A novel, potentially targetable TMEM106B-BRAF fusion in pleomorphic xanthoastrocytoma

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Abstract Pleomorphic xanthoastrocytoma (PXA) is a World Health Organization (WHO) Grade II glioma occurring primarily in children and young adults. Most PXAs harbor the known activating mutation *BRAF* V600E. We report a case of locally recurrent PXA with anaplastic features in a 10-yr-old female. The PXA was negative by immunohistochemical (IHC) staining for *BRAF* V600E mutation. Whole-exome and transcriptome sequencing of the tumor confirmed the absence of *BRAF* V600E, but identified copy-number alterations (including loss of the tumor suppressor *CDKN2A*) and a novel *TMEM106B*-*BRAF* fusion. Based on similar *BRAF* fusion proteins, this novel fusion is predicted to result in activation of *BRAF* signaling. Demonstration of positive IHC for phospho-ERK1/2 and phospho-MEK1/2 supported this prediction, and implicated MEK inhibitors as a potential therapeutic strategy.

CASE PRESENTATION

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Ontology term: neoplasm of the central nervous system

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This right-handed girl had onset of seizures at 2 years of age. Brain magnetic resonance imaging (MRI) was initially normal but was repeated at age 10 because of progressive headache and revealed a left temporal tip abnormality suggestive of ganglioglioma. She underwent resection of the lesion, which was reported as pleomorphic xanthoastrocytoma (PXA) with anaplastic features. Immunohistochemical (IHC) stain for *BRAF* V600E was negative. She remained seizure-free, but had local recurrence on MRI 6 months later, prompting re-resection. Pathology was identical, and IHC for *BRAF* V600E was again negative. She was referred for involved field radiation therapy, but following treatment had persistent tumor on MRI, with new contralateral disease, which was treated by stereotactic irradiation. Follow-up MRI showed further disease progression, and chemotherapy was initiated with temozolomide. Subsequent MRIs have shown stable disease in response to temozolomide.

METHODS

Whole-exome and transcriptome sequencing was performed on DNA and RNA from blood and tumor (second surgery). Exome sequences were captured using Agilent SureSelect

reagent V5+UTRs and sequenced on the Illumina HiSeq2500. An average coverage of 214- and 181-fold was obtained in the normal and tumor samples, respectively. Potential variants were filtered by known pathogenicity (ClinVar, Human Gene Mutation Database [HGMD], Online Mendelian Inheritance in Man [OMIM], Catalogue of Somatic Mutations in Cancer [COSMIC]) and frequency in the general population (The 1000 Genomes Project, Exome Variant Server, and our internal database). Copy-number changes were defined using EXCAVATOR software (Magi et al. 2013). Transcriptome sequencing was performed on RNA isolated from tumor. Transcriptome alignment was performed using the Tuxedo suite software package (Trapnell et al. 2012). One hundred and forty-eight million uniquely mapped reads were obtained. Translocations were detected using the FusionMap software. In view of *BRAF* V600E negativity, tumor was subjected to further IHC staining on formalin-fixed paraffin-embedded (FFPE) sections using standard procedures and the following antibodies: IgG control (sc-2027, Santa Cruz Biotechnology, Inc.), phospho-ERK1/2 (4370, Cell Signaling Technology), and phospho-MEK1/2 (2338, Cell Signaling Technology).

Genomic Analysis

A total of 124 somatic variants were identified and evaluated for pathogenicity and clinical significance. None were found to be clinically significant. Somatic copy-number changes identified included gain of Chromosome 7 (1–159,138,663), loss of Chromosome 13 (1–115,169,878), and copy-number neutral loss of heterozygosity of 9p (1–47,432,582) with copy-number loss of 9p21.3 (20,902,560–23,776,437, which includes the *CDKN2A* gene). Of note, transcriptome sequencing detected a novel *TMEM106B*-*BRAF* fusion on Chromosome 7, involving exons 1–3 of *TMEM106B* fused to exons 8–18 of *BRAF* (Fig. 1A; Table 1).

Clinical Interpretation

The primary treatment of PXA is surgical resection, and the outcome is generally favorable. Pathology is notable for anaplasia in ~15% of PXAs, but there is no consensus regarding any advantage of adjuvant postsurgical therapy (Perkins et al. 2012). *BRAF* V600E mutation is found in nearly 70% of PXAs (Dias-Santagata et al. 2011). Activation of *BRAF* by chromosomal rearrangement, such as the *KIAA1549*-*BRAF* fusion, which is common in pilocytic astrocytomas, is rare in PXA (Antonelli et al. 2015). *BRAF* is a serine/threonine kinase downstream from *RAS* in the mitogen-activated protein kinase (MAPK) signaling pathway. Mutation in *BRAF* constitutively activates the *RAS*/*RAF*/*MEK*/*ERK* signaling pathway. The possibility of targeted therapy for *BRAF* V600E mutated PXA has now been realized, with the report of transient single-agent activity of the selective *BRAF* V600E inhibitor vemurafenib (Chamberlain 2013). *TMEM106B* functions in dendrite morphogenesis and maintenance by regulating lysosomal trafficking and may be associated with frontotemporal dementia. The *TMEM106B*-*BRAF* fusion in our case results in replacement of the amino-terminal regulatory domain of *BRAF* with the amino-terminal region of *TMEM106B* while leaving the kinase domain of *BRAF* intact (Fig. 1B). Although not previously reported, this fusion is predicted to result in activation of *BRAF* signaling, based on the activity of similar *BRAF* fusion proteins. We used activation-state-specific immunohistochemical staining for phospho-ERK1/2 and phospho-MEK to demonstrate activation of the *MEK*-*ERK* pathway downstream from *BRAF* signaling (Fig. 1C). Given reports showing that tumors harboring the *KIAA1549*-*BRAF* fusion are not sensitive to vemurafenib and lead to paradoxical *MEK*-*ERK* activation, we expect PXA harboring *TMEM106B*-*BRAF* fusion will not be sensitive to vemurafenib as well, but could be sensitive to *MEK* inhibitors or second-generation *RAF* inhibitors such as PLX8394 (Sievert et al. 2013; Karajannis et al. 2014; Zhang et al. 2015).

Figure 1. *TMEM106B*-*BRAF* activates MEK-ERK pathway. (A) Transcriptional readout of the *TMEM106B* and *BRAF* loci from RNA-seq performed on primary tumor material. The site of translocation in each locus is indicated (red arrow). (B) Junctional sequence and reading frame. (C) Immunocytochemistry analysis of MEK/ERK pathway activity in patient's tumor utilizing phosphorylation-specific antibodies for phospho-MEK1/2 and phospho-ERK1/2. Scale bars, 100 μm.

ADDITIONAL INFORMATION

Data Deposition and Access

The data set supporting this article can be obtained from the cBioPortal for Cancer Genomics (<http://cbioportal.org>). The data set supporting the conclusions of this article can be obtained from the cBioPortal for Cancer Genomics (<http://cbioportal.org>) by selecting the DATA SETS tab and clicking on the citation listed in the Reference column. The

Table 1. *TMEM106B*-*BRAF* fusion found in patient

Gene1	Gene2	Position 1	Position 2	Transcript 1	Exon number 1	Transcript strand 1	Transcript 2	Exon number 2	Transcript strand 2	Fusion junction sequence	Frameshift class
<i>TMEM106B</i>	<i>BRAF</i>	Chr 7: 12258147	Chr 7: 140494267	NM_001134232_Ch7; NM_018374_Ch7	3;4	+	NM_004333_Ch7	8	-	TAGTGAT CAGAGAT TAAGGCC AAGAAG AACgcccc aaattctca ccagtccgt ctctcttc	In-frame

variant has been deposited in ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>) under accession number SCV000328979.

Competing Interest Statement

The authors have declared no competing interest.

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Ethics Statement

Written patient consent was obtained for this clinical testing. Publication was approved by the Columbia University Medical Center Institutional Review Board (IRB# AAAQ8170 and AAAP1200).

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