Review article

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Molecular alterations in ependymomas

Wojciech Biernat, Antoni Żawrocki

Department of Neuropathology and Molecular Pathology, Medical University of Gdańsk, Gdańsk, Poland

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Abstract

Ependymal tumours are uncommon neoplasms of the central nervous system. Basic molecular alterations underlying their development are not so well defined in contrast to the astrocytic tumours. We compiled literature data on the molecular changes in ependymomas to show clinical and pathological correlations and review the prognostic factors that may much better predict their clinical behaviour.

Key words: *ependymoma, anaplastic ependymoma, myxopapillary ependymoma, genetics, prognosis*

Introduction

Ependymal tumours are relatively uncommon primary neoplasms of the central nervous system (CNS) (3-9% of gliomas) arising from the lining of the ventricular system and from the remnants of the central canal of the spinal cord [37]. They are the third most common CNS malignancy in childhood, after astrocytomas and medulloblastomas. The current WHO classification recognizes ependymoma (WHO grade II), anaplastic ependymoma (WHO grade III), myxopapillary ependymoma (WHO grade I), and subependymoma (WHO grade I) [37]. Ependymomas may develop at any site along cerebral ventricles and the spinal canal. However, there is an interesting correlation between the site of their development and the age of patients: about 90% of paediatric ependymomas arise within the cranium, whereas most adult ependymomas develop in the spinal cord [9,20,23]. Myxopapillary ependymomas and subependymomas are rare variants of ependymal tumours and they usually develop in the cauda equina and at the ventricular wall, respectively [23,44].

Ependymomas affect mostly children and young adults, and are characterized by tremendous variability of their clinical behaviour. The overall 3-year survival rate in paediatric tumours is approximately 75% [70], and the overall 5-year survival rate for the adults is 60-70% [50]. Patients' age, anatomical location of the tumour, and the extent of surgical excision are all parameters of prognostic significance. In contrast, the prognostic significance of the specific microscopic features of those tumours, including their grading system, remains a controversial issue. In this setting, elucidation of the complex molecular changes may result in more precise understanding of their biology and, as a consequence, it may be of predictive and prognostic value. Therefore, we have decided to review the current state of knowledge on molecular alterations in ependymomas, with special regard to the pathological and clinical consequences of these aberrations.

Communicating author:

Prof. Wojciech Biernat, Department of Neuropathology and Molecular Biology, Medical University of Gdańsk, ul. Dębinki 7, PL 80-211 Gdańsk, Poland, tel./fax +48 58 349 16 50, Email: biernat@amg.gda.pl

Chromosomal abnormalities

Cytogenetic studies have shown that chromosomal abnormalities are relatively common in ependymomas [5,23,41,66,75]. Because of the rarity of ependymomas, and their clinical heterogeneity, the role of specific molecular alterations in the biological behaviour of these tumours remains unclear. Furthermore, when alterations are detected, they do not consistently help in distinguishing between low and high grade tumours [23].

Numerical aberrations of chromosomes in ependymomas show important differences between tumours developing in children and adults. About 40% of childhood ependymomas show a balanced chromosomal profile, in contrast to approximately 9% of adult tumours [11,15,48,78]. Likewise, there are differences between intracranial and spinal tumours: balanced chromosomal profiles are evident in 21-32% of intracranial ependymomas, and only in up to 3% of spinal ependymomas [11,24,68]. A common pattern of abnormalities in spinal (64%) and adult (56%) ependymomas is gain of multiple whole chromosomes.

A widespread imbalance was shown by comparative genomic hybridization (CGH) only in the myxopapillary ependymomas [11,48,61].

In total, abnormalities of the copy number of chromosomes in ependymomas as detected by classical cytogenetics and by CGH include chromosomes 1, 6, 7, 9, 10, 13, 17, 19 and 22. Deletions are more common, and losses of chromosome 22 are one of the most frequent (20-26%) [11,16,78]. The other chromosomal losses occurred at 1p, 4q, 6q, 9p, 10, 11q, 13q, 16, 17, 19q and 20q [4,6,16,24,35,36,41,48, 60,61,66,73,77,78].

Chromosome 22 and mutations of *NF2* gene

This is one of the most common chromosomal alterations in ependymomas. The role of chromosome 22 alterations in the pathogenesis of spinal ependymomas is emphasized by frequent development of that specific tumour in the setting of neurofibromatosis type 2 (NF2) syndrome. The molecular background of that family tumour syndrome depends on the germline mutations of the *NF2* gene, whose *locus* resides at chromosome 22q12. Indeed, cytogenetic studies of ependymomas have implicated chromosome 22 as an important site of nonrandom losses. By classical karyotyping, deletions and translocations involving chromosome 22q were identified in 56% of the adult and 31% of paediatric tumours [5,22,28,38, 38,47,53,75,80,81].

By means of other molecular techniques (loss of heterozygosity, CGH), frequency of allelic losses of chromosome 22 varied according to histological variant of ependymomas, their anatomical site, and age of the patient. In a large series of ependymal tumours, allelic losses on 22q were found in 0-100% of cases [6,11,16,25,28,30,47]. Loss of chromosome 22 was significantly associated with a spinal rather than an intracranial location [1,11,16,24]. It is not surprising that, due to the fact of a close relationship between the age of the patients and the location of the tumour (see above), analyses of that alteration in paediatric ependymomas revealed much lower frequency (9-28%) [16,34,77] than in the adult patients (54-56%) [16,38,39]. However, this observation and correlation has not been confirmed by Zheng et al., who identified only a slightly higher frequency of chromosome 22 loss in the intracranial than in the spinal ependymomas (78% vs. 60%) by means of microsatellite and CGH analysis [82]. More recent analysis disclosed preferential 22q loss in the adult infratentorial ependymomas in contrast to supratentorial and spinal ones, which are characterized by –9 and +2/+7/+12/–14q alterations, respectively [30].

NF2 mutations

Despite initial controversies, the *NF2* gene is clearly involved in ependymoma tumorigenesis, especially those tumours developing in the spinal cord [7,13,16,36,55,65,77]. The studies on paediatric and intracranial tumours failed to disclose *NF2* involvement [13,55,65,77]. However, in a large study of 62 ependymomas, Ebert et al. [16] showed *NF2* mutations in 43% of intramedullary tumours in contrast to none of the intracranial ependymomas. Interestingly, all the tumours bearing *NF2* mutations disclosed LOH 22 [16,36]; this indicates that *NF2* plays an important role in the oncogenesis of spinal ependymomas and shows genetically distinct subsets among WHO Grade II ependymomas [16].

Mutations of *NF2* in ependymomas affected splice sites in two tumours, frame shift mutations (two deletions and one insertion) with the introduction of premature stop codons in three tumours, and a non-

sense mutation creating an immediate stop codon in one tumour [16]. These *NF2* changes affected exons 1, 5, 7 (two instances), 10, and 13. A mutation reported by Alonso et al. [1] represented the first sequence duplication of *NF2* gene.

Other putative antioncogenes at chromosome 22

Analyses of non-NF2 families with ependymomas suggested a putative involvement of other tumour suppressor genes in the pathogenesis of these tumours independently of the *NF2* gene [27,42,59]. In support of this view, a case of anaplastic ependymoma was reported in a 5-year-old boy with a balanced reciprocal translocation of his constitutional karyotype t(1;22) (p22;q11.2) [45]. As the chromosomal breakpoint was located proximally to the *NF2 locus*, it seemingly did not alter the gene itself. The putative role of the *hSNF5/INI1* gene in the evolution of ependymomas was excluded [35]. Molecular analysis of 53 ependymal tumours from 48 patients failed to identify mutations or homozygous deletions of the *hSNF5/INI1* gene [35]. These findings corroborate the results of a study by Sevenet et al. [63], who did not detect alterations of the *hSNF5/INI1* gene in 25 ependymomas.

Chromosome 1

Gain of chromosome 1q was a frequent finding in intracranial ependymomas and this alteration was significantly associated with posterior fossa location and anaplastic histological features (WHO grade III) [11,15,24,39,48,61]. Recently, fluorescence in situ hybridization (FISH) analysis determined gain of 1q25 as an independent prognostic marker for either recurrence-free survival or overall survival in ependymomas [39].

Chromosome 6

Rearrangements and loss of chromosome 6q are common findings in a number of cases of adult and paediatric ependymomas [22,26,34,38,41,48,53,75]. No tumour suppressor gene has yet been identified at that *locus*. Structural abnormalities of 6q in ependymomas occurred in association with other chromosome abnormalities [34,41,53,65]. A strong association of loss of chromosome 6q with infratentorial location was reported by Hirose et al. [24] and later confirmed by Carter et al. [11]; all of the tumours with that alteration were located in the posterior fossa.

Allelotyping studies of ependymomas defined a hot spot deletion region at chromosome 6 (6q25.2--27) [73]. Frequent aberrations were also detected at other chromosomal regions: 6q15-16, 6q24 and 6q21-22.1 [26].

Chromosome 9

Several conventional cytogenetic studies [4,5,12, 14,41,47,53,66,69,81] described gains of chromosome 9 or translocations involving chromosome 9 in approximately 15% of ependymomas. Gain of 9p24.3--qter was identified as one of the most common alterations in ependymomas (58%) [39]. Comparing cases with alterations of chromosome 6q and/or chromosome 9, there appears to be a mutually exclusive correlation between these chromosomal aberrations [24,26]. On the other hand, loss of the whole chromosome 9 was associated with gain on 1q [24]. Furthermore, loss of whole 9 and 6q were identified in ependymomas with different anatomical location; the former alteration was seen in supratentorial lesions, while the latter was found in infratentorial tumours [24]. Loss of chromosome 9 is regarded as a hallmark of clear cell ependymomas, which preferably show this alteration in 40% of WHO grade II and 100% of WHO grade III lesions [51].

The *INK4A/ARF/INK4B* (*CDKN2A/ARF/CDKN2B*) *locus* is mapped to chromosome 9p21 and it is mutated in many cancers. It encodes three polypeptides that regulate cell proliferation via the RB and P53 tumour suppressor pathways. *CDKN2A* and *CDKN2B* encode the $P16^{INK4a}$ and $P15^{INK4b}$ polypeptides, respectively. These genes have a highly conserved amino acid sequence and seemingly they result from the duplication of the same gene [64]. They are inhibitors of CDK4 and CDK6 and, thereby, they block phosphorylation of RB [62]. The third product derived from that *locus* is ARF (for Alternative Reading Frame). As its name implies, it has no isoforms and structural homology with P16 and P15 [19,64]. It regulates P53 activity in response to unscheduled growth response signals generated by oncogenes. The alternative exons designated as 1α and 1β are spliced into common exons 2 and 3. $P16^{INK4a}$ is composed of the transcript exon 1α-exon 2-exon3, while ARF is encoded by exon 1β-exon 2-exon3 transcript and it has its own promoter [64].

Deletions and mutations in the *CDKN2A* gene are uncommon in ependymomas [10,58]. Loss of P16 expression was uncommonly found by immunohistochemistry (6.25%) [8]. The results presented by Bouvier et al. [10] suggest that in ependymomas, lack of $P16^{INK4a}$ is not associated with anaplasia and is inversely correlated with the Ki-67 labelling index (LI). It was also shown that $P16^{INK4a}$ was expressed only when cellular proliferation reached a threshold level [10]. Despite these studies that suggested an insignificant role of P16 alterations in ependymomas, Taylor et al. identified a preferential *CDKN2A* deletion in supratentorial tumours by array comparative genomic hybridization and FISH [68].

Although the significance of P16 inactivation for the pathogenesis of ependymomas is not clear, inactivation of P14ARF appears to play a role in ependymoma progression as it was shown in about 30% of these tumours [2,32].

Chromosome 5

CGH analysis revealed high incidence of gains on chromosome 5 (46%) with an overlapping region of DNA gain mapped to 5q21-22 [82]. Recurrent gains at 5p15.33 were determined as an adverse prognostic factor with resultant overexpression of hTERT leading to an increase of telomerase activity [39].

Chromosome 7

In contrast to glioblastomas, gain of chromosome 7 has been less commonly reported in ependymal tumours. Karyotyping revealed gain of chromosome 7 in a number of cases of ependymomas; most of them were anaplastic (WHO grade III) [4,21,47,53,60,75,81]. The frequency of whole chromosome 7 gains differed significantly between spinal and intracranial ependymomas; furthermore, intraspinal location was preferentially seen in adult patients [24]. A recent study confirmed these results, as high frequency of gains on 7q11.23-22.1 (58%) was identified in spinal tumours; gains of chromosome 7 were also one of the most common chromosomal imbalances independently of the anatomical location [39].

Gains of chromosome 7 were shown as a common genetic characteristic not only of spinal WHO grade II/III ependymomas but myxopapillary ependymomas as well [24]. These tumours differed in the profile of other chromosomal changes, as loss on 22q and gains of 15q and 12 had not occurred in myxopapillary tumours, in contrast to losses of chromosomes 1, 2, and 10, which occurred solely in the myxopapillary group [24].

Chromosome 10

Losses of chromosome 10 were reported in about 9-19% of ependymomas [3,21,53,60,61,66,81]. Similarly to oligodendrogliomas, it has been suggested that chromosome 10 loss may represent a final step in the malignant evolution of ependymomas [23].

In a study of spinal ependymomas, losses on chromosome 10 were seen only in myxopapillary tumours [11].

Chromosome 11

Monosomy of chromosome 11 has been described uncommonly in ependymomas [3,29,30,47,60,80,81]. Rearrangements involving 11q13 were described in a few paediatric cases [12,41,57]. The *locus* 11q13 is known to contain the oncogenes *BCL1*, *HST* and *INT2*, which are amplified in some human cancers [12], and it is likely that one of these genes plays a role in pathogenesis of ependymomas. *MEN1* mutation at 11q13 was identified in the recurrences of ependymoma WHO Grade II, that presented with LOH11q only [36]. This finding suggests a possible role of that alteration in ependymoma progression to higher grades [36,74].

Chromosome 13

With conventional cytogenetics, losses of chromosome 13 were described in approximately 5% of ependymomas [47,60,66].

Chromosome 16

Loss of chromosome 16 has not been reported as a consistent marker in ependymomas and the data on that subject are controversial. Monosomy of chromosome 16 was reported in one out of four ependymomas [60]. Much higher frequency (50-57%) of chromosome 16p loss was reported in more recent publications with the overlapped deletion regions mapped at loci 16p13.1-13.3 and 16q22-q24 [30,82].

Chromosome 17

Deletion of chromosome 17 is of particular interest because of the presence of a well-known tumour suppressor gene, *TP53* (17p13), and *NF1* (17q11.2). Monosomy 17 is one of the most common chromosomal abnormalities in ependymomas, especially in paediatric patients [21,47,53,66,81]. In a microsatellite analysis, von Haken et al. demonstrated that 50% of ependymomas harboured 17p arm loss, preferentially at the terminal end of 17p [77]. CGH data reported by Zheng et al. [82] indicated DNA losses in both arms of chromosome 17. However, other studies did not find convincing evidence of chromosome 17 abnormalities in ependymomas [6,24,28,30,34,38,47]. A recent study by Mendrzyk et al. suggested a candidate gene *PRKCA* responsible for ependymoma development, that is lost from the chromosome *locus* 17q24.2 [39].

Although a *TP53* germline mutation has been described in one patient with anaplastic ependymoma [40], somatic mutations of *TP53*, mapped to 17p13.1, are rarely affected by LOH or point mutation [17,34,43,71,77], indicating that tumour suppressor genes other than *TP53* are most likely involved in the aetiology of ependymomas.

Other genetic abnormalities

Ependymal tumours do not show amplification at classical amplified loci (*MYCC*, *MYCN* and *EGFR*) [22]. One study detected low accumulation of *MYCN* transcript in ependymoma without elevated *MYCN* gene copy number [18]. In another small series of ependymomas no amplification of *MYCN* was identified by Southern blotting [79].

EGFR overexpression, as determined by RT-PCR, was observed in 3 of 3 spinal and 6 of 7 intracranial ependymomas at similar levels and independently of DNA copy number [39]. Immunohistochemical EGFR overexpression was frequently detected and was correlated with adverse outcome in intracranial tumours. No correlation between EGFR overexpression and overall survival was observed in the spinal ependymomas [39].

Data on *MDM2* amplification in ependymomas are contradictory. The gene was mapped to chromosome 12q13-q14. It encodes the protein that specifically binds and inactivates P53. Suzuki et al. [67] detected *MDM2* gene amplification in 35% of ependymomas by differential PCR. Using the same technique, Tong et al. found *MDM2* gene amplification in only one case in their series of 26 ependymomas [72]. Another study performed by Southern blotting in 8 ependymomas did not reveal *MDM2* gene amplification in any of the tumours [49].

Gene silencing by CpG island hypermethylation seems to be rather non-operative in ependymomas as it was uncommonly identified in the ten genes analyzed by Alonso et al. [2]: 28% for *MGMT*; 28% for *GSTP1*; 57% for *DAPK*; 28% for $TP14^{ARF}$; 0% for *THBS1*; 28% for *TIMP3*; 14% for *TP73*; 0% for $CDKN2A/P16^{INK4A}$; 14% for *RB1*; and 0% for *TP53*. In another study, promoter methylation for *CDKN2A*, *CDKN2B* and $P14^{ARF}$ was identified in 21%, 32% and 21% of ependymomas, respectively [54]. In posterior fossa ependymomas all three genes were less frequently methylated in paediatric patients than in the adults. For *CDKN2B*, extracranial tumours were more frequently methylated (50%) than intracranial ones (23%). For *CDKN2B* and $P14^{ARF}$, methylation was more frequent in low-grade tumours; the reverse was observed for *CDKN2A* [54].

Histogenesis of ependymoma subsets as defined by gene expression profiles

A new technique of gene expression profiling enables simultaneous estimation of thousands of genes at the mRNA level. In a recent study, Taylor et al. have shown that ependymomas from various anatomical locations (the supratentorial region, the posterior fossa and the spinal cord) exhibit distinct patterns of gene expression and chromosomal losses and gains [68]. Interestingly, neither clinical nor histological features correlated with these molecular profiles. An important molecular hallmark of the supratentorial ependymomas was identified as an increase of expression of the members of EPHB-EPHRIN and NOTCH signalling pathways. In contrast, spinal ependymomas showed preferential expression of homeobox (HOX) family members. Interestingly, the genetic signature of these subgroups precisely correlates with the gene expression profiles of the normal ependymal cells developing from the embryonic radial glial cells (RGCs) in the subventricular zone of the lateral ventricles and spinal canal, respectively. This confirms earlier observations that supratentorial and spinal ependymomas may arise from different populations of neural progenitor cells [68]. Taylor et al. have defined these progenitor cells

more closely. The subset of RGCs was identified in the population of ependymoma cells (phenotype CD133+/RC2+/BLPB+) and the orthotopic transplants composed of these cells were capable of tumour formation, in contrast to CD133-negative and unsorted ependymoma cells. Taylor et al. [69] concluded that in ependymomas these stem cells have properties of self-renewal and multipotency and may represent the cellular targets of primary mutations that promote disease.

Prognostic significance of molecular markers

Several prognostic studies indicate that ependymomas developing in children fare worse than in the adults [37]. This difference may derive to some extent from the preferential location of paediatric ependymomas in the infratentorial compartment in contrast to the spinal ependymomas that prevail in adults.

The recent histological classification of ependymomas has thus far proven to be an unreliable predictor of clinical outcome. Likewise, the relationship between ependymoma grade and specific chromosomal aberrations is also controversial [11,15,24,30,31]. Although some age-related immunohistochemical patterns [33] and genetic alterations [11,15,24] have been found to be associated with clinical outcome in ependymoma patients, the underlying biological mechanisms remain unclear.

The relationships between ependymoma grade, specific chromosomal aberrations [11,24,30,61] and clinical outcome [33,46,52] are also controversial. Some reports indicate that gain of 1q may be a potential marker of poor prognosis in paediatric ependymomas [11,15]. The effect of gain of lq on survival of patients with intracranial ependymomas was examined. Survival curves of intracranial tumours split into classic and anaplastic groups, and those of intracranial tumours with and without gain of lq also showed clear differences. The difference between patients with anaplastic ependymomas showing gain of 1q and other tumours was even more significant [11].

Dyer et al. distinguished ependymal tumours according to the number of the chromosomal imbalances into "numerical" tumours (a total of 13 or more chromosome imbalances), "structural" tumours (a total of six or fewer imbalances) and a "balanced" group (no genetic imbalances) [15]. In multivariate analysis the structural tumours had a significantly worse outcome when compared with the other two genetic groups.

TP53 gene mutations were rarely detected in ependymal tumours [6,17,23], whereas aberrant P53 overexpression was closely correlated with both high-grade ependymomas and poor prognosis [56,76].

A high level of P14ARF expression is independently associated with prolonged progression-free survival in high-grade ependymomas [33].

Conclusions

Overall, the data indicate that spinal ependymomas, which present almost exclusively in adult patients, and intracranial childhood tumours differ significantly in their genetic profiles. Categorization of these tumours by cytogenetic aberrations may help establish a classification system that predicts patient outcome. Intracranial ependymomas may also be discriminated by molecular analyses into supratentorial and infratentorial lesions [24]. The former show preferentially loss of the whole chromosome 9, while loss on 6q is a hallmark of the latter tumours. Among spinal ependymal tumours, molecular studies disclosed basic differences between WHO grade II/III lesions and myxopapillary ependymomas (WHO grade I), despite commonly shared chromosomal 7 gains. The latter tumours did not show loss of chromosome 22 or gains of 15q and 12, but had losses of chromosomes 1, 2 and 10 [24].

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