Activating NRF1-BRAF and ATG7-RAF1 fusions in anaplastic pleomorphic xanthoastrocytoma without BRAF p.V600E mutation

Joanna J. Phillips[1,2,3,*], Henry Gong[1], Katharine Chen[1], Nancy M. Joseph[4,5], Jessica van Ziffle[4], Lee-Way Jin[6,7], Boris C. Bastian[3,4], Andrew W. Bollen[2,3], Arie Perry[1,2,3], Theodore Nicolaides[1,8], David A. Solomon[2,3,4], Joseph T.C. Shieh[9,10]

[1]Department of Neurological Surgery, University of California, San Francisco, CA

[2]Division of Neuropathology, Department of Pathology, University of California, San Francisco, CA

[3]Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, CA

[4]Clinical Cancer Genomics Laboratory, University of California, San Francisco, CA

[5]Department of Pathology, University of California, San Francisco, CA

[6]Department of Pathology and Laboratory Medicine, UC Davis Medical Center, Sacramento, CA

[7]M.I.N.D. (Medical Investigation of Neurodevelopmental Disorders) Institute, UC Davis Medical Center, Sacramento, CA

[8]Division of Pediatric Hematology/Oncology, Department of Pediatrics, University of California, San Francisco, CA

[9]Department of Pediatrics, Division of Medical Genetics, University of California, San Francisco, CA

[10]Institute for Human Genetics, University of California, San Francisco, CA

*Corresponding author:
University of California San Francisco
Department of Neurological Surgery
Helen Diller Family Cancer Building, HD492B
San Francisco, CA 94143
Phone 415-514-4929
FAX 415-514-9792
joanna.phillips@ucsf.edu

SUPPLEMENTAL INFORMATION

METHODS

Immunohistochemistry. Immunohistochemical assessment was performed using antibodies against: BRAFV600E (1:50, Spring Bioscience, Pleasanton, CA) and p-MAPK (#4370, 1:10000, Cell Signaling Technology, Inc, Danvers, MA). The presence or absence of staining and staining localization patterns were noted. For p-MAPK immunostained sections were scored as follows: 0, denotes no tumor cells positive; 1, less than 25% positive; 2, 25% - 75% positive; and 3, greater than 75% positive. Digital photomicrographs were taken using an Olympus DP72 camera.

Genetic evaluation. Capture-based next-generation sequencing (NGS) was performed at the UCSF Clinical Cancer Genomics Laboratory to target and analyze the coding regions of 510 cancer-related genes, select introns from 40 genes, and the *TERT* promoter, with a total sequencing footprint of 2.8 Mb (UCSF500 panel). Sanger sequencing validation of *NRF1-BRAF* fusion and KEAP1 variant in PXA#1 was performed using the primers listed in **Supplemental Table 4**.

Tumor samples. Cases were identified from the Brain Tumor Center Biorepository (Tissue Core) and the Department of Pathology, Division of Neuropathology at the University of California San Francisco. PXA#1 FFPE tumor tissue from two prior resections were obtained from the Department of Pathology and Laboratory Medicine, University of California Davis Medical Center, Sacramento, CA.

Ethics statement. The ethics approval number for the use of de-identified human biospecimens and autopsy material is 10-01318. These studies were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments. This article does not contain any studies with animals.

SUPPLEMENTAL FIGURE

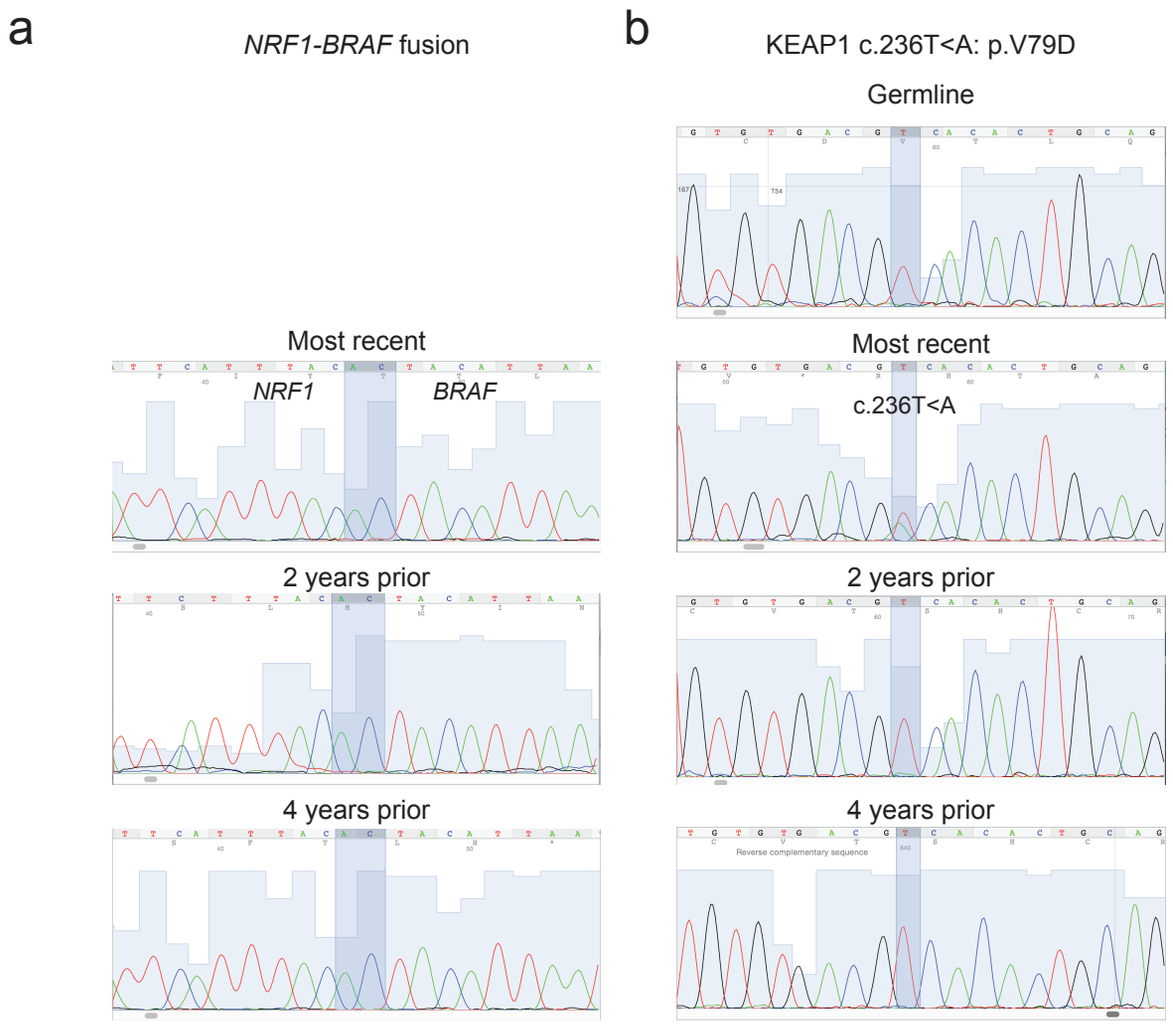
Supplemental Fig 1. Sanger sequencing validation of *NRF1-BRAF* fusion and KEAP1 variant in PXA#1 (**a,d**) and tumor tissue from resections 2 years (**b,d**) and 4 years (**c,f**) prior to the current resection. Only the *NRF1-BRAF* fusion was detected in prior resections.

Supplemental Table 1. Summary of the clinical, histopathologic features, genomic analysis, and MAPK analysis of the anaplastic PXA.

Supplemental Table 2. Somatic single nucleotide variants (SNVs) identified in tumors.

Supplemental Table 3. Primers used for variant analysis.

Supplemental Figure 1, Phillips et al.



Supplemental Table 1. Summary of the clinical, histopathologic, and genomic features of anaplastic PXA

PXA#	WHO Grade	Age at first diagnosis	Gender	Location	LM disease*	EGBs	Tumor cell infiltration#	Treatment	Prior resections	Recurrence/ Progression	Follow-up (months)	Alive	BRAF p.V600E	RAF alteration	CDKN2A	p-MAPK
1	III	9	F	T	Present	Y	Present	GTR	2	Yes	48	No	Absent	NRF1-BRAF	Deep deletion	+++
2	III	19	M	P	Present	Y	Present	GTR	None	Yes	15	Yes	Absent	ATG7-RAF1	Deep deletion	+++

*Denoted by histology or imaging.

#As determined by histopatholgic and/or immunohistologic evaluation.

Abbreviations: T = temporal, P = parietal; GTR=gross total resection; LM = leptomeningeal; EGB = Eosinophilic granular body

Supplemental Table 2. Single nucleotide variants (SNVs) identified in tumors.

PXA#	CHROM	POS	REF	ALT	Function	Gene	Variant	TranscriptID	Classification	Total Reads	Tumor mutant allele frequency	Exonic function	COSMIC
1	chr19	10,610,474	A	T	exonic	*KEAP1*	KEAP1 p.V79D	NM_203500	VUS	310	11%	nonsynonymous SNV	No
1	chr19	11,101,922	C	T	exonic	*SMARCA4*	SMARCA4 p.R448C	NM_001128844	VUS	91	4%	nonsynonymous SNV	No
2	chr17	7,577,120	C	T	exonic	*TP53*	TP53 p.R273H	NM_000546	Pathogenic	181	8%	nonsynonymous SNV	Yes

Supplemental Table 3. Primers used for variant analysis.

Gene	Variant	Primers used
KEAP1	*KEAP1* p.V79D	Forward: CCTTCAGCTACACCCTGGAG
KEAP1	*KEAP1* p.V79D	Reverse: AACATGGCCTTGAAGACAGG
NRF1-BRAF	Fusion	Forward: TTGGAGGAGGTTGAGATAAAGC Nested: GAGGTTGAGATAAAGCTTAGCAA
NRF1-BRAF	Fusion	Reverse: AGAGAACAGATGATGCTAGAAGTGT Nested: AACAGATGATGCTAGAAGTGTAAAAA