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Recent perspectives of ependymomas (childhood brain tumors)

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Abstract. – Ependymomas are childhood brain tumors that occur throughout the central nervous system, but are most common in the hindbrain, also known as the posterior fossa (PF). Current standard therapy comprises maximal safe surgery, and there is no scope for further increase in survival. Despite the histological similarity, ependymomas from throughout the neuroaxis likely comprise multiple independent entities, each with a distinct molecular pathogenesis. The present review article would discuss both genetics and epigenetics of ependymomas.

Key Words:
Ependymomas, Epigenetics, Genetics, Pediatrics.

Introduction

Ependymomas are rare, chemo-resistant, central nervous system tumors, arising in both children and adults[1]. Ependymomas could arise along the entire neuro-axis occurring in the supratentorial (ST) brain comprising the cerebral hemispheres, the posterior fossa (PF) comprising the cerebellum and brain stem, and along the entire spinal cord (SP)[2]. In children, 90% of ependymomas arise intracranially, with nearly two-thirds occurring in the PF[3]. The occurrence of relapse is significantly greater in the pediatric population, and is clinically variable with recurrences observed in some cases 10-15 years following treatment of the primary tumor[4].

Histopathology

The hallmark histological features of ependymoma include: (1) perivascular pseudorosettes, composed of ependymal cell processes radially arranged towards blood vessels; (2) true ependymal rosettes, consisting of tumor cells arranged radially surrounding an empty lumen. Regarding immunohistochemical staining, ependymomas are commonly positive for glial fibrillary acidic protein (GFAP), neural cell adhesion molecule protein (NCAM), and epithelial membrane antigen protein (EMA), which allows for further delineation from other histologically similar brain tumors[5]. The WHO recognizes three grades for ependymomas:

Grade I – comprising subependymomas and myxopapillary ependymomas, which are easily recognizable histological entities associated with a better survival and increased age;
Grade II – pertains to ependymomas, which lack Grade III features described below;
Grade III – also described as anaplastic, this classification corresponds to ependymomas with "increased cellularity and brisk mitotic activity.

While the Grade I criteria for ependymoma are relatively clear, the parameters used to distinguish Grade II and III ependymomas are highly debated as prognostic differences have been observed in some tumor cohorts, but not others[5]. This discrepancy occurs even after controlling potentially confounding factors such as age or tumor location. The lack of reproducibility and reliability of histopathological grading is demonstrated in a systemic review of three independent European trial cohorts by five leading neuropathologists, in which a consensus agreement on the classification of Grade II or III ependymoma was reached in less than half of 221 total cases examined[6]. While the prognostic utility of histopathological grading is still debated, immunohistochemistry (IHC) and molecular markers have been proposed as potential solutions e.g. Telomerase (TERT) protein expression[7], V-erb-b2 erythroblastic leukemia viral oncogene homolog 2/4 (ERBB2/4) protein expression, or fluorescence in situ hybridization (FISH) of recurrent chromosomal alterations[8,9]. These IHC and molecular markers remain to be validated in inde-

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pendent and prospective ependymoma cohorts, a challenge necessitating multi-center collaborative efforts.

Treatment Strategies

To this date, treatment for ependymoma remains aggressive surgical intervention and adjuvant radiotherapy (10). In spite of the histological challenges in identifying high- *vs.* low-risk ependymoma patients, the extent of surgical resection is the most frequently and consistently reported prognostic indicator of ependymoma patient survival. In other pediatric brain tumors, such as medulloblastoma, radiotherapy is typically avoided in children less than 3 years of age, due to increased risk of long-term neurological and neuroendocrine sequelae. However, the aggressive nature of ependymoma in infants and young children combined with a lack of effective chemotherapies has provided a rationale for the use of conformal and intensity- modulated radiotherapy in infants. Early evidence in prospective clinical trials has demonstrated that conformal radiation is both effective and associated with minimal short-term neurological side effects in the 5-years following treatment[11]. Whether these outcomes are maintained for long-term is in a queue for further evaluation in the near future. Despite improvements in surgical techniques and advances in conformal radiation, recurrence rates remain high in ependymoma patients, particularly in the pediatric population[12]. Although chemotherapy has been used extensively in the treatment of children with intracranial ependymoma, clinical trial response rates to numerous single agents are less than 12%, with less than 5% of patients experiencing complete responses[13]. Results from several multi-center ependymoma clinical trials suggested that there is a little evidence that chemotherapy is effective in treatment for this tumor type[14]. As result, the current standard of care for patients with recurrent ependymoma is maximal-safe surgical resection followed by re-irradiation. Despite prolonged overall survival observed from re-irradiation of recurrent ependymoma, the risks of secondary tumors and neurological impairments have yet to be adequately assessed. The high recurrence rate of pediatric ependymomas, lack of prognostic histological and molecular markers and dearth of chemotherapeutic avenues underscore the importance of understanding the biological basis of ependymoma such that rationale molecular targets could be identified and rapidly translated into the clinic[2].

The Genetic Basis of Ependymoma

Efforts to identify driver oncogenes and tumor suppressor genes (TSG) of ependymoma began largely with characterization of these tumors at a DNA copy number level using cytogenetic approaches, DNA-based microarrays, whole-genome and whole exome sequencing[15]. Despite higher resolution array technologies, the vast majority of recurrent somatic copy number alterations (SCNA) are broad and involve losses of chromosome: 1p, 3, 6q, 9p, 10q, 13q, 16p, 17, 21 and 22q, and gains of chromosome: 1q, 4q, 5, 7, 8, 9, 12q, and 2016 (16). The most frequent and focal SCNA in ependymoma is a homozygous deletion encompassing the CDKN2A/Ink4a locus, which is restricted to supratentorial ependymomas (ST) ([17]). In case of posterior fossa (PF) ependymomas, recurrent and focal copy number alterations pinpointing driver genes have yet to be discovered, highlighting the difficulty in understanding the biological basis, and identifying novel therapeutic targets for this anatomical subtype of ependymoma[17]. Chromosome 22 loss has been shown to be the most frequent gross copy number alteration in ependymoma with a frequency ranging from 26% to 71%[18,19] Further, chromosome 22q loss has been observed preferentially in spinal *vs.* intracranial ependymoma, and in adult *vs.* pediatric cases[19]. The Neurofibromatosis II (NF2) gene is thought to be the candidate TSG of this region, as patients with Neurofibromatosis type II disorder develop a variety of central nervous system tumors including ependymoma, schwannoma, and meningioma. However, NF2 is mutated exclusively in spinal ependymomas, thus suggesting alternate mechanisms of down-regulation, or another putative chromosome 22q TSG in the case of intracranial ependymoma. Another broad chromosomal abnormality frequently observed in ependymoma is monosomy 17, with complete or partial loss of both chromosomal p and q arms[18,19]. Chromosome 1q gain has also been consistently reported as a frequent genomic alteration occurring in nearly 22% of cases of intracranial ependymoma. Further, an increased incidence of 1q gain has been observed in posterior fossa ependymoma and associated with poor clinical outcome[20,21]. It is thought that the chromosome 1q25 locus harbors bonafide oncogene involved in the initiation, maintenance, or progression of ependymoma. Efforts have been made to correlate this region of chromosomal gain with gene expression and have identified

CHI3L1 and a family of S100 genes as up regulated and potential driver oncogenes; however, these candidates remain to be functionally validated[20,21].

Inter-Tumoral Heterogeneity and Putative Cells of Origin

Using gene expression profiling and unsupervised clustering, ependymomas have been divided into three principal molecular subgroups, which are separated largely according to anatomical location: (1) Supratentorial (ST); (2) Posterior Fossa (PF); 3) Spinal cord (SP). These three subgroups have been further divided into molecularly and biologically distinct subtypes of ST and PF ependymoma as defined by distinct clinical features[22,23]. The genes distinguishing supratentorial, posterior fossa, and spinal ependymomas involve mainly families of genes regulating neural precursor cell proliferation and differentiation. Supratentorial ependymomas have elevated EPHB-EPHRIN, NOTCH and cell cycle related genes, while posterior fossa ependymomas express many inhibitors of differentiation (ID1/2/4), and the aquaporin family of genes (AQP1/3/4). Spinal ependymomas, however, are characterized by the expression of various homeobox genes including HOXA7/9, HOXB6/7, and HOXC6/10[24]. While these subgroup gene signatures revealed distinct tumorigenic pathways, Taylor et al[20] proposed potential signatures of anatomically distinct cells of origin giving rise to different subgroups of ependymoma. They further suggested that ependymoma might originate from radial glial cells (RGCs), a population of primitive neural and multi-potent precursor cells important for neurogenesis. Further evidence implicating RGCs as cells of origin of ependymoma was demonstrated by Johnson et al[21], in which *ex-vivo* over-expression of EPHB2 in p16/INK4A deficient forebrain RGCs led to the formation of the first mouse model of ST ependymoma.

Cell Line and Animal Models of Ependymoma

In the past, lack of clear driver alterations in ependymoma has hampered the ability to generate animal models of this disease. Laboratories have thus relied upon patient-derived ependymoma cultures grown *in vitro* and orthotopic xenograft models generated with limited success particularly in the case of PF ependymoma[25]. Identification of the putative cell-of-origin of ependymoma and drivers of ST ependymoma, have led to the first animal models of this disease[26]. As novel ependymoma targets are discovered, validation and prioritization of candidates would require accurate pre-clinical models of ependymoma. Atkinson et al[27] demonstrate the utility and promise of this approach in ST ependymoma cultures, generated by EPHB2 over expression and CDKN2A/Ink4a deletion in forebrain radial glia.

The Epigenetic Basis of Ependymoma

Aberrant promoter methylation of CpG di-nucleotides is a well-recognized feature seen in numerous solid and liquid cancers[28]. TSG is reported in up to 100% of ependymomas, and occurring in all clinical and pathological subtypes[29]. HIC1 is also commonly methylated, in up to 83% of ependymomas, with a higher incidence of intracranial tumors[30]. Furthermore, the CDKN2A/INK4a locus, which is focally and recurrently deleted in supratentorial ependymoma18, has been shown to be hypermethylated in 21% of cases, followed by CDKN2B and p14ARF in 32% and 33% of tumors, respectively[31]. To a lesser extent, putative TSGs found to be hypermethylated in ependymoma include ZMYD10, GSTP1, DAPK, FHIT, MGMT, DNAJC15, RARB, TIMP3, THBS1, TP73, the Tumour Necrosis Factor Related Apoptosis-Inducing Ligand (TRAIL) gene family, CASP8, TNFRSF10C and TNFRSF10D[32,33]. Despite the frequency of DNA methylation of these potential TSGs in ependymoma, their role in tumor development remains unclear and requires further investigation in appropriate ependymoma models.

Aberrant DNA Methylation

In the last 5 years, the expansion of microarray and next generation sequencing technologies, has allowed for genome-wide investigations of DNA methylation and histone modifications at unprecedented resolution and throughput. Using the Illumina Golden Gate Methylation Cancer Panel 1 (1505 CpG sites), Rogers et al[29] profiled a series of 73 primary and 25 recurrent ependymomas. Here they reported that the DNA methylation profiles of ependymoma are distinguished largely according to their location in the central nervous system, supporting the notion that ependymomas arising from different anatomic compartments are molecularly distinct[20,22]. Furthermore, they demonstrated that ST and SP ependymomas, together, exhibited a larger number of hypermethylated and down-regulated genes in comparison to PF tumors. These changes in DNA methylation

were shown to be associated with alterations in gene expression of *de novo* and maintenance of DNA methyltransferases DNMT1, DNMT3A and DNMT3B. Interestingly, genes involved in immune cell response (NOD2, IRF7, IRAK3, OSM and PI3), cell growth and death (MAPK10, and TP73), and the c-Jun N-terminal kinases (JNK) pathway were found to be hypermethylated. Understanding the contribution of epigenetic alterations in these pathways might reveal mechanisms of ependymoma tumorigenesis, and potential actionable targets for therapeutic intervention. In contrast to hypermethylation of CpG island promoters in cancer, global hypomethylation is a trend observed in numerous tumor types and is associated with cancer progression. A global decrease in methylation has been observed predominantly at repetitive elements such as LINEs, SINEs and LTRs, which are important for maintaining genomic stability[34]. To elucidate the contribution of DNA methylation alterations at repetitive sequences in ependymoma, Xie et al[35] developed a novel genome-wide approach to generate methylation profiles for thousands of Alu elements (the most abundant class of repetitive elements) and their flanking sequences[35]. Here they demonstrated that while the majority of Alu elements and flanking sequences remain unaltered in ependymoma genesis, a small subset of Alu flanking sequences, with low CpG density, exhibited variable methylation patterns. These sequences tended to be hypermethylated in ependymoma at regions proximal to CpG islands and hypomethylated in intergenic regions. Importantly, several of these patterns were shown to be associated with aggressive primary ependymomas and tumor relapse. However, the impact that these epigenetic alterations on genomic stability and their respective pathways are remains to be elucidated.

Potential Applications of Epigenetic Modifiers for Ependymoma Treatment

Characterizing the epigenome of ependymoma might hold therapeutic promise, as these marks such as CpG DNA methylation and histone modification are generally reversible by pharmacologic inhibition. Importantly, inhibitors of DNA methylation (decitabine) and histone deacetylation (Vorinostat) are FDA approved and have shown efficacy in hematological malignancies[36,37]. These findings were also supported by Rahman et al[38], demonstrating that the ependymoma cell line nEPN2 underwent apoptosis in response to treatment with the HDACi, Trichostatin-A. Given the rapid development of novel pharmacologic inhibitors of epigenetic marks, it raises the question as to whether these, or at least some, epigenetic modifications are central to ependymoma pathogenesis and whether they might represent novel avenues for therapeutic inhibition[39].

Future Steps

Although genomic and transcriptomic profiling efforts have identified distinct molecular subtypes of ependymoma revealing potential drivers of the disease, the vast majority of ependymal tumors is characterized by either large chromosomal alterations, hampering the identification of driver events, or are characterized by very few genomic abnormalities occurring in the youngest patient population[40]. It remains to be seen whether recurrent somatic single nucleotide variants (SNVs) or structural rearrangements (i.e. fusion transcripts) might contribute to the pathogenesis of ependymoma, as reported in several other adults and pediatric CNS neoplasms[40-42]. DNA methylation profiling efforts have also been important in the molecular stratification of CNS tumors[43]. They have also shown promise in distinguishing the principle molecular subgroups of ependymoma as well as the identification of pathways targeted by DNA hypermethylation. As a future step, expanding DNA methylation profiling to platforms with higher CpG coverage could reveal novel targets, pathways, and mechanisms of epigenetic alteration. Also, given the contributions of aberrant methylation near repeat elements in the ependymoma epigenome, more global investigations beyond gene promoters might be needed, and could be readily examined with whole-genome bisulfite sequencing.

Conclusions

The contribution of genetic and epigenetic changes in ependymoma pathogenesis might not only improve our understanding of the biology of this disease, but also reveal actionable pathways that could be rapidly translated to the clinic.

Conflict of Interest
The Authors declare that they have no conflict of interests.

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