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BRAF V600E and *MAP2K1* Mutations in Hairy Cell Leukemia and Splenic Marginal Zone Lymphoma Cases

Sang-Yong Shin, M.D.[1], Seung-Tae Lee, M.D.[1], Hee-Jin Kim, M.D.[1], Chang-Seok Ki, M.D.[1], Chul Won Jung, M.D.[2], Jong-Won Kim, M.D.[1], and Sun-Hee Kim, M.D.[1]

Department of Laboratory Medicine & Genetics[1] and Department of Medicine[2], Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Dear Editor,

Differentiation of classic hairy cell leukemia (HCL-c) from HCL-variant (HCL-v) or splenic marginal zone lymphoma (SMZL) is important owing to their different treatment strategies and prognostic implications. Recently, testing for *BRAF* V600E mutations was suggested as an important diagnostic option for HCL-considering that it was exclusively detected in almost all cases [1]. The *BRAF* V600E mutation has been reported to be absent in most cases of immunoglobulin variable heavy chain rearrangements 4-34 (IGHV4-34)-positive HCL-c, HCL-v, and SMZL [2]. However, it was recently reported that high prevalence of *MAP2K1* mutation is observed in IGHV-34-positive HCL-c (5/7, 71.4%) [3].

We investigated the presence of *BRAF* V600E and *MAP2K1* mutations in four HCL-c, two HCL-v, and four SMZL cases involving the bone marrow that were diagnosed between June 2005 and June 2014 at our hospital. HCL and SMZL was diagnosed in accordance with the 2008 WHO classification of tumors of hematopoietic and lymphoid tissues [4]. HCL-c was defined as the expression of Annexin A1, CD20, CD22, CD11c, CD103, and CD25. HCL-v was defined as the negative expression of CD25 and Annexin A1 [4]. Real-time PCR was performed by using the Real Q *BRAF* V600E Detection Kits (BioSewoom Inc., Seoul, Korea) on the 7500 Fast Real-Time System (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions [5]. Mutant enrichment 3′-modified oligonucleotide (MEMO)-PCR and sequencing analysis for the *BRAF* V600E mutation were performed as previously described [6]. We designed the sequencing primers for *MAP2K*: exon 2 (forward) 5′-TTCTCTGGTGACAGTATTGACTTG-3′, (reverse) 5′-CCCTGAGAAATAATCCAATTACC-′ and exon 3 (forward) 5′-CATCCCTTCCTCCCTCTTTC-3′, (reverse) 5′-CTCTTAAGGCCATTGCTCCA-3′. Sequencing was performed by using the BigDye Terminator Cycle Sequencing Ready Reaction Kit on the ABI Prism 3130 Genetic Analyzer (Applied Biosystems). The DNA extracted from bone marrow aspirate slide was used for sequencing analysis.

We detected the *BRAF* V600E mutation in all HCL-c cases ei-

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Corresponding author: Sun-Hee Kim
Department of Laboratory Medicine & Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 135-710, Korea
Tel: +82-2-3410-2704, Fax: +82-2-3410-2719, E-mail: sunnyhk@skku.edu

Co-corresponding author: Seung-Tae Lee
Department of Laboratory Medicine & Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 135-710, Korea
Tel: +82-2-3410-0290, Fax: +82-2-3410-2719,
E-mail: nb.seungtae.lee@gmail.com

Table 1. Basic characteristics and results of *BRAF* V600E and *MAP2K1* mutation analyses

No.	Age	Sex	Diagnosis	Hb (g/dL)	WBC ($\times 10^9$/L)	PLT ($\times 10^9$/L)	*BRAF* V600E	*MAP2K1*	CD25	CD11c	CD103	Annexin A1	Treatment	F/U (yr)	Clinical status
1	48	F	HCL-c	7.7	1.79	46	+	-	+	NA	+	+	Cladribine	3.5	alive
2	75	F	HCL-c F/U*	7.6	1.18	20	+	-	NA	NA	NA	+	Cladribine	11	dead
3	50	M	HCL-c	9.2	4.96	30	+	-	+	+	+	+	Cladribine	2.9	alive
4	27	M	HCL-c F/U†	9.7	1.32	19	+	NA	+	+	+	+	Cladribine	2.5	alive
5	40	M	HCL-v	15.9	12.71	148	-	-	-	+	+	-	Cladribine	6	alive
6	75	M	HCL-v	14.6	26.30	131	-	NA	-	+	+	-	R-CVP	0.4	alive
7	30	F	SMZL	6.6	1.60	52	-	NA	NA	NA	NA	NA	Fludaribine R-CHOP	2	dead
8	74	M	SMZL	10.5	2.00	74	-	-	NA	NA	NA	NA	Gastrectomy	2.5	dead
9	75	M	SMZL	10.6	8.48	142	-	-	NA	NA	NA	NA	Splenectomy R-CVP	2.6	alive
10	62	F	SMZL	11.1	16.76	473	-	-	NA	-	NA	NA	Splenectomy R-CVP	2	alive

*At initial diagnosis; Hb-WBC-PLT: 5.2 g/dL-1.01×10^9/L-36×10^9/L; †At initial diagnosis; Hb-WBC-PLT: 9.7 g/dL-1.32×10^9/L–19×10^9/L.
Abbreviations: WBC, white blood cell; PLT, platelet; F/U, follow-up; HCL, hairy cell leukemia; SMZL, splenic marginal zone lymphoma; NA, not available; R-CHOP, Rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; R-CVP, rituximab with cyclophosphamide, vincristine, and prednisone.

ther by real-time PCR or by the MEMO-sequencing method (Table 1). All SMZL and HCL-v cases were negative for *BRAF* V600E on both real-time PCR and MEMO-sequencing analyses. The *MAP2K1* mutation analysis of three HCL-c cases, one HCL-v case, and three SMZL cases revealed negative results.

The most common types of *BRAF* mutation involve exon 15, and the substitution from valine to glutamate at the 600th amino acid (V600E) constitutes >90% of all reported cases of mutation. *BRAF* mutations in hematological malignancy are relatively rare [1, 7, 8]. In 2011, Tiacci *et al*. [1] reported that nearly all cases of HCL-c harbored the *BRAF* V600E mutation, although this mutation was not detected in any other B cell lymphomas including SMZL; this finding is in agreement with that of other studies [8-10]. Presently, *BRAF* V600E mutation analysis is considered to be the most useful diagnostic tool for differentiating HCL from related lymphomas. We also confirmed the presence of *BRAF* V600E in all HCL-c cases, but not in HCL-v or SMZL cases.

Recently, *MAP2K1* mutation was identified in a subset of HCL patients: 6/15 of IGHV-34-negative HCL-v, 4/9 of IGHV-34-positive HCL-v, and 5/7 of IGHV-34-positive HCL-c cases [3]. *MAP2K1* encodes mitogen-activated protein kinase kinase 1, which is a component of the MAP kinase signal transduction pathway. Somatic mutations were detected in HCL-v- and IGHV-34-positive HCL clusters in exons 2 and 3, which encode the N-terminal autoregulatory domain [3]. We detected negative results in all cases, which may be attributed to the small sample size and the low incidence of *MAP2K1* mutation.

In conclusion, we analyzed *BRAF* V600E and *MAP2K1* mutations in a small series of HCL-c, HCL-v, and SMZL cases. The *BRAF* V600E mutation was detected in all cases of HCL-c, but in none of the HCL-v or SMZL cases. This observation is consistent with that of a previous study, confirming the diagnostic utility of *BRAF* testing in HCL. Considering the rarity of HCL cases, further studies are needed for drawing conclusive observations from a sufficiently large sample size.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

REFERENCES

1. Tiacci E, Trifonov V, Schiavoni G, Holmes A, Kern W, Martelli MP, et al. BRAF mutations in hairy-cell leukemia. N Engl J Med 2011;364:2305-15.
2. Xi L, Arons E, Navarro W, Calvo KR, Stetler-Stevenson M, Raffeld M, et al. Both variant and IGHV4-34-expressing hairy cell leukemia lack the BRAF V600E mutation. Blood 2012;119:3330-2.
3. Waterfall JJ, Arons E, Walker RL, Pineda M, Roth L, Killian JK, et al. High prevalence of MAP2K1 mutations in variant and IGHV4-34-expressing hairy-cell leukemias. Nat Genet 2014;46:8-10.

http://dx.doi.org/10.3343/alm.2015.35.2.257

4. Foucar K, Falini B, Catovsky D, Stein H. Hairy cell leukaemia. In: Swerdlow S, Campo E, Harris NL, et al., eds. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon, France: International Agency for Research on Cancer, 2008:188-90.
5. Park SJ, Sun JY, Hong K, Kwak JY, Kim EK, Chung WY, et al. Application of BRAF, NRAS, KRAS mutations as markers for the detection of papillary thyroid cancer from FNAB specimens by pyrosequencing analysis. Clin Chem Lab Med 2013;51:1673-80.
6. Lee ST, Kim JY, Kown MJ, Kim SW, Chung JH, Ahn MJ, et al. Mutant enrichment with 3'-modified oligonucleotides a practical PCR method for detecting trace mutant DNAs. J Mol Diagn 2011;13:657-68.
7. Davidsson J, Lilljebjorn H, Panagopoulos I, Fioretos T, Johansson B. BRAF mutations are very rare in B- and T-cell pediatric acute lymphoblastic leukemias. Leukemia 2008;22:1619-21.
8. Trifa AP, Popp RA, Cucuianu A, Coada CA, Urian LG, Militaru MS, et al. Absence of BRAF V600E mutation in a cohort of 402 patients with various chronic and acute myeloid neoplasms. Leuk Lymphoma 2012; 53:2496-7.
9. Verma S, Greaves WO, Ravandi F, Reddy N, Bueso-Ramos CE, O'Brien S, et al. Rapid detection and quantitation of BRAF mutations in hairy cell leukemia using a sensitive pyrosequencing assay. Am J Clin Pathol 2012;138:153-6.
10. Laurini JA, Aoun P, Iqbal J, Chan W, Greiner TC. Investigation of the BRAF V600E mutation by pyrosequencing in lymphoproliferative disorders. Am J Clin Pathol 2012;138:877-83.