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Original contribution

BRAF and *MAP2K1* mutations in Langerhans cell histiocytosis: a study of 50 cases☆,☆☆

Khaled Alayed MD[a,b], L. Jeffrey Medeiros MD[a], Keyur P. Patel MD, PhD[a], Zhuang Zuo MD, PhD[a], Shaoying Li MD[a], Shalini Verma MD[a], John Galbincea MS[a], R. Craig Cason MS[a], Rajyalakshmi Luthra PhD[a], C. Cameron Yin MD, PhD[a,*]

[a]*Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, TX, 77030*
[b]*Department of Pathology, King Saud University, Riyadh, 11461, Saudi Arabia*

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Summary Langerhans cell histiocytosis (LCH) is a proliferation of Langerhans cells, often associated with lymphocytes, eosinophils, macrophages, and giant cells. *BRAF* mutations, usually V600E, have been reported in 40%-70% of cases, and recently, *MAP2K1* mutations have been reported in *BRAF*-negative cases. We assessed 50 cases of LCH for *BRAF* mutations and assessed a subset of cases for *MAP2K1* mutations. The study group included 28 men and 22 women (median age, 36.5 years; range, 1-78 years). *BRAF* V600E mutation was detected in 8 (16%) cases including 3 (30%) skin, 2 (11%) bone, 1 (50%) colon, 1 (20%) lung, and 1 (33%) extradural, intracranial mass. *MAP2K1* mutations were detected in 6 of 13 (46%) *BRAF*-negative cases including 2 (100%) lymph node, 2 (50%) bone, 1 (25%) skin, and 1 (100%) orbit. Patients with *BRAF* mutation were younger than patients with wild-type *BRAF* (median age, 28 versus 38 years; $P = .026$). The median age of *MAP2K1*-mutated patients was 34.5 years, similar to patients without *MAP2K1* mutation (41 years; $P = .368$). In agreement with 2 recent studies, we showed a high frequency of *MAP2K1* mutations in *BRAF*-negative LCH cases. Unlike other studies, the overall frequency of *BRAF* mutation in this cohort is substantially lower than what has been reported in pediatric patients, perhaps because most patients in this study were adults. Moreover, we showed a high concordance between mutational and immunohistochemical analysis for *BRAF* mutation. There was no statistically significant association between *BRAF* or *MAP2K1* mutation and anatomic site, unifocal versus multifocal presentation, or clinical outcome.
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1. Introduction

Langerhans cells are specialized dendritic cells located in the epidermis that are involved in antigen presentation [1]. Langerhans cell histiocytosis (LCH) is a rare disease defined morphologically as a proliferation of Langerhans cells that is often associated with lymphocytes, macrophages, eosinophils, giant cells, and necrosis. The presentation and clinical course of patients with LCH are highly heterogeneous. Affected patients show a wide age range, and a variety of

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* Corresponding author. Department of Hematopathology, Unit 72, 1515 Holcombe Blvd, Houston, TX 77030.
E-mail address: cyin@mdanderosn.org (C. C. Yin).

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anatomic sites can be involved. Patients can present with multifocal or unifocal disease, and the clinical course is also highly variable, ranging from spontaneous resolution to fatal dissemination [2,3]. As a result, LCH has been known by a number of designations in the past including *localized eosinophilic granuloma of bone*, *Letterer-Siwe disease*, *Hand-Schuller-Christian disease*, and *histiocytosis X*. The latter term was proposed as a unifying umbrella term in the 1950s based on the morphologic recognition of Langerhans cells at many different anatomic sites of disease. The term *Langerhans cell histiocytosis* was proposed by the Histiocyte Society in 1985. In 1994, 2 groups showed that a subset of cases of LCH is monoclonal using a human androgen receptor assay or X-chromosome inactivation [4,5].

V-Raf murine sarcoma viral homolog B1 (BRAF), located on chromosome 7q34, is a member of the RAF kinase family that is activated by RAS proteins and RAS-coupled receptor tyrosine kinases. Activated complexes of RAS and RAF transmit signals downstream to other parts of the mitogen-activated protein kinase (MAPK) cascade including MEK/ERK kinases [6–8]. The RAS-RAF-MAPK pathway is a key regulator of many cellular functions involving cell growth, proliferation, differentiation, and apoptosis by transmitting signals to nuclear, cytoplasmic, and cell membrane targets [6,7]. Mutations of *BRAF* have been identified in a large number of solid tumors, most commonly melanoma, papillary carcinoma of the thyroid gland, and colorectal carcinoma [9,10]. *BRAF* mutations also have been identified in hematologic diseases, particularly hairy cell leukemia and LCH [6–8,11]. Specifically regarding LCH, studies in recent years have shown *BRAF* V600E mutations in 35% to 69% of LCH cases [12–17]. These data further support the concept that many cases of LCH are monoclonal, in accord with earlier studies using other methods [12–17].

Mitogen-activated protein kinase kinase 1 (*MAP2K1*), also called *MEK1*, is located on chromosome 15q22.1-q22.33 and encodes a MEK1 protein kinase that is a known downstream target of RAF and is upstream of ERK1 and ERK2 [18]. Mutations of *MAP2K1* have been implicated in several human cancers including melanomas, lung and colon adenocarcinomas, and hairy cell leukemia [18–22]. Mutations in *MAP2K1* almost always occur in exons 2 and 3, and most mutations cause constitutive activation of MAP2K1 kinase [18]. Recently, targeted genomic sequencing revealed a high frequency of *MAP2K1* mutations in LCH, implicating oncogenic MAP kinase pathway signaling in LCH pathogenesis [23,24]. Moreover, *MAP2K1* mutations have been reported to be mutually exclusive with *BRAF* mutations in LCH [23,24].

In this study, we assessed the frequency of *BRAF*-mutations in a group of primarily adult LCH cases and assessed for *MAP2K1* mutations in a subset of *BRAF* negative cases. We also correlated mutation status with clinicopathologic features.

2. Materials and methods

2.1. Study group and immunohistochemistry

The archives of the Department of Hematopathology at The University of Texas MD Anderson Cancer Center from January 2000 to June 2015 were searched for cases diagnosed as LCH. Cases with available paraffin-embedded tissue blocks or unstained slides were selected. Clinical and laboratory data were retrieved from the medical records.

Routinely prepared hematoxylin-eosin–stained slides for all cases were reviewed. Most cases had been assessed using immunohistochemical (IHC) methods to confirm the diagnosis, but for this study, we performed limited IHC analysis on some cases using antibodies specific for CD1a (Leica, Newcastle, UK), S-100 protein (BioGenex, Fremont, CA), or langerin (Leica Biosystems, Buffalo Grove, IL). In addition, in 21 cases, we used the VE-1 antibody (dilution 1:50; Spring Bioscience, Pleasanton, CA) to assess for cytoplasmic staining supportive of the presence of *BRAF* V600E mutation. As shown by others, this antibody is highly specific for this mutation [25]. The study was conducted under an Institutional Review Board–approved protocol.

2.2. *BRAF* mutation analysis by pyrosequencing

DNA was extracted from fixed, paraffin-embedded tissue and analyzed for *BRAF* mutations. Cases with a low percentage involvement by LCH were microdissected before DNA extraction. We used a polymerase chain reaction (PCR)–based pyrosequencing assay developed at our institution that covers mutation hotspots in exons 11 (codon 468) and 15 (codons 595-600), as described previously [26]. This assay was chosen for *BRAF* analysis because almost all previously identified mutations in LCH have been clustered in exon 11 or 15 of the gene, altering the kinase domain of the protein [27].

Briefly, DNA was initially subjected to conventional PCR. A forward primer, 5′-TCC TGT ATC CCT CTC AGG CAT AAG GTA A-3′, and a reverse biotinylated primer, 5′-biotin-CGA ACA GTG AAT ATT CCT TTG AT-3′, were used to amplify a 322–base pair (bp) amplicon including codon 468 of exon 11. For exon 15 (codons 595-600), a 231-bp amplicon was amplified using a forward primer, 5′-CAT AAT GCT TGC TCT GAT AGG A-3′, and a reverse biotinylated primer, 5′-biotin-GGC CAA AAA TTT AAT CAG TGG A-3′. PCR amplification was performed in duplicate on an ABI 2720 Thermocycler (Applied Biosystems, Grand Island, NY). The PCR products underwent electrophoresis on agarose gels to confirm successful amplification. Fifteen microliters of the PCR products were then sequenced in duplicate using primer 5′-TTG GAT CTG GAT CAT TT-3′ (for exon 11) or 5′-GAA GAC CTC ACA GTA AAA ATA-3′ (for exon 15) and the pyrosequencing PSQ96 HS System (Biotage AB,