et al. Delineation of two clinically and molecularly distinct subgroups of posterior fossa ependymoma. *Cancer Cell.* 2011 Aug 16;20(2):143-57.
- [65] Wani K, Armstrong TS, Vera-Bolanos E, Raghunathan A, Ellison D, Gilbertson R, et al. A prognostic gene expression signature in infratentorial ependymoma. *Acta Neuropathol.* 2012 May;123(5):727-38.
- [66] Dubuc AM, Mack S, Unterberger A, Northcott PA, Taylor MD. The epigenetics of brain tumors. *Methods Mol Biol.* 2012;863:139-53.
- [67] Rogers HA, Kilday JP, Mayne C, Ward J, Adamowicz-Brice M, Schwalbe EC, et al. Supratentorial and spinal pediatric ependymomas display a hypermethylated phenotype which includes the loss of tumor suppressor genes involved in the control of cell growth and death. *Acta Neuropathol.* 2012 May;123(5):711-25.
- [68] Xie H, Wang M, Bonaldo Mde F, Rajaram V, Stellpflug W, Smith C, et al. Epigenomic analysis of Alu repeats in human ependymomas. *Proc Natl Acad Sci U S A.* 2010 Apr 13;107(15):6952-7.

