Chinese Journal of Cancer

Review

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.

Key words 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 children[1,2]. 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 age[3,4]. 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 cord [5,6]. Over 90% of pediatric ependymomas are intracranial, with two thirds occurring in the posterior fossa[4]. Many adult cases, on the other hand, occur in the spinal cord[7].

The primary treatment for ependymoma remains surgical resection followed by radiotherapy, with gross total resection frequently reported as the most important prognostic factor[8-12]. Although postoperative radiotherapy may induce stabilization and, occasionally, regression of residual disease, most incompletely resected tumors ultimately progress[13]. 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 radiotherapy[4,14]. No chemotherapy regimen has prolonged overall survival in children with ependymoma [9,11,13,15-18]. 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%, respectively[4,19]. Survivors are often left with serious physical and neurocognitive disabilities secondary to the disease and its treatment[9,20,21]. 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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doi: 10.5732/cjc.011.10129

radiotherapy for those under 3 years of age [19]. 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 [8,22,23], and that tumors of the same histological type often display variable clinical behaviors[6,23], 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) [24,25]. The *NF2* gene is located on chromosome 22q, which is frequently lost in patients with spinal ependymomas [25-27]. 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 [25-27]. 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[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 spinally [29,30]. Both intracranial and spinal ependymomas have also been observed in patients with the multiple endocrine neoplasia type I (MEN1) syndrome [31-33]. 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[34-36]. 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[34,36].

DNA sequences similar to SV40 virus and the virus-encoded large T-antigen have also been found in some ependymomas[37-39]. Furthermore, ependymoma can be induced in rodents through intracerebral inoculation of the SV40 virus [40,41]. Nevertheless, several studies have disqualified the SV40 tumor virus as a causative agent of ependymoma [42-44]. 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[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 [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[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 [4]. 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[4]. 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 genetic profiles, whereas a balanced genomic profile is observed in less than 10% of adult cases [4,49,53]. 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 [55,57-61]. Within the frequently deleted region 22q12.3–q13.33, Karakoula *et al.*[51] found *RAC2* and *C22ORF2* to be deleted in 38% and 32% of the 47 pediatric intracranial ependymomas analyzed, respectively. In over 60% of these ependymomas, *C22ORF2* 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.*[62] found the transcripts of four genes mapping to 22q12.3–22q13.33, namely *C22ORF2*, 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 $p16^{Ink4a}$/Rb and the Arf/p53 pathways [63]. The role of these pathways in ependymoma is unclear, though their deregulation is central to many types of cancer, including gliomas [64-67]. Furthermore, deletion and hypermethylation of *CDKN2A/P16* at 9q21.3 and *RB* at 13q14.2 have been reported in

ependymomas[68-71]. In the same study by Suarez-Merino *et al*. [62], 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 [72]. Furthermore, by integrating the genomic and expression profiles of 24 primary intracranial ependymomas, Modena *et al*. [73] 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[4,49,74]. Importantly, this genetic aberration is preferentially 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[4,23,49,53,54,74-78]. Interestingly, 1q gain is occasionally the only observable alteration, with few other chromosome imbalances detected in ependymomas [53,54,74,77]; yet, in some cases, it marks tumor recurrence [54,78]. 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*. [74] 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*.[78] 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[78,79]. *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 [80]. 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*, *SHC1*, and *S100A10* and other S100 family members [4,51,62,81,82]. 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[81].

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 [77,78,83]. 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 *ARHGEF5* at 7q34[73,78]. *EGFR* in particular exhibits frequent gains and high-level amplifications in intracranial ependymomas, and its over-expression predicts poor patient outcome[78]. Loss of chromosome 6q is found mostly in infratentorial tumors, whereas deletions on chromosome 9 occur more frequently in supratentorial tumors [53,76,77,84]. 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[83,85]. Locus 6q25.3, containing the *SNX9* and *SYNJ2* genes, was found to be the most frequently deleted [85]. 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[85]. 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*.[62] 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[6,75]. The molecular staging system developed by Korshunov *et al*. [75] 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 $P14^{ARF}$ protein by immunohistochemistry in 103 intracranial ependymomas revealed that decreased $P14^{ARF}$ expression is associated with tumor aggressiveness in terms of higher tumor grade, elevated growth fraction, and P53 protein accumulation[86]. Using microsatellite analysis, Schneider *et al*.[84] 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 [87,88]; *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 [6,49,57,73,78,94-97] 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 [94]. 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[6,73,78,95-97] 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[78,98-100]. 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

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, 16p13.3, 16pter, 17q21, 18, 19p13.1–13.3, 20p12, Xp21.2, 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, 20q13.2–13.3, 22q12, and 22q13.3

Table 2. Putative oncogenes and tumor suppressor genes in ependymoma

Oncogenes	*DUSP12* (1q23.3), *MYCN* (2p24), *DNASE1L3* (3q25.2), *hTERT* (5p15.33), *NOTCH4* (6p21.32), *EGFR* (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), *MTA1* (14q32.33), *SLC6A10* (16p11.2), *PRM1* (16q12.2), *CDC6* (17p13.3), *VAV1* (19p13.3), and *JAG1* (20p12.2)
Tumor suppressor genes	*ZNF262* (1p34.3), *AJAP1* (1p36.32), *CDKN2A* (9p21.3), *FOXD4* (9p24.31), *GRID1* (10q23.2), *MINPP1* (10q23.31), *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.*[102] therefore investigated the methylation status of the hypermethylated in cancer 1 (*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[73,102]. 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[102]. The Ras association domain family 1 isoform 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%[103]. *RASSF1A* is a recently well-recognized tumor suppressor gene whose inactivation through promoter methylation is implicated in the development of many human cancers[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[104]. Furthermore, Michalowski *et al.*[56] 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, TIMP3, TP73, MGMT, GSTP1, CDKN2A, FHIT, RARB, BLU*, and *MCJ*, with incidence ranging from 10% to 57%[56,69,70,103,105].

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.*[62] 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 *NPC1*, *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[6,48,73,106]. Taylor *et al.*[6] 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.*[73] and Palm *et al.*[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[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[107]. In addition to over-expression of the Notch ligands *JAGGED 1/2* shown by Taylor *et al.*[6], 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[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[96]. These mutations cause the Notch1 receptor to be constitutively active in a ligand-independent manner [109]. Moreover, inhibition of Notch signaling with γ-secretase inhibitor GSI-IX impaired ependymoma primary cell culture growth[96]. Gilbertson *et al.*[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 ERBB2 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[110].

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.*[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.*[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.*[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, ITGBL1, 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.*[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, like the primary ependymomas which can be subgrouped based on location, Peyre *et al.*[106] 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, EXTL1, 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[106,112].

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.* [6] 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 [6,113]. 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) [6]. Taylor *et al.* [6] 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.* [47] 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

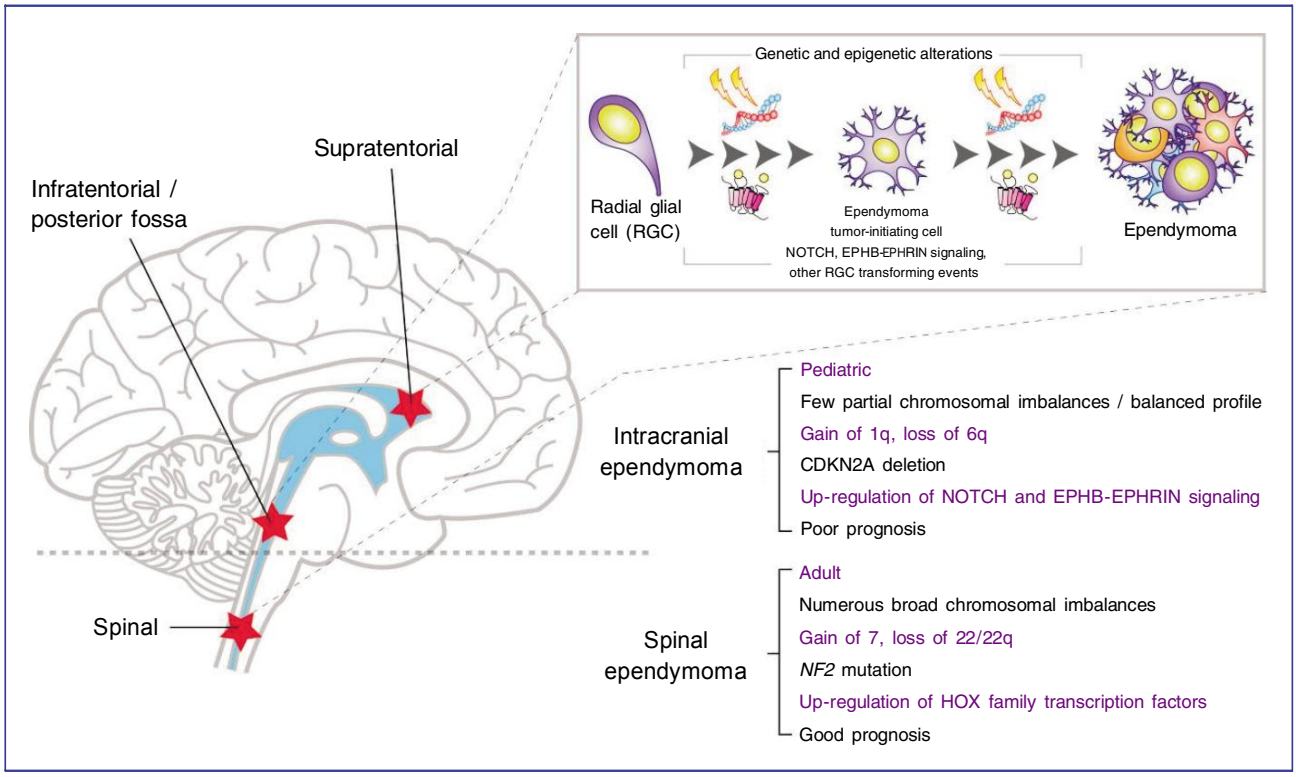

Figure 1. **Radial glial cells as the cells of origin for ependymoma, and the characteristic features distinguishing between intracranial versus spinal ependymomas.**

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 tumorigenesis 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.

Received: 2011-03-31; revised: 2011-04-27; accepted: 2011-05-30.

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