BRAFV600E Mutation in Melanotic Neuroectodermal Tumor of Infancy: Toward Personalized Medicine?

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The melanotic neuroectodermal tumor of infancy (MNTI) is a rare neoplasm that primarily affects the maxilla of infants during their first year of life. Complete resection is the conventional treatment and recurrence rates vary from 10% to 60%. The recurrent tumors grow more aggressively and can invade other anatomic structures, such as the nasal cavity, the orbit, and the skull base. The aggressive behavior of MNTIs may require radical resection, which may not be possible in some cases because of its rapid and invading growth together with invasion of vital structures. In these situations, adjunct radiotherapy or chemotherapy has been used. However, as there are no conclusive data regarding the molecular profile of this tumor, currently there is no targeted therapy that may be used in the treatment of selected aggressive cases. On the basis of MNTI similarities with melanomas, such as derivation from the neural crest cells and presence of large melanin-containing cells, we hypothesized that MNTIs also may harbor the BRAFV600E oncogenic mutation. We show for the first time that this important pediatric tumor may harbor the oncogenic BRAFV600E mutation, providing the first insights to their personalized treatment.

Melanotic neuroectodermal tumor of infancy (MNTI) is a rare neoplasm that primarily affects the maxilla of infants during their first year of life.

MNTI histology shows 2 distinct populations of cells that form nests or alveolar structures: large polygonal epithelioid cells, containing melanin, resembling melanocytes, and small, round neuroblastlike cells.[1] MNTI appears as an expansive dark-pigmented tumor mass that may cause tooth displacement. Despite being benign, MNTI grows rapidly, clinically presenting as a painless expansive unencapsulated tumor.[2] Radiographs show an intrabony radiolucent lesion with a poorly defined radiolucent area.[3] Complete resection is the conventional treatment and recurrence rates vary from 10% to 60%.[2] The recurrent tumors grow more aggressively and can invade other anatomic structures, such as the nasal cavity, the orbit, and the skull base.[4]

The Raf-MEK-ERK mitogen-activated protein kinase pathway is activated by growth factors, hormones, and cytokines and regulates proliferation, differentiation, survival, senescence, and migration of cells.[5] Among other isoforms of the Raf serine/threonine kinases, B-Raf (encoded by the *BRAF* gene) has a dominant role in signaling to the ERK pathway.[5] The most common *BRAF* oncogenic mutation detected in human cancer is characterized by a T>A transversion, leading to a valine (V) to glutamic acid (E) amino acid substitution: V600E. The identification of the BRAFV600E mutations in melanoma changed the

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Dr Gomes designed the study, revised the histopathology of the cases, worked on results interpretation, and critically revised the manuscript; Dr Diniz designed the study, worked on the molecular experiments and interpretation of the results, and critically revised the manuscript; Ms Menezes worked on the molecular experiments and data collection, and critically revised the manuscript; Dr Castro revised the clinical data of the cases, worked on data collection, selected the clinical images, and critically revised the manuscript; Dr Gomez conceptualized and designed the study, revised the clinical cases and histopathology, supervised the molecular study, and drafted the initial manuscript; and all authors approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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TABLE 1 Clinical and Molecular Data of the MNTI Cases Included in the Study

Case	Age	Gender	BRAF Mutation (Codon 600)
1	75 d	Girl	V600E
2	5 mo	Girl	Wild-type
3	12 mo	Boy	Inconclusive[a]

[a] The assays were repeated twice; however, as the tumor had been decalcified, DNA integrity might have been affected and results were not straightforward.

pathologic comprehension of the tumor and new treatment options emerged from this finding.[6,7]

On the basis of MNTI similarities with melanomas, such as derivation from the neural crest cells[8] and presence of large melanin-containing cells, we hypothesized that MNTIs also may harbor the BRAFV600E mutation.

CASE SERIES

We studied 3 cases of MNTI. Details of these cases are listed in Table 1. Clinical and histologic images of case 3 are shown in Fig 1. The local human investigations committee approved this study and formalin-fixed paraffin-embedded (FFPE) tumor samples were retrieved from the files of the university.

We retrieved 4 cases from the files; however, only 3 had enough material for DNA extraction and were included in the study. None of the 3 patients had any medical condition or has been submitted to any relevant medical intervention. The main sign reported by the parents was presence of an expansion in the anterior maxilla of the child. The 3 tumors were surgically excised and did not recur after a 5-year follow-up period. All 3 tumors presented the biphasic cell population formed by large melanin-pigmented epithelioid cells and small, round neuroblastlike cells.

Genomic DNA was extracted from FFPE tissue samples by using QIamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany). To access the BRAFV600E mutation, TaqMan Mutation Detection Assays (Life Technologies, Carlsbad, CA) and Sanger sequencing with primers forward 3′ TCATAATGCTTGCTCTGATAGGA 5′ and reverse 3′ CCAAAAATTTAATCAGTGGA 5′, were performed. We detected and confirmed BRAFV600E mutation in 1 sample (Fig 1), another was wild-type, and the third was inconclusive (Table 1). We repeated the third case reactions, but as it was decalcified, this might have affected DNA quality/integrity.

DISCUSSION

Despite being a benign tumor, MNTI affects very young children and may be potentially lethal. The aggressive behavior of MNTIs may require

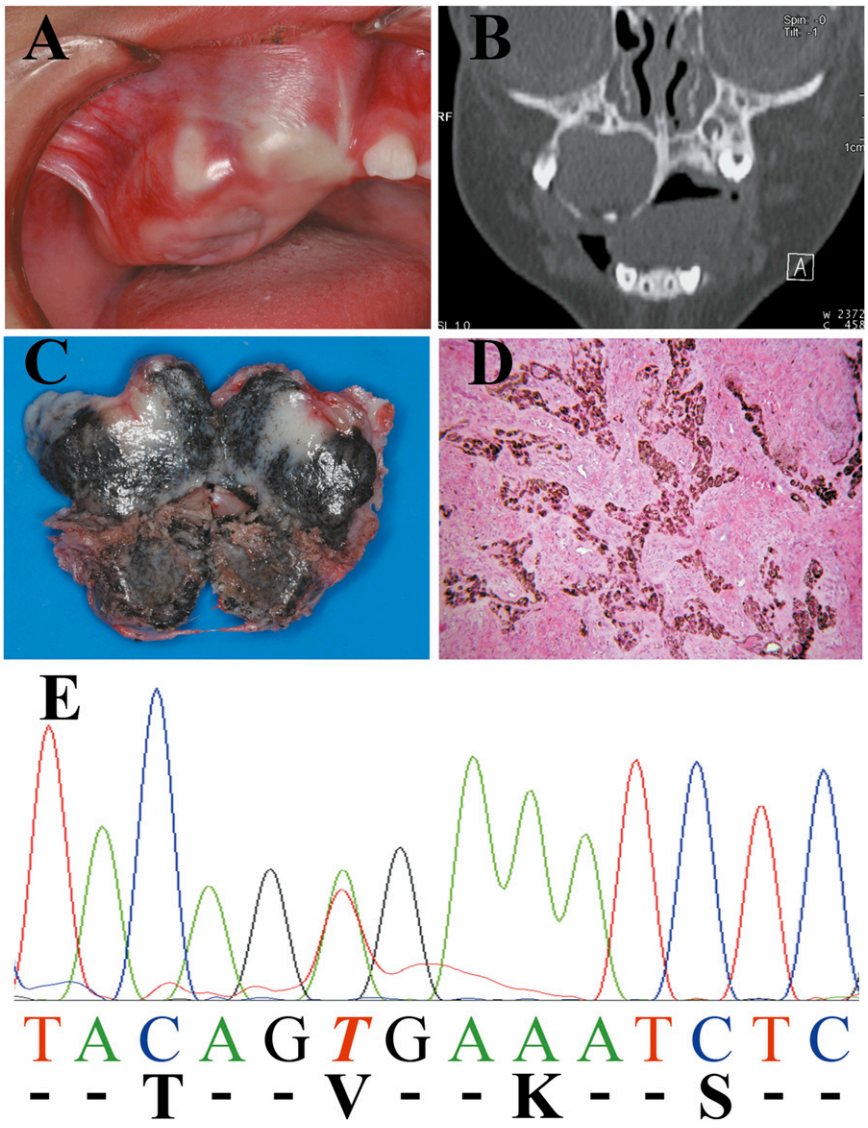

FIGURE 1
Clinical features of case 3 and Sanger traces of case 1. A, Clinical picture of the MNTI showing an expansive tumor mass causing tooth displacement. B, Computed tomography image showing a hypodense lesion causing bone resorption and tooth displacement. C, Gross aspect shows a dark-pigmented tumor. D, Section stained with hematoxylin and eosin showing the biphasic cell population formed by large melanin-pigmented epithelioid cells and small, round neuroblastlike cells. Original magnification ×20. E, Case 1 showed a heterozygous transversion T>A at codon 600, characterizing the BRAFV600E mutation.

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radical resection, which may be not possible in some cases because of its rapid and invading growth together with invasion of vital structures.[2] In these situations, adjunct radiotherapy or chemotherapy has been used. However, as there are no conclusive data regarding the molecular profile of this tumor, there is no targeted therapy that may be used in the treatment of selected aggressive cases.

Attempts to find molecular alterations linking MNTIs to other pediatric small cell tumors with neuroectodermal features (ie, neuroblastoma, Ewing sarcoma/peripheral primitive neuroectodermal tumor, and desmoplastic small round cell tumor) have not succeeded.[9] Based on the finding that the oncogenic *BRAF* mutation is an early and fundamental event in a group of melanocytic neoplasms,[10] we searched for the most common oncogenic *BRAF* alteration, BRAFV600E, in MNTI. We show that some of the MNTIs are prone to harbor the BRAFV600E mutation (Table 1) and identify a possible new MNTI molecular target, especially to treat cases not amenable to surgical management or to minimize facial mutilation. It is interestingly that the case with the *BRAF* mutation is from a 75-day-old infant.

Small molecule drugs targeting the BRAF or MEK kinases have been approved for the treatment of BRAF-mutant melanoma, including the immune checkpoint inhibitor ipilimumab, the selective type 1 BRAF inhibitors vemurafenib and dabrafenib, and the MEK inhibitor trametinib. Treatment with these drugs has proven effective in decreasing tumor size, but development of resistance is pointed out as the major challenge to the success of melanoma treatment.[7] Such drug resistance may be triggered by genomic instability leading to tumor heterogeneity, which is inherent of cancer progression. In this sense, as MNTI is a benign tumor, we hypothesize that the use of *BRAF*-targeted therapies has a higher chance of being successful and represent potential alternative treatments for aggressive tumors harboring *BRAF* mutations.

Our finding is important in terms of understanding MNTI tumor biology; however, caution must be exerted when bringing our results from bench to the clinics. There are scarce reports of pediatric patients with BRAFV600E-mutant tumors successfully treated with vemurafenib.[11,12] Although vemurafenib has been proven safe in melanoma clinical trials, there are important side effects of its treatment, varying from rash, arthralgia, and nausea, to cutaneous squamous cell carcinoma and liver function abnormalities.[13]

CONCLUSIONS

We show for the first time that this important pediatric tumor may harbor the oncogenic BRAFV600E mutation, providing the first insights to their personalized treatment.

ABBREVIATIONS

FFPE: formalin-fixed paraffin-embedded
MNTI: melanotic neuroectodermal tumor of infancy

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