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Case report

Relationship among clinical, pathological and bio-molecular features in low-grade epilepsy-associated neuroepithelial tumors

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ABSTRACT

The aim of this study was to evaluate the relationship between molecular markers and clinicopathological features in patients operated on for low-grade epilepsy-associated neuroepithelial tumors. Molecular-genetic signatures are becoming increasingly important in characterizing these lesions, which represent the second most common cause of focal epilepsy in patients undergoing epilepsy surgery. Data from 22 patients operated on for histopathologically confirmed low-grade epilepsy-associated neuroepithelial tumors were retrospectively collected. All specimens were examined for *BRAF* and *IDH* mutational status, 1p/19q codeletion and CD34 expression. The relationship between bio-molecular markers and several demographic, clinical and pathological features were analyzed. *BRAF* mutation was found in 11 (50.0%) patients and CD34 expression in 13 (59.1%). No patients presented *IDH* mutation or 1p/19q codeletion. Multiple seizure types were present in 5 (45.5%) patients with *BRAF* mutation and in none of those with BRAF wild type ($p = 0.035$). Moreover, *BRAF* mutation was predominant in right-sided lesions ($p = 0.004$) and CD34 expression was significantly associated with a longer duration of epilepsy ($p = 0.027$). Several other clinicopathological features, such as association with focal cortical dysplasia and postoperative seizure outcome, showed no significant correlation with molecular markers. Further studies are necessary both to confirm these data in larger cohort of patients and to investigate possible relationships between molecular markers and other clinicopathological features.

1. Introduction

Low-grade epilepsy-associated neuroepithelial tumors (LEATs) encompass a large spectrum of low grade glial and glioneuronal tumors (GNT) frequently encountered in epilepsy surgery practice, which respond very well to surgical treatment [1–10]. In the field of epilepsy surgery a low grade brain tumor is the second most common cause of focal epilepsy and it could be encountered in approximately 25–30% of patients operated on for refractory focal epilepsy [3,5,11].

Molecular-genetic signatures are becoming indispensable for the characterization of these lesions [11–16]. The increasing knowledge of molecular aspects of LEATs may allow more precise histological definition and a better comprehension of oncological behavior [11,12,14,15] and epileptological mechanisms involved [17–19].

While there are many studies focusing on bio-molecular markers [2,14,18,20–22], until now only a few examined their correlation with clinicopathological aspects [17,23,24]. In the present study, we investigated the relationship among clinical, pathological and bio-molecular features of 22 patients submitted to epilepsy surgery for low-grade epilepsy-associated neuroepithelial tumors.

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2. Materials and methods

We retrospectively reviewed the records of 22 consecutive patients affected with epilepsy, who underwent surgery between January 2009 and December 2015 at the Epilepsy Surgery Center of the Institute of Neurological Sciences of Bologna for histopathologically confirmed LEATs.

All operated patients underwent an individualized presurgical evaluation, which included a detailed history, careful evaluation of seizure semiology, interictal EEG and high-resolution (1.5 or 3 T) magnetic resonance imaging (MRI). Additionally, scalp video-EEG monitoring and neuropsychological testing were performed as needed.

Microsurgical resections were either limited to the macroscopic lesion (lesionectomy) or extended beyond the tumor to include the adjacent epileptogenic zone (tailored resection). In all cases a written preoperative informed consent was obtained.

Seizure outcome was assessed by periodic neurological evaluation in the outpatient setting and was graded according to Engel classification [25]. Postsurgical follow-up ranged from 1.5 to 7.5 years (mean 4 years).

Patients were divided according to *BRAF* mutational status and CD34 expression in order to assess differences in gender, age at seizure onset, age at surgery, epilepsy duration before surgery, seizure type, seizure frequency, anti-epileptic drugs (AEDs) intake at the time of surgery, neuropsychological deficits, tumor location, tumor type and presence of associated focal cortical dysplasia (FCD).

2.1. Pathological examination

Whenever provided "en bloc" and "spatially oriented", the surgical specimens were submitted to a proper standardized cutting procedure using anatomic landmarks. All cases were histologically reviewed and diagnosed according to the WHO classification of tumors of the central nervous system [13] and the ILAE classifications for FCD. Tissue was fixed in 10% buffered formalin, embedded in paraffin (FFPE) and routinely processed for histologic evaluation.

For immunohistochemistry, serial 4-μm-thick FFPE sections mounted on precoated slides were processed using standardized automated procedures with prediluted antibodies (Ventana-Benchmark). The areas identified as dysplastic were carefully evaluated with CD34, MAP2, p53, Ki67, and IDH1 antisera in order to rule out the possibility of tumor infiltration misdiagnosed as dysplastic tissue.

2.2. BRAF and IDH analysis by Next Generation Sequencing

All specimens were investigated for *BRAF* and *IDH* mutational status. DNA was extracted from FFPE material (two-four 10 μm-thick sections deposited on glass slides, according to the amount of lesional tissue present in the paraffin block). The areas of interest were scraped under microscopic guidance using a sterile blade. DNA was extracted using the Quick Extract Kit (Epicentre, Madison, WI, USA).

BRAF (exon 15) analysis was performed using the previously described primers [12].

IDH1 (exon 4, codons 96–138) and *IDH2* (exon 4, codons 151–178) analysis was performed using the following primers: IDH1-Exon4-Fw 5′-gAAACAAATgTggAAATCACCA-3′ and IDH1-Exon4-Rv 5′-TCACATTATTgCCAACATgACT-3′; IDH2-Exon4-Fw 5′-AgCCCAT CATCTgCAAAAA-3′ and IDH2-Exon4-Rv 5′-TgTggCCTTgTACTg CAgA-3′.

Amplicons were sequenced using the 454 GS-Junior Next Generation Sequencer (NGS – Roche, Mannheim, Germany) according to previously described protocols [26]. Based on previously published data [27] we established as criteria to define a sample mutated the identification of the mutation in at least 10 reads and in at least 2% of the total number of reads analyzed. The sequences obtained were analyzed using the Amplicon Variant Analyzer (AVA) Software (Roche Diagnostics, Mannheim, Germany). Only nucleotide variations observed in both strands were considered for mutational calls. Ambiguous base calls associated to stretches of homopolymer four-base-pair or longer were not considered mutated due to the limitations of the pyrosequencing chemistry that is used by 454-NGS [28].

2.3. FISH analysis for chromosomes 1p and 19q

Identification of the 1p/19q allelic status was obtained using a dual-color fluorescence in situ hybridization (FISH) analysis that was performed as previously described [29].

Briefly, Vysis LSI 1p36/LSI 1q25 and LSI 19q13/19p13 dual-color probes (Vysis, Inc, Downers Grove, IL) were applied onto the selected area based on the presence of neoplastic foci on each slide and analysis was carried out using an Olympus BX61 epifluorescence microscope (Olympus, Melville, NY). For each case, at least 100 neoplastic nuclei were counted, and the copy numbers of 1p36/1q25 and 19q13/19p25 were recorded for each nucleus. Cut-off values for the designation of the deletion were reported following the recommendations of Smith et al. [30].

2.4. Statistical analysis

Continuous variables were presented as median and interquartile range (IQR), while categorical variables as absolute frequency and relative frequency (%). Univariate associations were evaluated by using Fisher's exact test for categorical variables and Wilcoxon rank-sum test for continuous variables. All p-values were based on two-sided tests and $p < 0.05$ was considered significant. Statistical analysis was performed using Stata SE, 14.0.

3. Results

A total of 22 patients (11 females and 11 males) were included in this study, 4 (18.2%) were pediatric patients (age < 18 years at surgery). Median age at epilepsy onset was 15.5 years (IQR 7 – 21.75 years), median age at surgery was 28.5 years (IQR 21.25 – 49 years) and median duration of epilepsy was 12.5 years (IQR 2.5 – 19 years). Tumor location was temporal in 21 (95.5%) patients and frontal in one (4.5%). In 15 (68.2%) patients the lesion was on the right side and in 7 (31.8%) it was on the left side.

At the time of surgery, seizures occurred multiple times per day in 3 (13.6%) patients, more than once a week in 6 (27.3%) and more than once a month in 10 (45.5%). Three (13.6%) patients presented with sporadic seizures (once a month or less). Secondary generalization occurred in 3 (13.6%) patients and 5 (22.7%) presented two or more types of seizure. At the time of surgery, 7 (31.8%) patients were on anticonvulsant monotherapy while 16 (68.2%) were on polytherapy. Out of the 13 (59.1%) patients who underwent preoperative neuropsychological evaluation, 8 (61.5%) presented neuropsychological deficits.

Lesionectomy was performed in 7 patients (31.8%), while 15 (68.2%) underwent tailored resection. Complete tumor resection was achieved in all patients. No surgical complication nor postoperative neurological deficits were reported.

Histological examination of surgical specimens revealed 13 (59.1%) gangliogliomas (GG), 4 (18.2%) pleomorphic xanthoastrocytomas (PXA), 2 (9.1%) fibrillary astrocytomas, one (4.5%)

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