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BRIEF COMMUNICATION

Mutant *BRAF* in low-grade epilepsy-associated tumors and focal cortical dysplasia

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Abstract

BRAF alterations, namely *BRAF* fusion and *BRAF* V600E mutation, have been recently reported in low-grade epilepsy-associated tumors. Twenty low-grade epilepsy-associated tumors were retrieved to evaluate the *BRAF* mutational status. *BRAF* mutations were present in 10 tumors and concomitantly in associated dysplastic tissue of three patients. We here show for the first time that *BRAF* mutations are present not only in low-grade epilepsy-associated tumors but, in some cases, also in the associated focal cortical dysplasia.

Introduction

Low-grade epilepsy-associated tumors (LEAT) represent a common pathologic substrate in the setting of medically intractable chronic epilepsy.[1] These tumors most commonly arise in the temporal lobe, mainly in the temporo-anterior-basal-mesial site.[2] A significant part of these neoplasms, particularly ganglioglioma (GG) and dysembryoplastic neuroepithelial tumor (DNT), is associated with malformations of cortical development, in particular focal cortical dysplasia (FCD) (30–80% of cases).[3–5] Moreover, a case of GG associated with FCD type IIa has been recently reported, where findings suggest the dynamic-evolutive-oncogenic potential of cells comprising FCD type IIa.[6] A possible common origin of GG and FCD, from a precursor that undergoes abnormal glioneuronal development has been proposed.[7]

LEAT share the expression of CD34[8]: CD34-immunoreactive cells could represent dysplastic or undifferentiated neural precursors.[7]

Furthermore, *BRAF* alterations, namely *BRAF* fusion[9] and *BRAF* V600E mutation,[8,10] have been recorded in LEAT. Interestingly, it has been recently observed that mutant BRAF protein in GG is predominantly expressed by neuronal tumor cells.[11]

As a member of the RAF family of serine/threonine kinases, BRAF is a key mediator of the mitogen-activated protein kinase (MAPK) signaling pathway (also known as the RAF-MEK-ERK pathway). BRAF is involved in a wide variety of cellular functions, including cell proliferation, cell cycle arrest, terminal differentiation, and apoptosis.[12] The *BRAF* gene is activated by oncogenic mutations. Most BRAF mutations are missense mutations at amino acid position 600, resulting in an exchange of valine for glutamate (referred to as $BRAF^{V600E}$). A small-molecule inhibitor with antineoplastic activity (vemurafenib) that targets BRAF V600E mutant has been recently developed.[13]

We here show for the first time that *BRAF* mutations are present not only in LEAT but, in a significant

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proportion of cases, also in the FCD tissue associated with LEAT.

Methods

Twenty LEATs referred to the Bellaria Hospital, Bologna, Italy, were retrieved (Male/Female ratio 8/12) to evaluate the *BRAF* mutational status. Epilepsy onset mean age was 12 years (range 1 months to 51 years), mean age at surgery was 22.5 years (range 3–51 years), and mean duration of epilepsy was 5 years (range 6 months to 38 years). Mean follow-up was 6.5 years (range 1–11 years). All patients were submitted to a pre-surgical neurophysiological assessment by means of noninvasive long-term video-EEG monitoring aimed to define the epileptogenic area. Seizure semiology, obtained by clinical history and ictal video-EEG monitoring were consistent with focal temporal lobe seizures in all patients. In all cases magnetic resonance imaging was performed. All the patients were submitted to a tailored surgery, consisting in removing the tumor, the temporal pole, the anterior neocortical lateral cortex, the uncus–entorhinal area, and the hippocampus and parahippocampal gyrus.

LEATs were histologically classified according to the WHO classification of tumors of the central nervous system.[14] Namely the study included eight GG (40%), two gangliocytomas (GC) (10%), four DNT (20%), four pleomorphic xanthoastrocytomas (PXA) (20%), one papillary glioneuronal tumor (5%), and one grade II diffuse astrocytoma (5%).

Furthermore, all the specimens were also histologically classified according to the published criteria for mesial temporal sclerosis (MTS) diagnosis[15] and the most updated classification of FCD,[16] which has been shown to considerably improve the reproducibility of FCD diagnoses.[17]

In 10 cases (50%), the tumors were associated with FCD (associated cases), while in 10 cases (50%) FCD was not present (isolated tumoral cases). 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. Specifically, the group of cases with FCD showed an architectural cortical dysplasia, without dysmorphic neurons or ballon cells (FCD type IIIb), in 4/10 cases (40%). The remaining 6/10 (60%) patients had FCD type IIa. No patient presented concomitant MTS.

BRAF mutational status was separately analyzed in all 20 LEATS, in the 10 tumor-associated FCD specimens and in the 10 histologically normal cortical tissue samples adjacent to the LEATS that were not associated with FCD. For comparison 10 samples of "isolated FCD" (five of FCD type I, four of FCD type IIa and one of FCD type IIb) have also been tested for *BRAF* mutational status.

In order to assess the *BRAF* molecular status DNA was extracted from formalin-fixed paraffin-embedded (FFPE) material scraped under microscope guidance from five 10-μm thick sections, using the MasterPure DNA FFPE extraction kit (Roche Diagnostic, Manheim, Germany).

About 10 ng of DNA were used for amplification with primers specific for *BRAF* exon 15 (Fw 5′ – TGCTTGCTC TGATAGGAAAATGA – 3′; Rv 5′ – TGGATCCAGACAAC TGTTCAAA – 3′) modified with universal tail sequences and multiple identifier (MID) nucleotides. Sequencing was performed using the 454 GS-Junior next generation sequencer (Roche Diagnostic, Mannheim, Germany) according to established protocols (http://www.454.com/). Results were analyzed using Amplicon Variant Analyzer (AVA) software (Roche Diagnostic, Mannheim, Germany). Next generation sequencing of target DNA sequences (reads) not only allows to identify a mutation but also gives the percentage of mutated amplicons in the sample analyzed.

Results

Histopathological and molecular results of LEATs and of associated FCD are summarized in Table 1. Findings observed in isolated LEATs (without FCD) and in

Table 1. Histological and molecular results of LEATs with associated FCD.

Case	Histological diagnosis	*BRAF* status	% of mutated reads	Total number of reads
1	GG	V600E	2.5	571
	FCD type IIIb	WT		
2	GG	V600E	12	1091
	FCD type IIa	V600E	5	559
3	DNT	T599_V600InsT	2	1047
	FCD type IIIb	WT		
4	GG	V600E	3.8	1109
	FCD type IIa	WT		
5	GG	WT		
	FCD type IIa	WT		
6	DNT	WT		
	FCD type IIIb	WT		
7	GG	WT		
	FCD type IIa	WT		
8	PXA	WT		
	FCD type IIIb	WT		
9	PXA	V600E	33	940
	FCD type IIa	V600E	5	903
10	PXA	V600E	25	967
	FCD type IIa	V600E	3.5	820

DNT, dysembryoplastic neuroepithelial tumor; FCD, focal cortical dysplasia; GG, ganglioglioma; PXA, pleomorphic xanthoastrocytoma; WT, wild type.

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adjacent histologically normal cortex are summarized in Table 2. At least 519 consensual reads (analyzed sequences) were obtained with parallel sequencing (median number 922 reads, range 519–1352 reads). The *BRAF* V600E mutation was detected in eight neoplastic specimens. One GG sample had the *BRAF* V600K mutation. One DNT sample had a mutation resulting in the insertion of a threonine residue between codons 599 and 600 (*BRAF* T599_V600 InsT). Altogether *BRAF* mutations were found in three out of four PXA (75%), in four out of eight GG (50%) (including the case with the *BRAF* V600K mutation), in two out of four DNT (50%) (including the *BRAF* T599_V600insT mutation), and in the single case of grade II diffuse astrocytoma. The remaining 10 LEATs were not mutated for *BRAF*. Three dysplastic specimens, constituted by FCD type IIa, had the *BRAF* V600E mutation: all these three cases were associated with a *BRAF* V600E mutated tumor (one GG and two PXA) (see Fig. 1A and B). On the other hand, seven dysplastic cases (four FCD type IIIb and three FCD type IIa), the 10 tumor-associated brain samples without cortical dysplasia and the 10 cases of "isolated FCD" were not mutated for *BRAF*.

Table 2. Histological and molecular results of isolated LEATs (without FCD) and of adjacent histologically normal cortex.

Case	Histological diagnosis	*BRAF* status	% of mutated reads	Total number of reads
11	DNT	V600E	16.6	883
	NO FCD	WT		
12	DNT	WT		
	NO FCD	WT		
13	PGNT	WT		
	NO FCD	WT		
14	Gangliocytoma	WT		
	NO FCD	WT		
15	PXA	V600E	17.5	519
	NO FCD	WT		
16	Grade II diffuse astrocytoma	V600E	31	1352
	NO FCD	WT		
17	GG	WT		
	NO FCD	WT		
18	GG	V600K	26	630
	NO FCD	WT		
19	Gangliocytoma	WT		
	NO FCD	WT		
20	GG	WT		
	NO FCD	WT		

DNT, dysembryoplastic neuroepithelial tumor; FCD, focal cortical dysplasia; GG, ganglioglioma; PGNT, papillary glioneuronal tumor; PXA, pleomorphic xanthoastrocytoma; WT, wild type.

Discussion

We here show for the first time that *BRAF* mutations are present not only in LEATs, as recently shown[8,10] but also in the FCD that accompanies LEAT. These data are very intriguing, as a possible common origin of LEATs and FCD from a precursor that undergoes abnormal glioneuronal development, has been postulated[7] but not yet proven.

Lesional cells may represent a low proportion of FCD tissue requiring a highly sensitive method to identify genetic alterations. Next generation sequencing targeted to BRAF exon 15 was utilized for mutation detection.

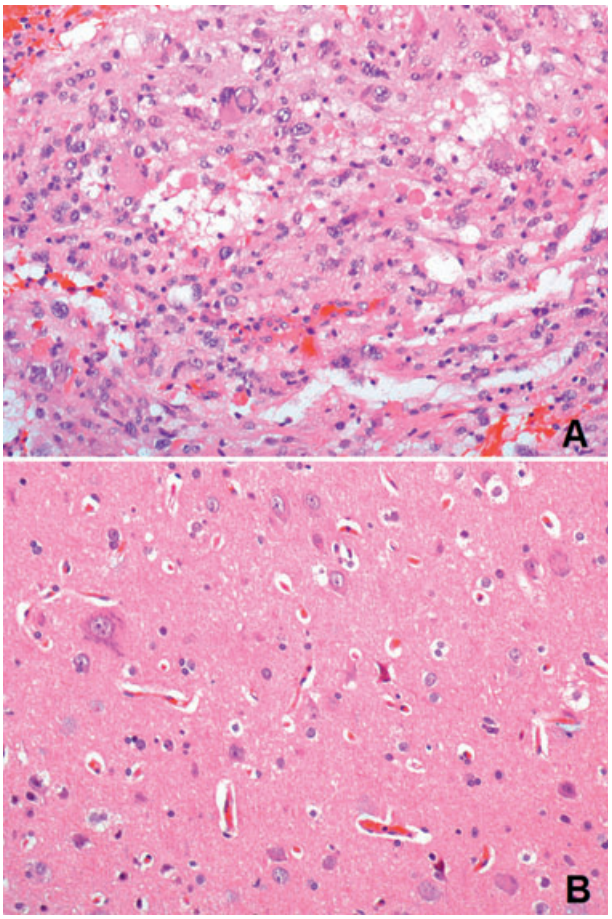

Figure 1. Case no. 9. (A) Representative BRAFV600E mutated LEAT: the tumor is a grade II pleomorphic xanthoastrocytoma composed of giant neoplastic cells showing nuclear pleomorphism and xanthomatous changes; nuclear inclusions and numerous eosinophilic granular bodies are present; in spite of marked cytological pleomorphism the tumor lacks microvascular proliferation and necrosis; mitotic figures are not common (H&E, 200× magnification); (B) corresponding BRAFV600E mutated associated FCD: dysmorphic neurons in focal cortical displasia type IIa (H&E, 200× magnification).

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In the FCD type IIa cases where we identified *BRAF* mutations, the diagnostic clue was represented by the presence of dysmorphic neurons. This observation is in line with the recent finding that the mutant BRAFV600E protein in GG is predominantly expressed by neuronal tumor cells.[11]

We considered the possibility that identification of BRAFV600E mutated alleles in FCD samples simply reflected the presence of rare neoplastic cells interspersed with the dysplastic elements. However, this possibility has been excluded by the careful histologic and immunohistochemical screening (with CD34, MAP2, p53, Ki67 and IDH1 antisera) of all FCD cases prior to molecular analysis. Importantly, the percentage of mutated target sequences (reads) in the BRAFV600E mutated FCD samples was 5% in two cases and 3.5% in the remaining case. Assuming that the BRAFV600E is heterozygous the amount of mutated cells in the sample analyzed was 7–10%, a proportion of possible neoplastic cells well above the threshold for histopathological diagnosis.

We did not find BRAF mutations in the cases of "isolated FCD," unlike "associated FCD." This difference could reflect a not common biological pathway. It is well known for example that isolated FCD type I represents a different disease, with different outcome, in comparison to FCD type I associated with another lesion (i.e., hippocampal sclerosis, tumor, vascular malformation), so that today it is diagnosed as FCD type III. Finally, the recent finding that expression of BRAF V600E is associated with a worse postoperative seizure outcome in glioneuronal tumors[18] could be explained by the presence, in some cases, of a not removed FCD in the adjacent cortex.

Thus, our results support the hypothesis that – in a significant proportion of cases – FCD and LEATs can show an evolutive oncogenic progression, similar to what has already been shown for other tumors.[19]

Interestingly, we found the *BRAF* V600E mutation also in a grade II diffuse astrocytoma, a tumor included in LEATs, but not having a glioneuronal nature. This finding, only rarely documented in large systematic studies,[10,20] is consistent with a recent report[21] in which the *BRAF* V600E mutation was identified in five (15%) out of 33 consecutive grade 2 diffuse gliomas. The authors observed that four out of these five *BRAF*-mutant grade 2 diffuse gliomas presented with long-standing, frequent, sometimes refractory seizures and all four tumors were located within the temporal lobe. They also reported two cases of glioblastoma with *BRAF* V600E, both in patients presenting with focal seizures. All these data indicate that *BRAF* mutations occur in a setting specifically linked to epileptogenesis.

To the best of our knowledge this is the first demonstration that *BRAF* mutations are present in the FCD associated with LEAT, suggesting a pathogenetic role. Our findings prompt further investigation into the potential role of BRAF mutations in cyto-architectural dysplasia and in the tumorigenesis of LEATs.

Author Contributions

G. M. designed the study, discussed and interpreted results, wrote the manuscript. D. d. B. performed molecular experiments, analyzed, and interpreted results. M. V. performed molecular experiments. M. G. performed tailored surgery, discussed results. M. M. performed tailored surgery. L. V. performed pre-surgical neurological assessment. P. R. contributed to pre-surgical neurological assessment. G. R. performed pre-surgical neurological assessment. R. M. discussed and interpreted results, supervised the work. G. T. discussed and interpreted results, coordinated, and supervised the work.

Conflict of Interest

None declared.

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