IMMUNOHISTOCHEMICAL AND BIOMOLECULAR ASPECTS IN CRANIOPHAR YNGIOMAS: IS THERE ANY SUGGESTION TO IMPROVE THEIR TREATMENT?

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Objective. Craniopharyngiomas (CP) are rare benign epithelial tumors of the sellar region, with two identified histological subtypes represented by the adamantinomatous variant (ACP) and the rarer papillary variant (PCP). Generally, they are locally infiltrative and surgically challenging tumors with severe long term morbidity. Current data suggest that both variants are defined by specific genetic alterations and influenced by distinct molecular pathways. The aim of the present study is to analyze histopathological, immunohistochemical and biomolecular characteristics in a series of CPs.

Materials and Methods. We retrieved from our database 37 CPs, 34 of which were ACP and only 3 PCP. The patients were 21 female and 16 male (age range 5-75 years, mean age=43.48 yrs); CPs samples included 7 childhood patients. 4mμ thick silane-coated sections were stained using a Ventana BenchMark ultraimmunostainer (Ventana, Tuscon, AZ, USA) and the following antibodies: monoclonal mouse-anti-β-catenin (Cell Marque, clone 14), Ki 67 (Dako, clone MIB-1) and monoclonal antibody recognizing the BRAF V600E mutant epitope (BRAF V600E-specific clone VE1, Ventana, USA). The staining protocol included pretreatment with cell conditioner 1 (pH 8,4) for 64 min, incubation with antibody at 36 °C for 16 minutes, primary antibody detection using the ultraView Universal DAB Detection Kit (Ventana), followed by counterstaining with hematoxylin for 4 minutes.

In order to evaluate BRAF mutational status, four 10 μm thick H&E-stained sections were microdissected by scalpel using an inverted microscope in order to collect only regions with the highest MPHC representation. DNA extraction was performed by QIAamp DNA FFPE Tissue kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's recommendations and DNA quantified by fluorometry with the Qubit® platform (Life Technologies, CA, USA). DNA samples were subjected to BRAF mutational analysis utilizing the BRAF Codon 600 Mutation Analysis Kit II (EntroGen, Inc, CA, USA) that allows to identify five BRAF somatic mutations in codons600 (V600D, V600E, V600K, V600M, V600R). The amplifications were carried out in a StepOnePlus™ Real-Time PCR system (Life Technologies) following the manufacturer's procedures and the recommendations of both the Italian Association of Medical Oncology (AIOM) and the Italian Society of Pathology and Cytology (SIAPEC).

Results. Twenty-five (73.52%) ACP cases showed β-catenin immunopositivity; interestingly, nine ACPs unreactive for β-catenin showed a Ki67 labelling index > 5%. Cytoplasmic immunopositivity for anti-VE1 antibody was observed only in all 3 PCPs, all of which also harboured *BRAF* V600E mutations. These latter cases exhibited a less favourable outcome, since 1 died for the disease and 2 presented recurrences.

Conclusions. The identification of hallmark immunohistochemical and biomolecular signatures in the two CPs histotypes may be utilized to identify alternate improved treatment modalities, taking also into consideration clinicopathological characteristics of these unusual brain tumors.

EXPRESSION OF TRKB RECEPTOR AND ITS LIGAND BDNF IN BRAIN METASTASES OF LUNG ADENOCARCINOMA.

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Introduction. Tropomyosin-related kinase B (TrkB) is a receptor for brain-derived neurotrophic factor (BDNF) and is highly expressed in various neoplasms. It plays an important role in tumor progression and metastasis in various cancers, including lung adenocarcinoma. The main TrkB isoforms, full-length (TrkB.FL) and truncated (TrkB.T1), mainly play opposite roles. We examined the distribution of both TrkB protein and mRNA isoforms (TrkB.FL and TrkB.T1) in non- brain- metastatic versus brain- metastatic adenocarcinoma of the lung. Moreover, in this second group, we compared TrkB protein and BDNF mRNA expression between primitive cancerous versus brain-metastatic cells.

Materials and Methods. Paraffin tissue sections of 10 non brain-metastatic lung adenocarcinoma (A group, follow-up=12 months) and of 16 samples from 8 patients with brain-metastatic lung adenocarcinoma (B group) were immunostained for TrkB. Moreover, expression of TrkB.FL and TrkB.T1 isoform mRNA were investigated by RT-PCR, in 9 patients from group A and in 5 from group B; furthermore, expression of BDNF mRNA, the ligand of TrkB, was evaluated in primary tumors and in metastatic samples.

Results. TrkB-protein was more expressed in metastatic tumors: 6 of 8 patients (75%, $p<0.05$) versus 3 of 10 patients (30%, $p<0.05$). Moreover, intensity of expression of TrkB-protein was 20% higher in brain metastasis vs primary samples. Finally expression of BDNF mRNA was 30% higher in brain-metastasis vs primitive samples ($p<0.05$).

Conclusions. Our findings provide preliminary evidence that the TrkB-receptor is more expressed in primary tumors of patients with metastatic lung adenocarcinoma. Interestingly, brain-metastasis express TrkB receptor and its ligand BDNF at higher levels than the corresponding primary tumors.