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Original Article

Immunohistochemical and Biomolecular Characteristics of Craniopharyngiomas: A Single Institution Experience from Southern Italy

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Abstract

In the present paper, The Authors discuss the histological and immunohistochemical aspects observed in a casuistry of craniopharyngiomas (CP), identifying not only subtypes (papillary or adamantinomatous), but also their proliferative activity and molecular pathways. For this purpose, a series of thirty-seven was retrieved during the last decade (2007-2017) from files of our pathology department; there were 21 female and 16 male patients (age range 5-75 years, mean age 43.48), but 7 cases were recorded in childhood. A prevalent adamantinomatous pattern (ACP) (34 cases) was encountered, while only 3 exhibited papillary variant (PCP).

After a pretreatment, the immunostaining protocol was performed by Ventana BenchMark ultraimmunostainer to investigate β-catenin, Ki67 and *BRAF* V600E mutant epitope. Successively, in order to evaluate the mutational status concerning *BRAF*, tissue sections were microdissected by scalpel, subjected to DNA extraction and subsequent analysis utilizing the *BRAF* codon 600 mutation analysis kit to identify five somatic mutations in codons 600.

Twenty-five ACP cases exhibited β-catenin immunostaining; moreover, nine additional cases of ACP were unreactive for β-catenin, but showed a Ki67 labelling index > 5%. Although none of PCP cases showed β-catenin immunoreaction, a cytoplasmic immunopositivity for *BRAF* V600E mutant epitope was recorded only in all PCP cases, which also harboured *BRAF* V600E mutations; in these latter cases, a less favorable outcome was documented since one patient died for the disease and two presented loco-regional recurrences. Therefore, we suggest that some immunohistochemical markers and biomolecular signatures in CP histologic variants may be utilized as hallmarks to identify improved treatment modalities.

Keywords

Craniopharyngioma, Histotype, Immunohistochemistry, *BRAF* status, Prognosis

Introduction

Craniopharyngiomas (CP) are benign epithelial tumors, histogenetically arising from Rathke's residues and frequently developed in the suprasellar region [1-5]. Nevertheless, intrasellar or sphenoidal osseous localizations have been reported [1-5]. It is well known that CP may develop in all ages, although young adults are the most frequent affected patients [6]. Grossly, the tumor exhibits a common cystic appearance with a lobular surface, but rarer totally solid presentation [7]. The neoplastic dimensions are ranging from few to many centimeters, although a reactive gliosis is always documented as well as a definite expansion and/or compression to adjacent nervous tissues, such as optic nerve, chiasm and the third ventricle [2-6]. This latter aspect greatly influences neurosurgical procedures and it may predict the final outcome [1,3]. Moreover, together with the local neoplastic infiltration, the identification of histological subtypes may also suggest a severe long term morbidity, stressing the attempt to identify immunohistochemical and biomolecular characteristics useful for the management of these unusual tumors as a peculiar aim of the present study.

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Materials and Methods

From archival files of our Department located in advanced teaching hospital (University of Messina & Azienda Ospedaliera Universitaria "Polyclinic G. Martino") 37 CPs were retrieved with reference the period 2007-2017. Total or sub-total surgically resected specimens were obtained from adult and pediatric neurosurgical sections; in detail, patients were 21 female and 16 male (age range 5-75 years, mean age 43.48 yrs). 30 cases exhibited a cystic appearance and 7 were represented by childhood cases. Accordingly to WHO (2016), 34 CP were histologically classified as adamantinomatous variant (ACP), while only 3 presented a papillary picture (PCP). For all cases follow-up data were available.

From same neoplastic tissue blocks utilized for the routine H&E staining, 4 mμ thick seriate silane-coated sections were obtained and then immunostained using a Ventana BenchMark ultraimmunostainer (Ventana, Tucson, AZ, USA) with the following antibodies: monoclonal mouse anti-β-catenin (Cell Marque, clone 14, working dilution 1:100, Darmstadt, Germany), Ki 67 (Dako, clone MIB-1, w.d. 1:150, Glostrup, Denmark) and monoclonal mouse recognizing the *BRAF* V600E mutant epitope (*BRAF* V600E-specific clone VE1, Ventana, w.d. 1:200, Tucson, AZ, USA). The staining protocol included pretreatment with cell conditioner 1 (pH 8.4) for 64 minutes, incubations with antibodies at 36 °C for 16 minutes, followed by primary antibodies detection using the UltraView Universal DAB detection kit (Ventana, Tucson, AZ, USA). Finally, the nuclear counterstain by haematoxylin for 4 minutes. The stained sections were then dehydrated in the ascending alcohols, cleared in xylene and mounted with Permount (Sigma-Aldrich, Darmstadt, Germany).

In order to evaluate *BRAF* mutational status, four 10 mμ thick haematoxylin & eosin stained sections were microdissected by a scalpel using an inverted microscope to collect only regions with the highest neoplastic representation. DNA extraction was performed by QIAamp DNA FFPE Tissue kit (Qiagen, GmbH, Hilden, Germany) according to the manifacturer's recommendations and DNA quantified by fluorometry with the Qubit platform (Life Technologies, Rockville, Maryland, USA). DNA samples were then subjected to *BRAF* mutational analysis utilizing the *BRAF* Codon 600 Mutation Analysis Kit II (EntroGen Inc, Los Angeles, CA, USA) that allows to identify five *BRAF* somatic mutations in codons 600 (V600D, V600E, V600K, V600M, V600R). The amplifications were carried out in a StepOnePlus Real-Time PCR system (Life Technologies, Rockville, Maryland, USA), following the manifacturer's procedures as well as according to the recommendations of both the Italian Association of Medical Oncology (AIOM) and the Italian Society of Pathology and Cytopathology (SIAPEC) [8].

Results

Among 37 CP patients, sub-total excision was performed in 19 cases (51.35%), while a gross total excision was done in the remaining 18 cases (48.65%). The follow-up observational

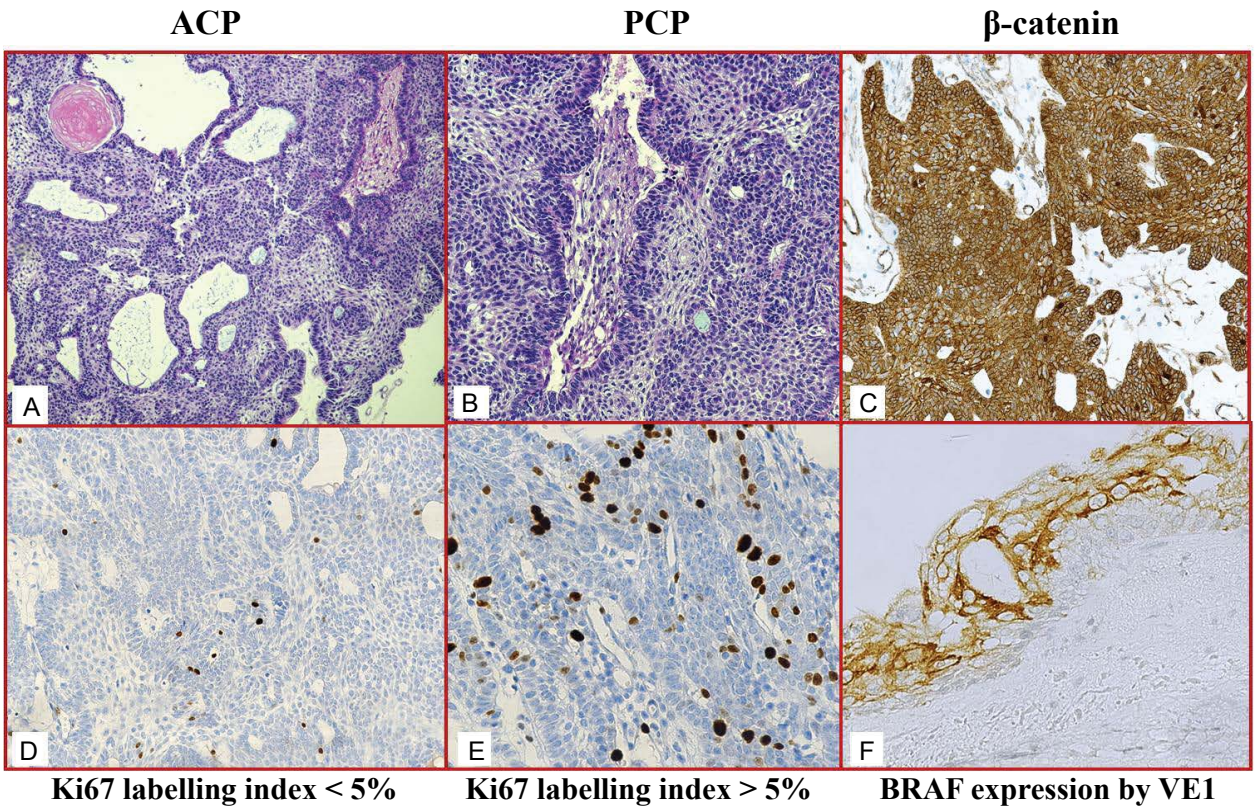

Figure 1: Histological characteristics of APC (a, X20) and PCP (b, X40). Note the intense and diffuse membranous β-catenin immunostaining in APC (c, X20). Low (d, X20) and high labelling index (e, X40) were encountered in different CP cases. A cytoplasmic immunopositivity for anti-VE1 antibody was encountered only in PCP (f, X40).

period ranged from 5-122 months (mean 49 months). During this interval, four patients experienced recurrences (2 ACP and 2 PCP), two patients died (1 ACP for neurosurgery related events, 1 PCP for the disease), while the remaining were alive, some of them with significant morbidities. Histologically ACP, representing the great majority of our casuistry, exhibited an adamantinomatous pattern characterized by stratified epithelium with a palisading arrangement of the basal cells, with presence of keratin and microcystic changes (Figure 1a). Three cases of PCP presented papillary areas composed by stratified squamous epithelium resting upon a connective stromal tissue (Figure 1b). A reactive, sometimes exuberant, gliosis with Rosenthal fibers was encountered in the adjacent cerebral tissue.

By immunohistochemistry, twenty-five (73.52%) ACP cases showed an evident β-catenin immunostaining with its peculiar cytoplasmic/membranous distribution and without any shift to nuclear accumulation (Figure 1c); in these cases, Ki67 immunoexpression rate was constantly < 5% (Figure 1d). interestingly, The additional 9 cases of ACP were unreactive for β-catenin, but showed a Ki67 labelling index > 5%, (Figure 1e). The two ACP cases that exhibited recurrences were present either in positive β-catenin group either in negative one, independently from the Ki growth fraction. None of the PCP cases showed β-catenin immunostaining.

Moreover, with the VE1 antibody cases were considered positive if there was unequivocal diffuse cytoplasmic staining in > 85% of tumour cells (Figure 1f). The intensity of staining of *BRAF* expression in tumour cells was recorded on a 0-3 scale. Strong cytoplasmic staining was scored as 3, medium cytoplasmic staining as 2, weak cytoplasmic staining as 1 and the absence of staining was scored as 0. Scores of 1-3 represented positive staining, while scores less than 1 were considered negative staining. A cytoplasmic immunopositivity for anti-VE1 antibody was encountered only in all 3 PCPs; these latter cases also harboured *BRAF* V600E mutations and presented a less favorable outcome, since 1 patient died for the disease and two manifested recurrences.

Discussion

In intracranial tumours, the management of CPs remains one of the most hot point due to their pattern of growth as well as high recurrence rate and co-morbidities [9-11]. Although a complete resection may be considered largely curative in order to preserve patient's long-term quality of life, the risk of involvement for other brain structures often represents an adverse event [12,13]. Moreover, recurrent CPs may result refractory to additional surgery as well as to adjuvant radio-chemotherapy; therefore, emerging molecular targeted therapy should be addressed to fight CP in reducing morbidity [14-18]. In particular, the discovery of new molecular pathways involved in CPs, such as mutations in the gene encoding β-catenin or in the *BRAF* gene, has raised a great interest to provide potential targets for new treatments [19-22].

It has been reported that nuclear translocation of β-catenin was associated to more aggressive CPs, as documented by an intense β-catenin expression in cases with generalized recurrences [23-26]. In our series, a β-catenin cytoplasmic/membranous immunostaining, without shift towards nuclear accumulation, was encountered in 73.52% ACP cases, while Ki67 immunoexpression rate was constantly < 5%. The additional 9 cases of ACP (26.48%), showing a Ki67 labelling index > 5%, were unreactive for β-catenin. Consequently, the occurrence of CTNNB1 mutation is not always present as rule in CPs. However, our immunohistochemical observations offered an advantage of Sanger sequencing in the detection of β-catenin gene mutations, even though a relationship between this marker and growth fraction performed by Ki67 was not revealed. In fact, the two ACP patients with recurrences belonged to positive β-catenin ACP group as well as to negative one. On the other hand, our data were not so surprising since other analyses failed to demonstrate such a predictive value [27].

It is well known the *BRAF* gene encoded a serine/threonine-protein kinase B-Raf (*BRAF*), which belongs to the family of growth signal transduction non-receptor protein kinases [28]. *BRAF* mutations have been identified in different types of cancer, such as colon carcinoma, melanoma, papillary thyroid carcinoma and some lymphomas [28]. Recently, a *BRAF* V600E mutant-specific antibody VE1 became available for immunohistochemical screening of such *BRAF* mutant tumors [29,30]. Testing by this specific antibody our CP cohort, we observed a cytoplasmic immunopositivity was recorded only in all 3 PCPs; moreover, these cases also harboured *BRAF* V600E mutations and they presented a less favorable outcome, since 1 patient died for the disease and two manifested recurrences. According to this molecular event, the presence of *BRAF* mutation in PCPs might suggest new therapeutic approaches; recently in fact, among different treatment options, some studies have shown relevant response of PCPs using *BRAF* inhibitors or combination therapy with *BRAF* (vemurafenib and dabrafenib) and MEK (trametinib) inhibitors [22,31]. These data may suggest the opportunity to surgically access either before or after targeted therapy in order to significantly lower chances of recurrence while maintaining superior quality of life. Specifically, trials with *BRAF* inhibitors such as vemurafenib and dabrafenib preceding or following surgical procedures for CPs (mainly PCPs) may lead to improved clinical outcomes.

Finally, further pluri-institutional prospective studies will help to elucidate the role of targeted therapy in CPs, improving the management in cases with aggressive or recurrent behavior, such as *BRAF* positive PCPs, according to the present although limited observations.

Conflict of Interest

The authors declare no conflict of interests.

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