Molecular genetics of ependymoma

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Abstract

Brain tumors are the leading cause of cancer death in children, with ependymoma being the third most common and posing a significant clinical burden. Its mechanism of pathogenesis, reliable prognostic indicators, and effective treatments other than surgical resection have all remained elusive. Until recently, ependymoma research was hindered by the small number of tumors available for study, low resolution of cytogenetic techniques, and lack of cell lines and animal models. Ependymoma heterogeneity, which manifests as variations in tumor location, patient age, histological grade, and clinical behavior, together with the observation of a balanced genomic profile in up to 50% of cases, presents additional challenges in understanding the development and progression of this disease. Despite these difficulties, we have made significant headway in the past decade in identifying the genetic alterations and pathways involved in ependymoma tumorigenesis through collaborative efforts and the application of microarray-based genetic (copy number) and transcriptome profiling platforms. Genetic characterization of ependymoma unraveled distinct mRNA-defined subclasses and led to the identification of radial glial cells as its cell type of origin. This review summarizes our current knowledge in the molecular genetics of ependymoma and proposes future research directions necessary to further advance this field.

Keywords Ependymoma, brain tumor, cytogenetics, genetics, epigenetics, gene expression, subgroups, cells of origin, radial glial cells

Brain tumors are the most common childhood solid malignancy and have become the leading cause of cancer mortality in children¹,². Ependymoma is the third most common pediatric brain tumor, following astrocytoma and medulloblastoma, with over 50% of cases arising in children under 5 years of age³,⁴. These tumors arise from the cells lining the wall of the ventricular system along the entire craniospinal axis and can occur in three distinct locations: the supratentorial brain comprising the cerebral hemispheres, the region around the brain stem and cerebellum known as the posterior fossa, and the spinal cord⁵,⁶. Over 90% of pediatric ependymomas are intracranial, with two thirds occurring in the posterior fossa⁷. Many adult cases, on the other hand, occur in the spinal cord⁷.

The primary treatment for ependymoma remains surgical resection followed by radiotherapy, with gross total resection frequently reported as the most important prognostic factor⁸-¹². Although postoperative radiotherapy may induce stabilization and, occasionally, regression of residual disease, most incompletely resected tumors ultimately progress¹³. Some studies have even shown that local tumor recurrence can still develop in up to 50% of cases despite complete tumor removal in conjunction with radiotherapy⁸-¹⁰. No chemotherapy regimen has prolonged overall survival in children with ependymoma¹⁰,¹¹,¹³-¹⁵. Due to the lack of salvage therapies for patients who relapse, the 5-year overall and progression-free survival rates for patients with ependymoma are merely 60% and 30%, respectively¹⁶. Survivors are often left with serious physical and neurocognitive disabilities secondary to the disease and its treatment¹⁵,¹⁷-¹⁹. Furthermore, very dismal outcome is often observed in younger patients. This is possibly due to a higher incidence of high-grade ependymomas; a higher frequency of tumors of the lateral posterior fossa, which tend to infiltrate into neighboring vital structures and therefore complicate gross total resection; and the necessary delay in initiating

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radiotherapy for those under 3 years of age \cite{28}. Indeed, ependymoma presents a heavy clinical burden, as the mechanism of its pathogenesis, reliable prognostic indicators, and effective chemotherapy and targeted therapies all remain elusive.

Ependymoma, like other cancers, is a genetic disease. Given that the relationship between ependymoma tumor grade, histological appearance and prognosis is highly controversial \cite{8,22}, and that tumors of the same histological type often display variable clinical behaviors \cite{8,23}, it is imperative to probe deeper into the molecular genetic basis of ependymoma initiation and progression in search of reliable prognostic markers and therapeutic targets. Here, we review knowledge accumulated through the years on the molecular genetics of ependymoma, from the familial genetic risk factors and early cytogenetic detections of broad chromosomal anomalies to the identification of candidate driver genetic events and pathways in ependymoma tumorigenesis. We also present our most current understanding of ependymoma heterogeneity and its cells of origin. Finally, we conclude by suggesting possible future directions in ependymoma research.

**Familial Syndromes and Risk Factors**

No clear etiology has been associated with most ependymomas to date. Unlike many other cancers for which existing familial cancer syndromes provided important clues for our initial understanding of tumorigenic mechanisms, there are few known familial ependymoma syndromes. We do know, however, that there is increased incidence of spinal intramedullary ependymomas in patients with neurofibromatosis type 2 (NF2) \cite{24,25}. The NF2 gene is located on chromosome 22q, which is frequently lost in patients with spinal ependymomas \cite{25,26,27}. However, many of these tumors, especially those that occur intracranially, do not harbor NF2 mutations. Thus, despite that the NF2 gene may be important in the formation of some spinal ependymomas, it is probably not the critical tumor suppressor gene on chromosome 22q that is involved in sporadic intracranial ependymoma tumorigenesis \cite{25,26,27}. Ependymoma has also been reported in patients with Li-Fraumeni syndrome, i.e. germline mutation of the TP53 tumor suppressor gene, but such occurrences as well as somatic mutations of TP53 in sporadic ependymomas are rare, thus diminishing the role of p53 in ependymoma tumorigenesis \cite{28}. There has been one report of a patient with Turcot syndrome, i.e. germline mutation of the adenomatous polyposis coli (APC) gene, whose loss of function activates the Wnt pathway and predisposes the patient to colorectal cancer, who developed multiple ependymomas located intracranially and spinaly \cite{29,30}.

Both intracranial and spinal ependymomas have also been observed in patients with the multiple endocrine neoplasia type I (MEN1) syndrome \cite{29,30}. However, the role of the APC gene/Wnt signaling activation and that of MEN1 in sporadic ependymoma tumorigenesis remain unknown. Furthermore, there are a few families with increased ependymoma incidence but without any currently known familial cancer syndromes \cite{31,32}. Two such families have loss of 22q but lack NF2 mutation, further suggesting the presence of another crucial tumor suppressor gene at that chromosomal region \cite{33,34}.

DNA sequences similar to SV40 virus and the virus-encoded large T-antigen have also been found in some ependymomas \cite{41,42}. Furthermore, ependymoma can be induced in rodents through intracerebral inoculation of the SV40 virus \cite{43,44}. Nevertheless, several studies have disqualified the SV40 tumor virus as a causative agent of ependymoma \cite{45,46}. The strongest opposing argument is based on epidemiologic studies that showed no increase in the incidence of ependymoma and other cancers in the years following the massive introduction of SV40-contaminated polio vaccines into the human population \cite{45,46}. To date, knowledge of whether SV40 virus contributes to ependymoma tumorigenesis remains unknown; and if it does, the oncogenic pathways on which it acts remain to be elucidated.

**Cytogenetic Abnormalities**

Over the years, cytogenetic studies using karyotyping and comparative genomic hybridization (CGH) have reported numerous broad chromosomal abnormalities in ependymoma. Results varied considerably among early studies largely due to their small sample sizes and the variations among sample sets in terms of patient age and anatomical tumor location. However, pediatric and adult ependymomas were soon realized to be biologically distinct \cite{4}. In fact, increasing evidence supports that ependymomas are heterogeneous and can be classified as distinct disease subtypes based on patient age, anatomical tumor location, and genetic alterations \cite{6,47,48}.

Frequently observed genomic anomalies in pediatric ependymomas include loss of chromosomes 1p, 2, 3, 6/6q, 9p, 13q, 17, and 22 as well as gains of 1q, 5, 7, 8, 9, 11, 18, and 20, with the gain of 1q occurring in over 20% of cases being the most common \cite{49}. In adult ependymomas, chromosomes 6, 10, 13q, 14q, 16, and 22/22q are frequently lost whereas chromosomes 2, 5, 7, 9, 12, 18, and X are gained, with gains of 7 and 9 and loss of 22q being the most frequently observed, though with each occurring in only 30% of cases or less \cite{49}. Location-specific genomic anomalies observed in intracranial versus spinal ependymomas roughly correspond to those seen in children versus adults, as
most pediatric ependymomas occur intracranially whereas adult cases predominantly occur within the spinal cord [4]. Aside from genomic gains and losses, cytogenetic studies have also identified translocations within the ependymoma genome, often involving chromosomes 1, 11, and 22 [49-52]. Adult ependymomas have been observed to display more frequent and broader chromosomal aberrations than pediatric tumors. Based on a meta-analysis of all CGH studies performed on more than 300 primary ependymomas, Kilday et al. [4] calculated that there are on average 7.5 and 3.8 genomic anomalies per adult and pediatric tumor, respectively. This finding is reinforced given that over 40% of pediatric ependymomas exhibit balanced profiles, whereas a balanced genomic profile is observed in less than 10% of adult cases [49,53]. Interestingly, the large number of genomic aberrations often seen in adult spinal ependymomas is associated with tumors of lower histological grades and favorable patient outcome [50,53,54]. Furthermore, according to the CGH analysis done by Dyer et al. [54] on pediatric intracranial ependymomas, tumors can be subdivided into three distinct subgroups based on the number of chromosomal anomalies detected per tumor. Tumors with a balanced genetic profile make up the “balanced” group, which is significantly associated with an infant age at diagnosis. The second “structural” group shows few and mainly partial genomic imbalances. Lastly, the third “numerical” group exhibits 13 or more primarily whole chromosome imbalances similar to those often seen in adult ependymomas. These subdivisions are significantly associated with prognosis, with the numerical group demonstrating the best patient outcome and the structural group doing the worst. Consistent with this observation, almost all recurrent ependymomas exhibit genetic profiles characteristic of the structural group [50,53,54].

**Molecular Genetic Aberrations**

Despite the identification of the aforementioned common genomic gains and losses in ependymomas and their cytogenetic profile-based stratification, few insights into the oncogenes, tumor suppressors, and molecular pathways responsible for the development of ependymoma could be obtained from these findings. Furthermore, specific genetic events could not be identified as prognostic markers for this disease at only chromosome-level resolution. These chromosome-level aberrations are broad and typically span numerous genes, making it difficult to discriminate driver genetic events from passenger events. Recently, array CGH (aCGH) has been adopted by the research community to fine-map copy number variations in cancer at much higher resolutions. The list of genes within the common regions of amplification or deletion identified using aCGH can be further narrowed through correlation with their expression levels. This permits the discovery of candidate driver genes for ependymoma development, with putative oncogenes and tumor suppressor genes exhibiting copy number-driven expression. Indeed, the advances in microarray and next generation sequencing technologies have permitted the examination of ependymoma genetics in terms of copy number variations and gene expression levels in much greater detail.

Irrespective of anatomical tumor location or patient age, monosomy 22 and allelic losses on chromosome 22q have been found in numerous studies to be the most common genetic abnormalities in sporadic ependymoma, with frequencies ranging from 26% to 71% [3,49]. Initial quests for tumor suppressor genes present on 22q focused on NF2 located at 22q12; however, NF2 mutation is not associated with the majority of ependymoma cases [25-27]. Another potential tumor suppressor gene is hSNF5/INI1 at 22q11.23. Kraus et al. [55] found no mutations or homozygous deletions of this gene in a series of 53 ependymomas, and this gene has not been shown to be silenced by DNA promoter methylation [56]. Mapping of deletions and translocation breakpoints on 22q using high-resolution techniques revealed 22pter–22q11.2, 22q11, 22q11.21–12.2, and 22q13.1–13.3 to be the “hotspots” where the elusive tumor suppressor gene is likely to be found [57-59]. Within the frequently deleted region 22q12.3–q13.3, Karakoula et al. [51] found RAC2 and C22orf22 to be deleted in 38% and 32% of the 47 pediatric intracranial ependymomas analyzed, respectively. In over 60% of these ependymomas, C22orf22 was found to be transcriptionally inactive, indicating its potential importance in the development of pediatric intracranial ependymomas. Loss of RAC2, on the other hand, was shown to be a prognostic factor significantly associated with shorter overall survival in patients younger than 2 years. Using gene expression microarray technology, Suarez-Merino et al. [60] found the transcripts of four genes mapping to 22q12.3–22q13.33, namely C22orf22, as identified by Karakoula et al. [51] mentioned above, FBX7, CBX7, and SBF1, to be under-expressed in pediatric ependymomas as compared to normal brain controls. Allelic loss of one of these genes, CBX7 located at 22q13.1, could be detected in 55% of ependymoma cases. Interestingly, CBX7 controls cellular lifespan through regulating both the p16INK4a/Rb and the Arf/p53 pathways [61]. The role of these pathways in ependymoma is unclear, though their deregulation is central to many types of cancer, including gliomas [62-64]. Furthermore, deletion and hypermethylation of CDKN2A/P16 at 9q21.3 and RB at 13q14.2 have been reported in
ependymomas. In the same study by Suarez-Merino et al., the expression of SCHIP-1 was significantly down-regulated in pediatric ependymomas. SCHIP-1 is known to interact with the NF2 gene product merlin, and their interaction is regulated by conformational changes in merlin induced by post-translational modifications, alternative splicing, or mutations. Furthermore, by integrating the genomic and expression profiles of 24 primary intracranial ependymomas, Modena et al. identified down-regulation of the SULT4A1 gene located at 22q13.3. In pediatric intracranial ependymomas, the most common genomic aberration is the gain of chromosome 1q, which is associated with tumors in the posterior fossa location in children and with anaplastic histological features. It is also a significant predictor of tumor aggressiveness and poor patient outcome. Interestingly, 1q gain is occasionally the only observable alteration, with few other chromosome imbalances detected in ependymomas; yet, in some cases, it marks tumor recurrence. This suggests the presence of genes located on 1q that may be involved in the initiation, progression, and/or therapeutic resistance of ependymoma. Thus, efforts have been made to determine the critical region on chromosome 1q for the identification of these crucial genes. Ward et al. reported a minimal overlapping region with high-copy amplification at 1q24–31 in pediatric ependymomas. Subsequently, an aCGH study done on 49 sporadic intracranial ependymomas by Mendrzyk et al. identified two commonly gained regions on 1q, one at 1q21.3–23.1 and another at 1q31.1–31.3. They also found that gains of 1q21.1–32.1 were correlated with tumor recurrence and identified the gain of 1q25 as an independent prognostic marker for significantly lower recurrence-free or overall survival rate. Additionally, they identified DUSP12, found to be over-expressed in all their tested samples, as a candidate gene located at 1q23.3. The mRNA level of DUSP12 correlates with that of cyclin D1 throughout the cell cycle, suggesting its role in regulating cell division and potentially in neoplastic transformation. DUSP12 was also found to be important for cell survival in response to heat-shock-induced cell death, which further supports its proposed oncogenic function. Gene expression analyses correlated with copy number variations have since uncovered additional candidate oncogenes located within 1q21–32, including laminin, PRELP, HSPA6, GAC1, CHI3L1, TPR, JTB, SCH1, and S100A10 and other S100 family members. Among these, GAC1 amplification-driven over-expression has also been implicated in the pathogenesis of other malignant gliomas, suggesting its likely importance in ependymoma development.

In addition to chromosome 22q loss and 1q gain, other commonly identified chromosomal aberrations include deletion of chromosomes 6q and 9 and gain of chromosome 7, notably the region from 7q11.23–22.1, which is associated almost exclusively with spinal ependymomas. Candidate oncogenes proposed by analyzing recurrent gains on chromosome 7 include EGFR (epidermal growth factor receptor) at 7p11.2, TWIST1 and HDAC9 at 7p21.1, and ARHGFE5 at 7q34. EGFR in particular exhibits frequent gains and high-level amplifications in intracranial ependymomas, and its over-expression predicts poor patient outcome. Loss of chromosome 6q is found mostly in infratentorial tumors, whereas deletions on chromosome 9 occur more frequently in supratentorial tumors. With microsatellite analysis, LOH hotspots on chromosome 6 were determined to be 6q15–16, 6q21–22.1, and 6q24.3–25.3, which were further limited to 6q24.3 and 6q25.2–25.3. Locus 6q25.3, containing the SNX9 and SYNJ2 genes, was found to be the most frequently deleted. However, loss of 6q25.3 was a favorable prognostic marker for overall survival of patients with anaplastic intracranial ependymomas, as the deletion of the SNX9 and SYNJ2 genes, which are known to regulate cell migration and invasion, could inhibit tumor progression. Furthermore, the polyamine biosynthesis gene AMD1 and the cyclin-dependent kinase CDK11, both located at 6q21, as well as the tumor suppressor gene SASH1 at 6q24.3 were found to be under-expressed by Suarez-Merino et al. using microarray gene expression analysis. On chromosome 9, which is also frequently deleted in patients with ependymomas, homozygous deletion spanning the CDKN2A locus at 9q21.3 has been detected and is a characteristic of anaplastic supratentorial tumors. The molecular staging system developed by Korshunov et al. highlighted that CDKN2A deletion together with young age at diagnosis and gain of 1q comprise the most reliable independent indicators of unfavorable patient outcome. In contrast, gains of chromosomes 9, 15q, and 18 and loss of chromosome 6 are features indicating excellent chance of survival. Furthermore, detection of the expression of P14ARF protein by immunohistochemistry in 103 intracranial ependymomas revealed that decreased P14ARF expression is associated with tumor aggressiveness in terms of higher tumor grade, elevated growth fraction, and P53 protein accumulation. Using microsatellite analysis, Schneider et al. closely examined the aberrations on chromosome 9 in both adult and pediatric ependymomas and identified 9p21.1–22.3 and 9q31.3–33.2 to be the most commonly deleted regions on this chromosome. Potential tumor suppressor genes located within 9q31.3–33.2 include DBC1, which is frequently deleted in bladder cancer and also exhibits markedly reduced mRNA expression in gliomas, DEC1, whose
down-regulation driven by copy number loss is frequently seen in esophageal cancer and contributes to tumor cell motility [89,90]; LPAR1, which is known to mediate cell proliferation, differentiation, and migration among other functions [91]; and TXN, which inhibits apoptosis and enhances drug resistance in cancer cells [92,93].

Other frequently occurring regions of genomic imbalances have been revealed by profiling the ependymoma genome at high resolution [6,40,57,73,78,94–97] and are summarized in Table 1. Among these imbalances, the combined presence of 6p22-pter and 13q14.3-qter losses predicted significantly reduced survival in intracranial pediatric ependymomas [98]. Puget et al. [96] found that gains of 1q and 9qter and loss of 6q occurred more often in recurrent tumors. Interestingly, the specific 9qter region linked to tumor recurrence is associated with posterior fossa ependymomas, whereas chromosome 9 deletion is usually associated with supratentorial ependymomas. Candidate oncogenes and tumor suppressor genes proposed based on these copy number variation hotspots [6,73,78,94–97] are listed in Table 2.

Among the putative oncogenes in ependymoma are NOTCH1, NOTCH4, and JAG1, which are two of the membrane receptors and one of the ligands, respectively, of the Notch signaling pathway, suggesting the involvement of Notch signaling in ependymoma tumorigenesis. Furthermore, recurrent gains at 5p15.33, which includes the human telomerase reverse transcriptase (hTERT) gene, were validated by immunohistochemistry. Elevated hTERT expression has been shown to be associated with ependymoma progression and recurrence and is currently the most important predictor of survival for pediatric intracranial ependymomas independent of other clinicopathologic prognostic features [98,99,100]. Furthermore, hTERT expression relates with telomerase activity [99]. Recently, Wong et al. [101] proposed telomerase inhibition as a novel therapy for ependymoma after demonstrating its effects on reducing ependymoma cell viability by increasing DNA damage, decreasing proliferation, and increasing apoptosis.

In addition to fine-mapping genomic aberrations to identify candidate genes involved in ependymoma development, profiling studies have also been used to divide ependymomas into distinct subgroups that correlate with tumor location. Using the aCGH profiles of 103 ependymomas, Taylor et al. [6] categorized these tumors into three molecularly distinct subgroups that correlate with the anatomical location of the tumor, namely the supratentorial region, the posterior fossa, or the spine. Although ependymomas from these different anatomical regions are histologically indistinguishable, they are in fact molecularly distinct diseases that should be separately examined to determine the genetic events involved in tumorigenesis and progression, as well as prognostic factors and patient outcome. According to the results of their aCGH experiment, Taylor et al. [6] found that CDKN2A deletion occurred in >90% of supratentorial ependymomas but was rare in tumors from other regions of the central nervous system (CNS). Deletion of chromosome 22q12 was detected in mostly spinal but sometimes posterior fossa ependymomas. Furthermore, posterior fossa ependymomas could be further classified into three subgroups: tumors harboring multiple concurrent DNA amplifications, tumors with chromosome 1q gain, and tumors exhibiting a balanced genomic profile [6].

**Epigenetics**

Although aCGH analyses have considerably advanced our understanding of the genetic events in ependymomas, they do not provide insights into epigenetic alterations that can play a role in the tumorigenesis of these malignancies. In recent years, the use of epigenetic profiling has provided new understanding of ependymomas. Among the epigenetic abnormalities found in ependymomas, DNA methylation alterations are common. Abnormal DNA methylation may lead to inactivation of tumor suppressor genes, such as CDKN2A, CDKN1A, and CDKN1B. Furthermore, DNA hypomethylation may lead to the activation of oncogenes, such as MYC and RAS.

### Table 1. Regions of frequent gains and losses in the ependymoma genome

| Gains          | 1p34, 1q, 2p24, 2q23, 3p14, 3q29, 5p15.33, 6p21, 7p21, 7q11.23–22.1, 7q34, 7q35, 8q11.2, 9p24.3–qter, 9q22, 9qter, 10q25.2–26.3, 11q13–q23, 12p, 12q13.13–13.3, 13q21.1, 14q11.2, 14q32.2, 15q21.3, 16p11.2, 18p13.1–13.3, 20p12, Xq21.2, and Xq26.3 |
|----------------|--------------------------------------------------------------------------------|
| Losses         | 1p36, 3q23–qter, 4q33–qter, 5q31, 6p22–pter, 6q25.3, 6q26, 7q36, 9p21, 9p23, 9p24.31, 10q23–26, 12q13, 13q14.3–qter, 15q21.1, 16p12–13.1, 16q24, 17p13.3, 17q22–24, 18q22.2, 19p13.2–13.3, 20q12, Xq12, and Xq26.3 |

### Table 2. Putative oncogenes and tumor suppressor genes in ependymoma

| Oncogenes                  | DUSP12 (1p23.3), MYCN (2p24), DNAE1L3 (3q25.2), hTERT (5p15.33), NOTCH4 (6p21.32), EGRF (7p11.2), ARHGEF5 (7q34), EDG3 (9q22), SHC3 (9q22), TNC (9q33.1), NOTCH1 (9q34.3), STK32C (10q26.3), MDK (11p11.2), TYR (11p13), YAP1 (11q22), BIRC2 (11q22), BIRC3 (11q22), HOXC4 (12q13.13), MTAS (14q32.33), SLC6A10 (16p12.2), PRF7 (16q12.2), CDC6 (17p13.3), VAV1 (19p13.3), and JAG2 (20p12.2) |
|----------------------------|--------------------------------------------------------------------------------|
| Tumor suppressor genes     | ZNF262 (1p34.3), AJAP1 (1p36.32), CDN2A (9p21.3), FOXD4 (9p24.31), GRID1 (9q23.3), MINPP1 (9q23.31), TACC2 (10q26.13), TUBGCP2 (10q26.3), PRKCA (17q24.2), and SULT4A1 (22q13.3) |
Ependymoma tumorigenesis, almost half of ependymomas present a balanced aCGH profile, making it imperative to interrogate alternative mechanisms of gene regulation. Epigenetics in the form of promoter DNA (CpG) hypermethylation is an important route by which transcriptional inactivation can be achieved and, as in other cancers, likely plays a significant role in silencing tumor suppressor genes involved in ependymoma development. Unfortunately, epigenetic studies on ependymoma have been limited to candidate gene approaches, with the genes in question selected based on their roles as tumor suppressor genes and methylation status in other malignancies. Waha et al. \[\text{102}\] therefore investigated the methylation status of the hypermethylated in cancer1 (HIC-1) putative tumor suppressor gene, which exhibits hypermethylation and loss of expression in various tumors such as medulloblastoma and gliomas. Furthermore, the HIC-1 locus at chromosome 17p13.3 is frequently lost in ependymoma \[\text{92, 102}\]. They detected HIC-1 hypermethylation and down-regulation in 83% and 81% of ependymomas, respectively, and found that HIC-1 hypermethylation was significantly correlated with nonspinal localization and pediatric age \[\text{102}\]. The Ras association domain family 1 isom form A (RASSF1A) gene has also been found, independent of clinical and histological subtype, to be frequently silenced by methylation in ependymoma, with an incidence of 86% \[\text{100}\]. RASSF1A is a recently well-recognized tumor suppressor gene whose inactivation through promoter methylation is implicated in the development of many human cancers \[\text{104}\]. RNA interference experiments have shown that down-regulation of RASSF1A, an effector of Ras, results in loss of cell cycle control, enhanced genetic instability and cell motility, and resistance to K-Ras and tumor necrosis factor α (TNFα)-induced apoptosis \[\text{100}\]. Furthermore, Michalowski et al. \[\text{106}\] identified the TRAIL apoptosis pathway-related genes CASP8, TFRSF10C, TFRSF10D, and TNFRSF10C to be methylated in ependymoma, with incidences of 30%, 9.5%, 36.4%, and 9.5%, respectively. Other commonly methylated genes in ependymoma include DAPK, THBS1, TIP33, TP73, MGMT, GSTP1, CDKN2A, FHIT, RARB, BLU, and MCJ, with incidence ranging from 10% to 57% \[\text{96, 69, 70, 103, 105}\].

Gene Expression Profiles

Gene expression profiling employs microarray technology to capture gene expression levels of thousands of genes simultaneously. Integration of gene expression with copy number data allows one to determine the genes demonstrating copy number-driven expression as putative oncogenes and tumor suppressor genes. Moreover, by applying ontological analysis on the gene expression profiles, it is possible to uncover those aberrant cellular processes and pathways that contribute to ependymoma. Using microarray-based gene expression profiling to compare ependymoma with normal brain controls, Suarez-Merino et al. \[\text{102}\] identified 112 abnormally expressed genes in ependymoma. Genes with increased expression included the oncogene WNT5A, TP53 homologue TP63, and several cell cycle, proliferation, adhesion, and extracellular matrix genes such as the transcription factor ZIC1, the angiogenesis factor VEGF, and fibronectin 1 (FN1). Other putative oncogenes identified in this study that have been implicated in other cancers are COL4A1, IBP2, HOX7, WEE1, and GAC1. Genes that were found to be down-regulated included the NF2-interacting gene SCHIP-1, the APC-associated gene EB1, and genes that are involved in vesicle trafficking and recycling such as NCP1, RAB40B, TJ2, and SH3GL3.

Consistent with ependymoma subgroups based on aCGH profiles, ependymoma gene expression profiles are significantly associated with tumor location, patient age at disease onset, grade, and retrospective risk for relapse \[\text{6, 48, 73, 106}\]. Taylor et al. \[\text{9}\] found that supratentorial ependymomas expressed markedly elevated levels of members of the EphB-Ephrin (EPHB2/3/4 and EPHRIN A3/4) and Notch (JAGGED 1/2) signaling pathways, as well as genes involved in cell cycle regulation (Cyclin B2/D1/G2, CDK2/4, and CDKN1C/2C). On the other hand, the highly expressed genes that distinguished posterior fossa ependymomas were inhibitors of differentiation (ID1/2/4) and members of the aquaporin family (AQP1/3/4). Spinal ependymomas are characterized by the up-regulation of multiple homeobox (HOX) family members (HOXA7/9, HOXB6/7, and HOXC6/10) and insulin-like growth factor 1 (IGF1). Subsequently, gene expression profiling studies performed by Modena et al. \[\text{73}\] and Palm et al. \[\text{48}\] confirmed that intracranial ependymomas are indeed characterized by high expression levels of genes involved in Notch signaling and that spinal ependymomas are defined by over-expression of numerous HOX genes. Additionally, up-regulation of the sonic hedgehog (SHH) and bone morphogenetic protein (BMP) pathway members were also evident in intracranial ependymomas \[\text{48, 73}\].

Deregulated Notch signaling, which is crucial for neural development, is believed to play a significant role in ependymoma tumorigenesis, especially at the supratentorial location, since oncogenesis is thought to mirror normal development gone awry \[\text{107}\]. In addition to over-expression of the Notch ligands JAGGED 1/2 shown by Taylor et al. \[\text{10}\], there is consistent up-regulation of the Notch receptors (NOTCH1/2), ligands (JAGGED 1/2 and DLL1/3), and target genes (HES1/5, HEY2, c-MYC, and
ERBB2), whereas FBXW7, the major repressor of the Notch pathway, is consistently down-regulated\cite{48,73,96,108}. In an early study, missense mutations of NOTCH1, either in the heterodimerization domain C or the transactivation domain, were detected in 8.3% of pediatric intracranial ependymomas from the posterior fossa, thus making NOTCH1 the first oncogene found to be mutated in ependymomas\cite{98}. These mutations cause the Notch1 receptor to be constitutively active in a ligand-independent manner\cite{108}. Moreover, inhibition of Notch signaling with γ-secretase inhibitor 9-AC impaired ependymoma primary cell culture growth\cite{98}. Gilbertson et al.\cite{108} further demonstrated that high-level expression of ERBB receptors (ERBB2/4), which are direct targets of Notch signaling, could be found in over 75% of pediatric ependymomas and were significantly correlated to tumor proliferative activity as measured by the Ki-67 labeling index. Functional studies proved that activating ERBB receptor signaling in short-term ependymoma cell cultures resulted in AKT phosphorylation and cell proliferation, which could be effectively blocked in a dose-dependent manner with an inhibitor of ERBB tyrosine kinase activity. Coincidentally, we have recently learned that the SV40 virus, which was thought to be a causative agent for ependymoma, can in fact induce oncogenic transformation of human mesothelial cells through direct induction of NOTCH1 over-expression\cite{109}.

Currently, neither cell lines nor animal models are available to elucidate the sequential events in ependymoma development. Thus, researchers have compared the gene expression profiles of low grade versus high grade ependymomas and primary tumors versus recurrent tumors to better understand the molecular genetics of ependymoma progression. Palm et al.\cite{48} revealed that WHO grade 3 anaplastic ependymomas differed from grade 2 tumors by the over-expression of genes implicated in Wnt/β-catenin signaling activation, cell cycle regulation/cell proliferation (cyclin-dependent kinases CDK2/4, cell division cycle proteins CDC25A/25B/25C/2, and minimal chromosome maintenance proteins MCM2/3/5/6/7), apoptosis (tumor necrosis factor super family members TNFRSF11A/21 and caspases CASP1/4), angiogenesis (VEGF, VEGFR2, VEGFB, TNIP2, and DOC2), and remodeling of adherens junctions through E-cadherin destruction (MET, MN23H1, caveolin, RAB5/7 GTPases), as well as up-regulation of the transcription factors E2F1 and DP1 (TFDP1). Wnt signaling activation in grade 3 ependymomas is indicated by the over-expression of Wnt ligand (WNT11), Frizzled receptors (FZD2/5/8), and Dishevelled genes (DVL2/3). Furthermore, increased expression was detected for β-catenin (CTNNB1) and its associated transcription factor TCF3 and the Wnt target genes BIRC5, CCND1, FOSL1, c-MYC, and TP53. Similarly, to comprehend the molecular mechanisms underlying ependymoma recurrence, Peyre et al.\cite{106} performed a dual-color gene expression microarray analysis on 17 tumors at diagnosis co-hybridized with 27 corresponding tumors at first or subsequent relapses. They identified 87 genes collectively as the expression signature of ependymoma recurrence. Like the gene expression characteristics of high grade ependymomas noted by Palm et al.\cite{48}, the signature of ependymoma recurrence was also marked by Wnt pathway activation with over-expression of SFRP1, SFRP2, FZD2, FZD8, and WNT10B. Other frequently over-expressed genes in recurrent ependymomas included CD133, members of the Notch signaling pathway, and genes involved in the kinetochore (KIF14, KIF11, KIF1C, KIF2C, PRC1, BUB1B, ZWINT, ASPM, KNTC2, and CENPF). The genes that were significantly down-regulated were metallothionein genes (MT1L, MT1G, MT1E, MT1X, MT1B, MT2A, MT3), with reduced expression in up to 80% of recurrences, and genes involved in the immune system (CXCL5, CX3CL1, TRAF3IP2, ITGB1, SERPING1, IFT20, ENTPD3, HP, and HPR). The importance of immune function in hindering ependymoma progression and recurrence was also recognized through the study by Donson et al.\cite{111}. Their ontological analysis on gene expression profiles from pediatric ependymomas correlated with clinical outcome revealed that the up-regulation of immune function-related genes was associated with non-recurrent ependymomas and a longer time to progression in recurrent ependymomas. In addition, increased infiltration of CD4+ T cells were observed by immunohistochemistry in non-recurrent ependymoma samples. Furthermore, the primary ependymomas which can be subgrouped based on location, Peyre et al.\cite{109} found that supratentorial versus infratentorial ependymomas showed distinct changes in expression profile at recurrence. Recurrent supratentorial ependymomas were characterized by the up-regulation of genes related to cytoskeleton organization (gelsolin, SEMA5A, contactin-1, sarcoglycan, villin-like, scinderin) and extracellular matrix-cell interactions (gliomedin, EXT1, galectin-9, desmuslin, tetranectin, versican, COL21A1, COL16A1, CXCL12), which are functionally involved in the mesenchymal transition. Infratentorial ependymoma recurrences, on the other hand, were associated with over-expression of ribosomal protein genes, which are markers of oncogenic transformation in many human tumor types\cite{108,110}.

Cells of Origin of Ependymoma

One of the key questions to answer in the field of cancer research is to determine the normal cell type that gives rise to a particular malignancy. This is a crucial
step towards functionally identifying the successive oncogenic events leading to tumor onset and progression, which would be indispensable for developing targeted therapies and finding keys to prevention.

Growing evidence suggests that tumor subgroups may arise due to deregulation of cell signaling pathways involved in normal development of different precursor cell populations. Thus, the unique gene expression signatures of ependymoma subgroups might provide insight into their cells of origin. Indeed, Taylor et al. [9] showed that the signature genes which characterize supratentorial, posterior fossa, and spinal ependymomas are expressed in the matching regions in the developing CNS of embryonic mice. Moreover, many of these signature genes are members of signaling pathways that modulate neural precursor cell proliferation and differentiation in the corresponding regions of the CNS [8,113]. This confirmed the hypothesis that subgroups of ependymoma either maintain or recapitulate the developmental expression profiles of anatomically restricted progenitor cells, which were then identified to be radial glial cells (RGCs) [9]. Taylor et al. [4] further demonstrated that RGCs are likely the cells of origin for ependymoma by isolating a rare population of self-renewing and multipotent cancer stem cells from fresh samples of ependymoma. These cancer stem cells exhibited bipolar morphology resembling RGCs, expressed the RGC immunophenotype CD133+/Nestin+/RC2+/brain lipid-binding protein (BLBP)+, and were both required and sufficient to recapitulate the original tumor when transplanted into immunocompromised mice.

RGCs are a pivotal cell type in the developing CNS of all vertebrates and are a specific group of neural stem cells. They serve as ubiquitous precursors that generate neurons and glia, as guide cells for subsequent neuronal migration, and as key elements in patterning and region-specific differentiation of the CNS [114]. Studies have also shown that ependymal cells, from which ependymoma arises, are derived from RGCs during embryogenesis [115]. Genetic mutations in RGCs may therefore lead to their transformation into cancer stem cells of pediatric ependymomas [4,116]. Since supratentorial ependymomas are characterized by elevated expression of members of the Notch and EphB-Ephrin signaling pathways, it is likely that over-activation of these pathways may induce neoplastic transformation of RGCs in the cerebral subventricular zone. Likewise, up-regulation of the HOX family of transcription factors may be involved in spinal ependymoma development by transforming RGCs in the spinal region. Furthermore, there is evidence that RG-like cells are present not only during development but also persist in the adult CNS, specifically in the subventricular zone and the spinal cord. Thus, these RG-like cells may serve as the cells of origin for adult ependymomas [116-118].

Recently, Johnson et al. [4] catalogued DNA copy number alterations among 204 tumor samples, which is the largest cohort of ependymomas ever examined at the highest resolution. They further generated mRNA and microRNA expression profiles for 83 and 64 of these tumors, respectively. These profiles segregated ependymomas by CNS location and unmasked additional subgroups among supratentorial, posterior fossa, and spinal ependymomas. To test that distinct subgroups of ependymoma might arise due to oncogenic transformation of regionally and developmentally restricted populations of RGCs by characteristic genetic mutations, the gene expression profile of a subset of human supratentorial ependymomas was matched with that of embryonic cerebral RGCs taken from Ink4a/Arf (Cdkn2a)-null mice, as the CDKN2A locus is frequently deleted from human supratentorial ependymomas. These embryonic cerebral Ink4a/Arf (Cdkn2a)-null RGCs were first transduced with EphB2, which has been shown to be focally amplified in a subgroup-specific manner and to exhibit copy number-driven over-expression in supratentorial ependymomas, and were subsequently implanted into the cerebrum of immunocompromised mice. This established the first highly penetrant (over 70% incidence) murine allograft model of supratentorial ependymoma that accurately recapitulates the histological features and gene expression profile of the human tumor. Comparative gene expression analysis of matched mouse and human tumors revealed deregulation of genes in neural differentiation and maintenance, particularly ion transport and synaptogenesis, thus highlighting the importance of these events in the formation of this ependymoma subgroup. Thus, this study provided functional confirmation that ependymoma variants indeed arise from their matched populations of RGCs transformed with the subgroup-specific mutations.

Conclusion and Future Directions

Over the past decade, research has significantly advanced our knowledge on the molecular genetics of ependymoma. Key features of intracranial versus spinal ependymomas are summarized in Figure 1. Early cytogenetic studies identified broad chromosomal gains and losses, with loss of 22q being the most common. NF2 is recognized as a putative tumor suppressor gene in spinal ependymomas based on mutational analysis and increased incidence of ependymoma in patients with NF2 familial syndrome. It is, however, rarely mutated in pediatric intracranial ependymomas, for which much effort is still being made in identifying the elusive tumor suppressor gene(s) on chromosome 22q. Other common
Genomic imbalances include gain of 1q and losses of 6q and 9 in intracranial ependymomas, and gain of 7 in spinal ependymomas, among many others. With the advent of aCGH technology permitting the identification of genomic imbalances at much greater resolution, it became possible to uncover putative oncogenes and tumor suppressor genes, such as hTERT and CDKN2A, respectively, by testing candidates found in focal regions of amplifications and deletions for copy number-driven expression. Importantly, over the years, ependymoma tumor heterogeneity has become progressively more appreciated at the genetic level and can be subgrouped based on chromosomal abnormalities, aCGH, and, recently, gene expression profiles. It is now generally recognized that ependymomas from different regions of the CNS, i.e. the supratentorium, posterior fossa, and the spinal cord, are genetically distinct diseases marked by unique gene expression signatures, indicating the deregulation of different developmental pathways involved in tumorigenesis. Supratentorial ependymomas are characterized by Notch and EphB-Ephrin signaling, whereas spinal ependymomas show specific over-expression of HOX family transcription factors. Furthermore, comparing the expression profiles of ependymomas at first diagnosis versus at relapse and at low grade versus high grade revealed that ependymoma recurrence and progression likely result from the up-regulation of Wnt signaling and down-regulation of immune function-related genes. Recently, RCGs at various locations throughout the CNS have been identified to be the cells of origin for the corresponding ependymoma subgroups, as illustrated in Figure 1. The notion that subgroups of ependymoma arise from regionally and developmentally distinct RGCs that have undergone transformation by subgroup-specific genetic mutations was further confirmed functionally in the case of supratentorial ependymomas.

Despite these achievements in ependymoma research, greater progress is still urgently needed if we are to realize the ultimate goal of improving clinical outcome for patients. With newly developed microarray platforms able to detect copy number changes and gene expressions at even higher resolution, next-generation sequencing technologies and high-throughput techniques for unbiased epigenetic profiling, we can expect to gain unprecedented understanding of the molecular genetics of ependymoma. Posterior fossa ependymomas in particular deserve our attention, as they frequently occur in children of very young age, and complete surgical resection is often difficult to achieve owing to the involvement of multiple cranial nerves and branches of the vertebrobasilar arterial system at this location. In addition, up to half of posterior fossa ependymomas present a balanced genomic profile, making the
identification of genetic events contributing to their tumorgenesis especially challenging. Consequently, it is important to examine the genetics of posterior fossa ependymomas at a greater resolution to identify very focal amplifications and deletions, as well as to concentrate on decoding its epigenome.

Candidate oncogenes and tumor suppressor genes discovered to date should be promptly assessed for their diagnostic and therapeutic potential, with the aim to effectively translate our knowledge from laboratory to clinic. At present, however, ependymoma research is severely hampered by the lack of in vitro and in vivo systems to functionally examine the genetic events identified through aCGH and gene expression studies that potentially contribute to ependymoma development. Indeed, the identification of RGCs as the cells of origin for ependymoma was a significant breakthrough towards mapping out the pathogenic mechanisms of ependymoma. Similar to what has been done for one subset of supratentorial ependymomas, the next step will be to identify the distinct populations of RGCs for all ependymoma variants and functionally determine the subgroup-specific driver mutations necessary for transforming corresponding RGCs to ependymoma. This approach will allow us to functionally identify the key genetic events involved in the initiation and progression of all ependymoma subgroups, as well as to model these subgroups in vitro and in vivo. Unlike end-stage tumor samples which provide little information on the chronology and relative importance of the uncovered genetic events in the process of ependymoma pathogenesis, these functional models will be instrumental in deciphering the pathogenic mechanisms of the ependymoma subgroups, as well as in uncovering and verifying potential targets for therapy.

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References

[1] Bleyer WA. Epidemiologic impact of children with brain tumors [J]. Childs Nerv Syst, 1999,15(11-12):758–763.

[2] Baldwin RT, Preston-Martin S. Epidemiology of brain tumors in childhood—a review [J]. Toxicol Appl Pharmacol, 2004,199(2): 118–131.

[3] Hasselblatt M. Ependymal tumors [J]. Recent Results Cancer Res, 2009,171:51–66.

[4] Kilday JP, Rahman R, Dyer S, et al. Pediatric ependymoma: biological perspectives [J]. Mol Cancer Res, 2009,7(6):765–786.

[5] Hadjipanayis CG, Van Meir EG. Brain cancer propagating cells: biology, genetics and targeted therapies [J]. Trends Mol Med, 2009,15(11):519–530.

[6] Taylor MD, Poppleton H, Fuller C, et al. Radial glia cells are candidate stem cells of ependymoma [J]. Cancer Cell, 2009, 8(4):323–335.

[7] Gilbert MR, Ruda R, Soffietti R. Ependymomas in adults [J]. Curr Neurol Neurosci Rep, 2010,10(3):240–247.

[8] Kawabata Y, Takahashi JA, Arakawa Y, et al. Long-term outcome in patients harboring intracranial ependymoma [J]. J Neurosurg, 2005,103(1):31–37.

[9] Merchant TE, Fouladi M. Ependymoma: new therapeutic approaches including radiation and chemotherapy [J]. J Neurooncol, 2005,75(3):287–299.

[10] Kano H, Yang HC, Kondziolka D, et al. Stereotactic radiosurgery for pediatric recurrent intracranial ependymomas [J]. J Neurosurg Pediatr, 2010,6(5):417–423.

[11] van Veelen-Vincent ML, Pierre-Kahn A, Kalia C, et al. Ependymoma in childhood: prognostic factors, extent of surgery, and adjuvant therapy [J]. J Neurosurg, 2002,97(4): 827–835.

[12] Chamberlain MC. Ependymomas [J]. Curr Neurol Neurosci Rep, 2003,3(3):193–199.

[13] Pollack IF. Brain-tumors in children [J]. N Engl J Med, 1994,331(22):1500–1507.

[14] Hamilton RL, Pollack IF. The molecular biology of ependymomas [J]. Brain Pathol, 1997,7(2):807–822.

[15] Bouffet E, Foreman N. Chemotherapy for intracranial ependymomas [J]. Childs Nerv Syst, 1999,15(10):563–570.

[16] Souweidane MM, Bouffet E, Finlay J. The role of chemotherapy in newly diagnosed ependymoma of childhood [J]. Pediatr Neurosurg, 1998,28(5):273–278.

[17] Geyer JR, Sposo R, Jennings M, et al. Multiagent chemotherapy and deferred radiotherapy in infants with malignant brain tumors: a report from the children’s cancer group [J]. J Clin Oncol, 2005,23(30):7621–7631.

[18] Brandes AA, Cavallo G, Reni M, et al. A multicenter retrospective study of chemotherapy for recurrent intracranial ependymal tumors in adults by the Gruppo Italiano Cooperativo Di Neuro–Cncologia [J]. Cancer, 2005,104(1):143–148.

[19] Merchant TE. Current management of childhood ependymoma [J]. Oncology (Williston Park), 2002,16(5):629 –642, 644; discussion 645–646, 648.

[20] Mabbott DJ, Spiegler BJ, Greenberg ML, et al. Serial evaluation of academic and behavioral outcome after treatment with cranial radiation in childhood [J]. J Clin Oncol, 2006,23(10):2256–2263.

[21] Spiegler BJ, Bouffet E, Greenberg ML, et al. Change in neurocognitive functioning after treatment with cranial radiation in childhood [J]. J Clin Oncol, 2004,22(4):706–713.

[22] Pollack IF. Brain tumors in children [J]. N Engl J Med, 1994,331(22):1500–1507.

[23] Pfister S, Hartmann C, Korshunov A. Histology and molecular pathology of pediatric brain tumors [J]. J Child Neurol, 2009,24(11):1375–1388.

[24] Martuza RL, Eldridge R. Neurofibromatosis 2 (bilateral acoustic neurofibromatosis) [J]. N Engl J Med, 1988,319(11):684–688.

[25] Rubio MP, Correa KM, Ramesh V, et al. Analysis of the neurofibromatosis 2 gene in human ependymomas and astrocytomas [J]. Cancer Res, 1994,54(1):45–47.

[26] von Haken MS, White EC, Daneshvar-Styvsther L, et al. Molecular genetic analysis of chromosome arm 17p and chromosome arm 22q DNA sequences in sporadic pediatric ependymomas [J]. Genes Chromosomes Cancer, 1996,17(1):37–44.

[27] Buccoliero AM, Castiglia F, Degi’Innocoenti DR, et al. Merlin
expression in pediatric anaplastic ependymomas real time PCR study [J]. Fetal Pediatr Pathol, 2010,29(4):245–254.
[28] Albright AL, Adelson PD, Pollack IF. Principles and practice of pediatric neurosurgery [M]. Second edition. New York: Thieme, 2008.
[29] Mullins KJ, Rubio A, Myers SP, et al. Malignant ependymomas in a patient with turcot’s syndrome: case report and management guidelines [J]. Surg Neurol, 1998,49(3):299–304.
[30] Torres CF, Korones DN, Pilcher W. Multiple ependymomas in a patient with turcot’s syndrome [J]. Med Pediatr Oncol, 1997,28(1):59–61.
[31] Al-Salameh A, Francois P, Giraud S, et al. Intracranial ependymoma associated with multiple endocrine neoplasia type [J]. J Endocrinol Invest, 2010,33(5):353–356.
[32] Giraud S, Tajbakhsh S, Mascalchi M, et al. A large multiple endocrine neoplasia type 1 family with clinical expression suggestive of anticipation [J]. J Clin Endocrinol Metab, 1997,82(10):3487– 3492.
[33] Urioste M, Martinez-Ramirez A, Oigudosa JC, et al. Complex cytogenetic abnormalities including telomeric associations and MEN1 mutation in a pediatric ependymoma [J]. Cancer Genet Cytogenet, 2002,138(2):107–110.
[34] Yokota T, Tachibana T, Fukiko K, et al. A family with spinal anaplastic ependymoma: evidence of loss of chromosome 22q in tumor [J]. J Hum Genet, 2003,48(11):598–602.
[35] Dimopoulos VG, Fountas KN, Robinson JS. Familial intracranial ependymomas. Report of three cases in a family and review of the literature [J]. Neurosurg Focus, 2006,20(1):E8.
[36] Nijsen PC, Deprez RH, Tijssen CC, et al. Familial anaplastic ependymoma: evidence of loss of chromosome 22 in tumour cells [J]. J Neurol Neurosurg Psychiatry, 1994,57(10):1245– 1248.
[37] Bergsagel DJ, Finegold MJ, Butel JS, et al. DNA sequences similar to those of simian virus 40 in ependymomas and choroid plexus tumors of childhood [J]. N Engl J Med, 1992,326(15):988–993.
[38] Suzuki S, Miozuchi M, Iwai T. Detection of SV40 T antigen genome in human gliomas [J]. Brain Tumor Pathol, 1997,14(2): 125–129.
[39] Lednicky JA, Garcea RL, Bergsagel DJ, et al. Natural simian virus 40 strains are present in human choroid plexus and ependymoma tumors [J]. Virology, 1995,212(2):710–717.
[40] Kirschstein RL, Gerber P. Ependymomas produced after intraepithelial inoculation of SV40 into new-born hamsters [J]. Nature, 1962,195:299–300.
[41] Rabson AS, O’Conor GT, Kirschstein RL, et al. Papillary ependymomas produced in Rattus (Mastomys) natelensis inoculated with vacuolating virus (SV40) [J]. J Natl Cancer Inst, 1962,29:765–787.
[42] Reuther FJ, Lohter J, Hermas J, et al. Low incidence of SV40-like sequences in ependymal tumours [J]. J Pathol, 2001,195 (5):580–585.
[43] Engels EA, Sarkar C, Daniel RW, et al. Absence of simian virus 40 in human brain tumors from northern India [J]. Int J Cancer, 2002,101(4):348–352.
[44] Shah KV. SV40 and human cancer: a review of recent data [J]. Int J Cancer, 2007,120(2):215–223.
[45] Stricker HP, Rosenberg PS, Devesa SS, et al. Contamination of poliovirus vaccines with simian virus 40 (1955–1963) and subsequent cancer rates [J]. JAMA, 1998,279(4):292–296.
[46] Engels EA. Cancer risk associated with receipt of vaccines contaminated with simian virus 40: epidemiologic research [J]. Expert Rev Vaccines, 2005,4(2):197–206.
[47] Johnson RA, Wright KD, Poppleton H, et al. Cross-species genomics matches driver mutations and cell compartments to model ependymoma [J]. Nature, 2010,466(7306):632–636.
[48] Palm T, Figarella-Branger D, Chapon F, et al. Expression profiling of ependymomas unravels localization and tumor grade-specific tumorigenesis [J]. Cancer, 2009,115(17):3955–3968.
[49] Mack SC, Taylor MD. The genetic and epigenetic basis of ependymoma [J]. Childs Nerv Syst, 2009,25(10):1195–1201.
[50] de Bont JM, Packer RJ, Michiels EM, et al. Biological background of pediatric medulloblastoma and ependymoma: a review from a translational research perspective [J]. Neuro Oncol, 2008,10(6):1040–1060.
[51] Karakoulia K, Suarez-Merino B, Ward S, et al. Real-time quantitative PCR analysis of pediatric ependymoma identifies novel candidate genes including TP63 and CHIBBY at 22q12–q13 [J]. Genes Chromosomes Cancer, 2008,47(11): 1005–1022.
[52] Parzefall W, Valiero S, Morra I, et al. A complex karyotype including a 1(2;11) in a paediatric ependymoma: case report and review of the literature [J]. J Neurooncol, 2010,99(1):141–146.
[53] Carter M, Nicholson J, Ross F, et al. Genetic abnormalities detected in ependymomas by comparative genomic hybridisation [J]. Br J Cancer, 2002,86(6):929–939.
[54] Dyon S, Prebble E, Davison A. Genetic imbalances in pediatric intracranial ependymomas define clinically relevant groups [J]. Am J Pathol, 2002,161(6):2133–2141.
[55] Kraus JA, de Millas W, Sorensen N, et al. Indications for a tumor suppressor gene at 22q11 involved in the pathogenesis of ependymal tumors and distinct from HSNFS5/IN1 [J]. Acta Neuropathol, 2001,102(1):69–74.
[56] Michalowski MS, de Forest F, Michelland S, et al. Methylation of RASSF1A and TRAIL pathway-related genes is frequent in childhood intracranial ependymomas and benign choroid plexus papilloma [J]. Cancer Genet Cytogenet, 2006,166(1):74–81.
[57] Tong CY, Zheng PP, Pang JC, et al. Identification of novel regions of allelic loss in ependymomas by high-resolution allelotyping with 384 microsatellite markers [J]. J Neurosurg, 2001,95(1):9–14.
[58] Hulsebos TJ, Oskam NT, Bijleveld EH, et al. Evidence for an ependymoma tumor suppressor gene in chromosome region 22pter–22q11.2 [J]. Br J Cancer, 1999,81(7):1150–1154.
[59] Mazewski C, Soukup S, Ballard E, et al. Karyotype studies in 18 ependymomas with literature review of 107 cases [J]. Cancer Genet Cytogenet, 1999,113(1):1–8.
[60] Ammerlaan AC, de Bustos C, Aparicio A, et al. Localization of a putative low-penetration ependymoma susceptibility locus to 22q11 using a chromosome 22 tiling-path genomic microarray [J]. Genes Chromosomes Cancer, 2005,43(4):329–338.
[61] Huang B, Starostik P, Kuhl J, et al. Loss of heterozygosity on chromosome 22 in human ependymomas [J]. Acta Neuropathol, 2002,103(4):415–420.
[62] Suarez-Merino B, Hubank M, Revesz T, et al. Microarray analysis of pediatric ependymoma identifies a cluster of 112 candidate genes including four transcripts at 22q12.1–q13.3 [J]. Neuro Oncol, 2005,7(1):20–31.
[63] Gil J, Bernard D, Martinez D, et al. Polycym on polycomb CBX7 has a unifying role in cellular lifespan [J]. Nat Cell Biol, 2004,6(1): 67–72.
[64] Sherr CJ, McCormick F. The RB and p53 pathways in cancer [J]. Cancer Cell, 2002,2(2):103–112.
[65] Kato H, Kato S, Kumabe T, et al. Functional evaluation of p53 and PTEN gene mutations in gliomas [J]. Clin Cancer Res, 2000,6(10):3937–3943.
[66] Watanabe T, Yokoo H, Yokoo M, et al. Concurrent inactivation of RB1 and TP53 pathways in anaplastic oligodendrogliomas [J]. J Neuropathol Exp Neurol, 2001,60(12):1181–1189.
[67] Nozaki M, Tada M, Kobayashi H, et al. Roles of the functional loss of p53 and other genes in astrocytoma tumorigenesis and
Molecular genetics of ependymoma

Korshunov A, Golanov A, Timirgaz V. P14ARF protein (FL-132) immunoreactivity in intracranial ependymomas and its prognostic significance: an analysis of 103 cases [J]. Acta Neuropathol, 2001,102(3):271–277.

Nishiyama H, Hornigold N, Davies AM, et al. A sequence-ready 840-kb PAC contig spanning the candidate tumor suppressor locus DCB1 on human chromosome 9q32–q33 [J]. Genomics, 1999,59(3):335–338.

Beetz C, Broedel S, Patt S, et al. Low expression but infrequent genomic loss of the putative tumour suppressor BDCR1 in astrocytoma [J]. Oncol Rep, 2005,13(2):335–340.

Yang L, Leung AC, Ko JM, et al. Tumor suppressor role of a 2.4 Mb 9q33–q34 critical region and DECl in esophageal squamous cell carcinoma [J]. Oncogene, 2005,24(4):697–705.

Alonso Y, Nakano A, Katoaka H, et al. Deleted in esophageal cancer 1 (DEC1) is down-regulated and contributes to migration in head and neck squamous cell carcinoma cell lines [J]. ORL J Otorhinolaryngol Relat Spec, 2011,73(1):17–23.

Modenaar WH. Bioactive lysophospholipids and their G protein-coupled receptors [J]. Exp Cell Res, 1999,253(1):230–238.

Saltoh M, Nishihoh F, Fuji M, et al. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1 [J]. EMBO J, 1998,17(9):2596–2606.

Chen X, Tang W, Liu S, et al. Thioredoxin-1 phosphorylated at T100 is needed for its anti-apoptotic activity in HepG2 cancer cells [J]. Life Sci, 2010,87(7–8):254–260.

Rizzolo A, Capi V, Russo A, et al. Identification of novel chromosomal abnormalities and prognostic cytogenetics markers in intracranial pediatric ependymoma [J]. Cancer Lett, 2008,261(2):235–243.

Magrassi L, Marzilliano N, Inzani F, et al. EDG3 and SHC3 on chromosome 9q22 are co-amplified in human ependymomas [J]. Cancer Lett, 2010,290(1):36–42.

Puget S, Grill J, Valet A, et al. Candidate genes on chromosome 9q33–q34 involved in the progression of childhood ependymomas [J]. J Clin Oncol, 2009,27(11):1884–1892.

Milde T, Pfister S, Korshunov A, et al. Stepwise accumulation of distinct genomic aberrations in a patient with progressively metastasising ependymoma [J]. Genes Chromosomes Cancer, 2009,48(3):229–238.

Tabori U, Wong V, Ma J, et al. Telomere maintenance and dysfunction predict survival in pediatric ependymoma [J]. Br J Cancer, 2008,99(7):1129–1135.

Tabori U, Ma J, Carter M, et al. Human telomere reverse transcriptase expression predicts progression and survival in pediatric intracranial ependymoma [J]. J Clin Oncol, 2006,24(10):1522–1528.

Ridley L, Rahman R, Brundler MA, et al. Multifactorial analysis of predictors of outcome in pediatric intracranial ependymoma [J]. Neuro Oncol, 2008,10(5):675–689.

Wong VC, Morrison A, Tabori U, et al. Telomerase inhibition as a novel therapy for pediatric ependymoma [J]. Brain Pathol, 2010,20(4):780–786.

Waha A, Koch A, Hartmann W, et al. Analysis of HIC-1 methylation and transcription in human ependymomas [J]. Int J Cancer, 2004,110(4):542–549.

Hamilton DW, Lusher ME, Lindsey JC, et al. Epigenetic inactivation of the RASSF1A tumour suppressor gene in ependymoma [J]. Cancer Lett, 2005,227(1):75–81.

Donninger H, Vos MD, Clark GJ. The RASSF1A tumour suppressor [J]. J Cell Sci, 2007,120(Pt 18):3163–3172.

Lindsey JC, Lusher ME, Strathdee G, et al. Epigenetic inactivation of MCM4 (DNA D1) in malignant paediatric brain tumours [J]. Int J Cancer, 2006,118(2):346–352.

Peyre M, Commo F, Dantas-Barbosa C, et al. Portrait of
Ependymoma recurrence in children: biomarkers of tumor progression identified by dual-color microarray-based gene expression analysis [J]. PLoS One, 2010, 5(9): e12932.

[107] Louvi A, Artavanis-Tsakonas S. Notch signalling in vertebrate neural development [J]. Nat Rev Neurosci, 2006, 7(2): 93–102.

[108] Gilbertson RJ, Bentley L, Hernan R, et al. ERBB receptor signaling promotes ependymoma cell proliferation and represents a potential novel therapeutic target for this disease [J]. Clin Cancer Res, 2002, 8(10): 3054–3064.

[109] Weng AP, Ferrando AA, Lee W, et al. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia [J]. Science, 2004, 306(5694): 269–271.

[110] Bocchetta M, Miele L, Pass HI, et al. Notch-1 induction, a novel activity of SV40 required for growth of SV40-transformed human mesothelial cells [J]. Oncogene, 2003, 22(1): 81–89.

[111] Donson AM, Birks DK, Barton VN, et al. Immune gene and cell enrichment is associated with a good prognosis in ependymoma [J]. J Immunol, 2009, 183(11): 7428–7440.

[112] Montanaro L, Trere D, Derenzini M. Nucleolus, ribosomes, and cancer [J]. Am J Pathol, 2008, 173(2): 301–310.

[113] Gilbertson RJ. Brain tumors provide new clues to the source of cancer stem cells: does oncology recapitulate ontogeny? [J]. Cell Cycle, 2006, 5(2): 135–137.

[114] Campbell K, Gotz M. Radial glia: multi-purpose cells for vertebrate brain development [J]. Trends Neurosci, 2002, 25(5): 235–238.

[115] Spassky N, Merkle FT, Flames N, et al. Adult ependymal cells are postmitotic and are derived from radial glial cells during embryogenesis [J]. J Neurosci, 2005, 25(1): 10–18.

[116] Poppleton H, Gilbertson RJ. Stem cells of ependymoma [J]. Br J Cancer, 2007, 96(1): 6–10.

[117] Barry D, McDermott K. Differentiation of radial glia from radial precursor cells and transformation into astrocytes in the developing rat spinal cord [J]. Glia, 2005, 50(3): 187–197.

[118] Merkle FT, Tramontin AD, Garcia-Verdugo JM, et al. Radial glia give rise to adult neural stem cells in the subventricular zone [J]. Proc Nat Acad Sci U S A, 2004, 101(50): 17528–17532.