## **MYELOID NEOPLASIA**

# High prevalence of somatic *MAP2K1* mutations in *BRAF* V600E–negative Langerhans cell histiocytosis

Noah A. Brown,<sup>1</sup> Larissa V. Furtado,<sup>2</sup> Bryan L. Betz,<sup>1</sup> Mark J. Kiel,<sup>1</sup> Helmut C. Weigelin,<sup>1</sup> Megan S. Lim,<sup>1</sup> and Kojo S. J. Elenitoba-Johnson<sup>1</sup>

<sup>1</sup>Department of Pathology, University of Michigan Medical School, Ann Arbor, MI; and <sup>2</sup>Department of Pathology, University of Chicago, Chicago, IL

#### **Key Points**

- Targeted genome sequencing reveals high-frequency somatic *MAP2K1* mutations in Langerhans cell histiocytosis.
- MAP2K1 mutations are mutually exclusive with BRAF mutations and may have implications for the use of BRAF and MEK targeted therapy.

Langerhans cell histiocytosis (LCH) represents a clonal proliferation of Langerhans cells. *BRAF* V600E mutations have been identified in approximately 50% of cases. To discover other genetic mechanisms underlying LCH pathogenesis, we studied 8 cases of LCH using a targeted next-generation sequencing platform. An E102\_I103del mutation in *MAP2K1* was identified in one *BRAF* wild-type case and confirmed by Sanger sequencing. Analysis of 32 additional cases using *BRAF* V600E allele-specific polymerase chain reaction and Sanger sequencing of *MAP2K1* exons 2 and 3 revealed somatic, mutually exclusive *BRAF* and *MAP2K1* mutations in 18 of 40 (45.0%) and 11 of 40 (27.5%) cases, respectively. This is the first report of *MAP2K1* mutations in LCH that occur in 50% of *BRAF* wild-type cases. The mutually exclusive nature of *MAP2K1* and *BRAF* mutations implicates a critical role of oncogenic MAPK signaling in LCH. This finding may also have implications in the use of BRAF and MEK inhibitor therapy. (*Blood.* 2014;124(10):1655-1658)

#### Introduction

Langerhans cell histiocytosis (LCH) is characterized by a clonal proliferation of specialized cells with characteristics resembling antigen-presenting cells that reside in the skin and mucosa.<sup>1</sup> The disease manifests a broad clinical spectrum ranging from focal and self-limited disease to aggressive multisystem disease, with 20% mortality.<sup>2</sup> BRAF V600E mutations have been identified in 38% to 57% of LCH cases.<sup>3,4</sup> This mutation results in constitutive activation of the mitogen-activated protein kinase (MAPK) pathway. Since this discovery, patients with BRAF V600E-mutated LCH have been successfully treated with BRAF inhibitors.<sup>5</sup> Badalian-Very et al<sup>3</sup> noted that the intensity of Langerhans cell immunohistochemical staining for phosphorylated downstream mediators of the MAPK pathway did not vary with BRAF mutation status. This finding suggests another mechanism of MAPK pathway activation in BRAF wild-type (WT) cases. We sought to identify mutations that may contribute to the pathogenesis of LCH in cases without BRAF V600E mutations.

#### Methods

We studied cases of LCH within the archives of the Department of Pathology at the University of Michigan with institutional review board approval and in accordance with the Declaration of Helsinki. DNA was extracted from available nondecalcified, formalin-fixed, paraffin-embedded samples in which at least 30% neoplastic nuclei could be isolated using the Pinpoint Slide DNA Isolation System (Zymo Research). To discover genetic mechanisms that might explain ERK1 activation in the absence of *BRAF* V600E, we initially screened 8 LCH cases using both *BRAF* V600E allele–specific polymerase chain reaction (PCR) and the Ion AmpliSeq Comprehensive Cancer Panel. Allele-specific PCR was performed as previously described by Brown et al.<sup>6</sup>

For each of the 8 cases, sequencing libraries were generated using the Ion AmpliSeq Comprehensive Cancer Panel (Life Technologies). Approximately 40 ng of starting DNA from each sample block was amplified (10 ng per primer pool). Libraries were barcoded (IonXpress Barcode Kit, Life Technologies) and equalized (Ion Library Equalizer Kit) to a final concentration of approximately 100 pM. Emulsification PCR was performed using the OneTouch DL instrument, and template-positive Ion Sphere particles were enriched using the OneTouch ES instrument according to the manufacturer's instructions. Sequencing was performed on a 318 chip on the Ion Torrent PGM following the recommended protocol. Reads were aligned to hg19 and variants were called using the Torrent Suite, version 4.0.2.

Upon identification of a *MAP2K1* mutation within exon 3 in one of 8 cases using the Ion AmpliSeq Comprehensive Cancer panel, we evaluated all 8 cases using bidirectional Sanger sequencing of *MAP2K1* exons 2 and 3. An additional 32 cases of LCH were then evaluated using *BRAF* V600E allele–specific PCR and *MAP2K1* exon 2 and 3 bidirectional Sanger sequencing. DNA was sequenced using the BigDye Terminator V1.1 sequencing

Submitted May 27, 2014; accepted June 23, 2014. Prepublished online as *Blood* First Edition paper, June 30, 2014; DOI 10.1182/blood-2014-05-577361.

There is an Inside Blood Commentary on this article in this issue.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

© 2014 by The American Society of Hematology

The online version of this article contains a data supplement.



Figure 1. Somatic MAP2K1 mutations in LCH. (A) The frequency of mutually exclusive BRAF and MAP2K1 mutations in LCH is shown. (B) A portion of the MAP2K1 gene including exons 2 and 3 is depicted at the bottom, and regions of the MEK1 protein encoded by exons 2 and 3 are depicted above. Somatic mutations in LCH involve the N-terminal negative regulatory region encoded by exon 2 and the catalytic core encoded by exon 3. The circles above the protein denote substitutions and the bars below the protein indicate in-frame deletions. (C) A sequence electropherogram from case 26 (top) demonstrates the most common mutation identified in this study, E102\_1103del. The absence of this mutation in a sequence electropherogram from matched constitutional DNA (bottom) confirms the somatic nature of this mutation. (D) Sequence electropherogram from case 32 (top) and matched constitutional DNA (bottom) demonstrate 2 somatic missense mutations—C121S and G128V—at similar allele frequencies.

kit (Applied Biosystems) and the 3130xl DNA Analyzer (Applied Biosystems). Primers are listed in supplemental Table 1 on the *Blood* Web site. Sanger sequencing of exon 15 of the *BRAF* gene was performed for one case with a *BRAF* insertion mutation, as described by Hookim et al.<sup>7</sup>

#### **Results and discussion**

Similar to previous reports, a *BRAF* V600E mutation was detected in 3 of the 8 cases initially screened. An E102\_I103del mutation in *MAP2K1* was identified in one *BRAF* WT case using the Ion Comprehensive Cancer panel and confirmed by Sanger sequencing. Two additional *MAP2K1* mutations (c. 159\_173del, p.F53\_Q28delinsL and c. G140A, p. R47Q) were identified by Sanger sequencing of *MAP2K1* exons 2 and 3 that were not identified using the Ion AmpliSeq Comprehensive Cancer Panel.

Analysis of 32 additional cases of LCH using *BRAF* V600E allele–specific PCR and Sanger sequencing of *MAP2K1* exons 2 and 3 revealed *BRAF* and *MAP2K1* mutations in 18 of 40 (45.0%) and 11 of 40 (27.5%) cases, respectively (Figure 1A). The *MAPK2K1* mutations were mutually exclusive with *BRAF* mutations and were present in 11 of 22 (50%) *BRAF* WT cases. All *MAP2K1* mutations were somatic based on sequencing of matched constitutional DNA. No statistically significant association was found between *MAP2K1* mutation status and clinical indices such as age, sex, sites of involvement, or stage (supplemental Tables 2 and 3).

MAP2K1 encodes the dual-specificity kinase MEK1 protein. MEK1 is normally activated by BRAF within the MAPK pathway and is directly upstream of extracellular signal-regulated kinases ERK1 and ERK2. MAP2K1 mutations have been described in several neoplasms including melanoma<sup>8</sup> and lung carcinoma,<sup>9</sup> and recently in BRAF V600E-negative hairy cell leukemia.<sup>10</sup> The MAP2K1 mutations identified in this study were located in the negative regulatory region encoded by exon 2 and the catalytic core encoded by exon 3 (Figure 1B and Table 1).<sup>11,12</sup> Similar mutations affecting these sites have previously been demonstrated to result in constitutive activation of the MAPK pathway in nonhematologic neoplasms.9,13-15 Compared with the predominance of missense mutations observed in other neoplasms, the majority of MAP2K1 mutations in LCH were in-frame deletions. Six in-frame deletions involved exon 3, including residues E102 and I103 (Figure 1C). Two cases of mutations involving this site have been described in melanoma and lung adenocarcinoma.<sup>8,16</sup> Another 2 deletions (F53 O58delinsL and K57\_G61del) occurred in exon 2, affecting the helix A regulatory region.<sup>12</sup> Deletions involving this region have been reported to have increased MEK1 enzymatic activity 60-fold.<sup>13</sup> Five missense mutations were also identified-R47Q, R49C, A106T, C121S, and G128V. The C121S mutation has been shown to increase kinase activity and promote melanoma cell proliferation.<sup>15</sup> Of note, 2 of our cases demonstrated 2 separate missense mutations at similar allele frequencies-one case with C121S and G128V (Figure 1D) and one case with R49C and A106T. The former patient was diagnosed at

#### Table 1. BRAF V600E allele-specific PCR and MAP2K1 exons 2 and 3 Sanger sequencing results

Case #	BRAF V600E AS-PCR result	MAP2K1 Sanger sequencing		
		Exon 2	Exon 3	Confirmed somatic*
1	Negative	WT	c. 303_308del p. E102_I103del	Yes
2	Negative	c.140G>A p.R47Q	WT	Yes
3	Positive	WT	WT	N/A
4	Positive	WT	WT	N/A
5	Negative	WT	WT	N/A
6	Positive	WT	WT	N/A
7	Positive	WT	WT	N/A
8	Negative	c. 159_173del p.F53_Q58delinsL	WT	Yes
9	Positive	 WT	WT	N/A
10	Negative	WT	WT	N/A
11	Positive	WT	WT	N/A
12	Negative	c. 168_182del p.K57_G61del	WT	Yes
13	Positive	WT	WT	N/A
14	Positive	WT	WT	N/A
15	Negative	WT	WT	N/A
16	Positive†	WT	WT	N/A
17	Negative	WT	WT	N/A
18	Negative	WT	WT	N/A
19	Negative	WT	c.299_307delinsCTC p.H100_I103delinsPL	Yes
20	Positive	WT	WT	N/A
21	Negative	WT	c. 303_308del p. E102_1103del	Yes
22	Negative	WT	 WT	N/A
23	Positive	WT	WT	N/A
24	Negative	WT	WT	N/A
25	Negative	WT	WT	N/A
26	Negative	WT	c. 304_309del p. E102  103del	Yes
27	Negative	WT	WT	N/A
28	Positive	WT	WT	N/A
29	Negative	WT	WT	N/A
30	Positive	WT	WT	N/A
31	Negative	WT	c. 295_312del p. l99 K104del	Yes
32	Negative	WT	c. 361T>A p. C121S c. 383G>T p.G128V	Yes
33	Positive	WT	WT	N/A
34	Negative	WT	WT	N/A
35	Positive	WT	WT	N/A
36	Negative	WT	c. 304_309del p. E102 I103del	Yes
37	Negative	c. 145C>T, p. R49C	c. 316G>A p. A106T	Yes
38	Positive	WT	WT	N/A
39	Positive	WT	WT	N/A
40	Positive	WT	WT	N/A

AS-PCR, allele-specific polymerase chain reaction; N/A, not applicable.

\*MAP2K1 Sanger sequencing of germline DNA was performed to confirm the somatic nature of mutations where indicated.

tc. 1798\_1799insAGGCTACAG, p. T599\_V600insEAT was confirmed by Sanger sequencing of BRAF exon 15.

birth, suffered aggressive multisystem disease refractory to several therapies, and died at 19 months of age (supplemental Table 2).

Because MEK1 is downstream of BRAF within the MAPK pathway, LCH patients with *MAP2K1* mutations would not be expected to benefit from BRAF inhibitor therapy. Accordingly, *MAP2K1* mutations have been demonstrated to confer resistance to

BRAF inhibitor therapy in other neoplasms.<sup>17</sup> Several small-molecule inhibitors targeting MEK are Food and Drug Administration– approved or are in clinical trials for the treatment of neoplasms with activating MAPK pathway mutations, principally *BRAF*-mutated melanoma with and without *MAP2K1* mutations.<sup>18-21</sup> However, at least one mutation identified in this study—C121S—has been

shown to confer resistance to both BRAF inhibitors and current MEK inhibitors.<sup>15</sup> Nevertheless, MEK1 and its downstream kinase ERK remain attractive targets for therapy in LCH.

In conclusion, this is the first report of somatic *MAP2K1* mutations in LCH that occur in 50% of *BRAF* WT cases. The mutually exclusive nature of *MAP2K1* and *BRAF* mutations suggests that each of these mutations may function as an initiating mutation driving the proliferation of Langerhans cells through a common pathway. This finding may also have implications for the use of BRAF and MEK inhibitor therapy.

### Acknowledgments

This work was supported by the Division of Clinical Pathology at the University of Michigan.

#### References

- Jaffe R, Weiss LM. Facchetti. Tumours derived from Langerhans cells. In: Swerdlow SH, Harris NL, Jaffe ES, Pileri SA, Harald Stein, Thiele J, Vardiman JW, eds. World Health Organization Classification of Tumours of the Haematopoietic and Lymphoid Tissues. Lyon: IARC; 2008: 358-360.
- Minkov M. Multisystem Langerhans cell histiocytosis in children: current treatment and future directions. *Paediatr Drugs*. 2011;13(2): 75-86.
- Badalian-Very G, Vergilio JA, Degar BA, et al. Recurrent BRAF mutations in Langerhans cell histiocytosis. *Blood.* 2010;116(11):1919-1923.
- Sahm F, Capper D, Preusser M, et al. BRAFV600E mutant protein is expressed in cells of variable maturation in Langerhans cell histiocytosis. *Blood*. 2012;120(12): e28-e34.
- Haroche J, Cohen-Aubart F, Emile JF, et al. Dramatic efficacy of vemurafenib in both multisystemic and refractory Erdheim-Chester disease and Langerhans cell histiocytosis harboring the BRAF V600E mutation. *Blood.* 2013;121(9):1495-1500.
- Brown NA, Weigelin HC, Bailey N, et al. Requisite analytic and diagnostic performance characteristics for the clinical detection of BRAF V600E in hairy cell leukemia: a comparison of 2 allele-specific PCR assays. *Appl Immunohistochem Mol Morphol.* 2014 [epub ahead of print].

- Hookim K, Roh MH, Willman J, et al. Application of immunocytochemistry and BRAF mutational analysis to direct smears of metastatic melanoma. *Cancer Cytopathol.* 2012;120(1):52-61.
- Hodis E, Watson IR, Kryukov GV, et al. A landscape of driver mutations in melanoma. *Cell.* 2012;150(2):251-263.
- Marks JL, Gong Y, Chitale D, et al. Novel MEK1 mutation identified by mutational analysis of epidermal growth factor receptor signaling pathway genes in lung adenocarcinoma. *Cancer Res.* 2008;68(14):5524-5528.
- Waterfall JJ, Arons E, Walker RL, et al. High prevalence of MAP2K1 mutations in variant and IGHV4-34-expressing hairy-cell leukemias. *Nat Genet.* 2014;46(1):8-10.
- Bromberg-White JL, Andersen NJ, Duesbery NS. MEK genomics in development and disease. *Brief Funct Genomics*. 2012;11(4):300-310.
- Fischmann TO, Smith CK, Mayhood TW, et al. Crystal structures of MEK1 binary and ternary complexes with nucleotides and inhibitors. *Biochemistry*. 2009;48(12):2661-2674.
- Nikolaev SI, Rimoldi D, Iseli C, et al. Exome sequencing identifies recurrent somatic MAP2K1 and MAP2K2 mutations in melanoma. *Nat Genet.* 2012;44(2):133-139.
- Mansour SJ, Matten WT, Hermann AS, et al. Transformation of mammalian cells by constitutively active MAP kinase kinase. *Science*. 1994;265(5174):966-970.

### Authorship

Contribution: N.A.B. designed and performed research, analyzed data, and wrote the paper; L.V.F. and B.L.B. designed and performed research and analyzed data; M.J.K. analyzed next-generation sequencing data; H.C.W. performed research and analyzed data; and M.S.L. and K.S.J.E.-J. designed research, analyzed data, and wrote the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Kojo S. J. Elenitoba-Johnson, Department of Pathology, University of Michigan Medical School, BSRB 2037, 109 Zina Pitcher Place, Ann Arbor, MI 48109; e-mail: kojoelen@ med.umich.edu; or Megan S. Lim, Department of Pathology, University of Michigan Medical School, BSRB 2039, 109 Zina Pitcher Place, Ann Arbor, MI 48109; e-mail: meganlim@med.umich.edu.

- Wagle N, Emery C, Berger MF, et al. Dissecting therapeutic resistance to RAF inhibition in melanoma by tumor genomic profiling. *J Clin Oncol.* 2011;29(22):3085-3096.
- Imielinski M, Berger AH, Hammerman PS, et al. Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing. *Cell.* 2012; 150(6):1107-1120.
- Emery CM, Vijayendran KG, Zipser MC, et al. MEK1 mutations confer resistance to MEK and B-RAF inhibition. *Proc Natl Acad Sci USA*. 2009; 106(48):20411-20416.
- Flaherty KT, Infante JR, Daud A, et al. Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. *N Engl J Med.* 2012; 367(18):1694-1703.
- Boers-Sonderen MJ, Desar IM, Blokx W, Timmer-Bonte JN, van Herpen CM. A prolonged complete response in a patient with BRAF-mutated melanoma stage IV treated with the MEK1/2 inhibitor selumetinib (AZD6244). Anticancer Drugs. 2012;23(7):761-764.
- Park SJ, Hong SW, Moon JH, et al. The MEK1/2 inhibitor AS703026 circumvents resistance to the BRAF inhibitor PLX4032 in human malignant melanoma cells. *Am J Med Sci.* 2013;346(6): 494-498.
- Catalanotti F, Solit DB, Pulitzer MP, et al. Phase II trial of MEK inhibitor selumetinib (AZD6244, ARRY-142886) in patients with BRAFV600E/Kmutated melanoma. *Clin Cancer Res.* 2013;19(8): 2257-2264.