Letter to the Editor
Diagnostic Immunology

Ann Lab Med 2015;35:551-553
http://dx.doi.org/10.3343/alm.2015.35.5.551
ISSN 2234-3806 • eISSN 2234-3814

ANNALS OF LABORATORY MEDICINE

Loss of Mismatched HLA Detected in the Peripheral Blood of an AML Patient who Relapsed After Haploidentical Hematopoietic Stem Cell Transplantation

Borae G. Park, M.D.[1,*], Yong-Hak Sohn, M.D.[2], Heung-Bum Oh, M.D.[1], Eul-Ju Seo, M.D.[1], Seongsoo Jang, M.D.[1], and Sun Pyo Hong, Ph.D.[3]

Department of Laboratory Medicine[1], University of Ulsan College of Medicine and Asan Medical Center, Seoul; Department of Laboratory Medicine[2], Eulji University Hospital, Daejeon; R&D Center[3], GeneMatrix Inc., Seongnam, Korea

Dear Editor

Loss of patient-specific HLA was recently identified in AML patients who relapsed after haploidentical hematopoietic stem cell transplantation (haploHSCT) [1]. Several subsequent studies were reported [2-4], but they were all performed by using bone marrow (BM) specimens. However, peripheral blood (PB) samples are commonly used for hematopoietic chimerism (HC) analysis by short-tandem repeat (STR) amplification after HSCT. Therefore, the peripheral detection of patient-specific HLA loss along with HC analysis could be clinically useful. We report a case in which the loss of mismatched HLA was detected in the PB of an AML patient who relapsed after haploHSCT.

A 51-yr-old man was diagnosed as having refractory anemia with excess blasts that progressed to a normal karyotype AML after six months. The patient received haploHSCT from his first daughter two years after the diagnosis of AML, but the disease relapsed 14 months later, and he therefore received a second haploHSCT from his second daughter. The conditioning regimen consisted of fludarabine, busulfan, and antithymocyte globulin. A total of 7.2×10^6 cells/kg of CD34+ cells was transplanted, and 4.3×10^8 cells/kg of donor T lymphocytes was infused. One month after retransplantation, HC analysis performed by STR amplification revealed that all cells were derived from the donor, and BM examination showed normocellular marrow with trilineage regeneration. However, the patient eventually experienced a relapse of the disease four months after transplantation, with 76.4% of blasts in his BM. The immunophenotype and cytogenetic features of the BM blasts were the same as those at the time of the primary diagnosis and first relapse. HC analysis of PB revealed that all cells had changed to the patient type (Table 1).

At the time of the second relapse, HLA-DQB1 typing analysis performed by sequence-based typing in PB revealed a loss of mismatched HLA-DQB1. The remaining specimen volume was insufficient for all HLA-A, -B, -C, -DR, and -DQ analyses. The patient's original HLA-DQ type was DQB1*03:01, *03:03, and the

Received: October 30, 2014
Revision received: February 23, 2015
Accepted: June 1, 2015

Corresponding author: Yong-Hak Sohn
Department of Laboratory Medicine, Eulji University Hospital, 96 Dunsan-ro, Seo-gu, Daejeon 302-799, Korea
Tel: +82-42-611-3476, Fax: +82-42-611-3464
E-mail: medsohn@eulji.ac.kr

*Current affiliation of this author is Department of Laboratory Mecicine, Yonsei University College of Medicine, Seoul, Korea

donor's type was DQB1*03:01, *02:02; however, only DQB1 *03:01 was detected, not the patient-specific type (Table 1).

To verify this result, copy number variation was analyzed with BM specimens at relapse by using the HumanCytoSNP-12 bead chip (Illumina Inc. San Diego, CA, USA) and GenomeStudio software v2010.1/v1.6.3 (Illumina Inc.). B allele frequency (BAF) and Log R ratio (LRR) were used for copy number determination. LRR value of 0 represents two copies of alleles. In BAF plots a value of 0.5 indicates a heterozygous genotype, whereas 0 and 1 indicate homozygous genotypes. The percentage of BAF indicates the percentage of B allele raw copy number among the total allele raw copy number. A copy number-neutral loss of heterozygosity (CN-LOH) of 6p was identified, indicating that the acquired uniparental disomy (UPD) had caused the loss of the patient-specific HLA allele (Fig. 1). Additionally, CN-LOH of both 13q12.11-q34 and 21q21.3-q22.3 in all clones and subclones, respectively, were detected (Fig. 1). The patient was treated with salvage chemotherapy but he died three months later.

In the present case, acquired UPD on 6p resulted in the mismatched HLA loss. It was reported that 13q of the *FLT3* gene was the most frequent site of UPD in relapsed AML [5], and that CN-LOH of 21q of the *RUNX1* gene was responsible for the homozygosity of its mutation in normal karyotype AML [6].

Table 1. Loss of mismatched HLA detected in the peripheral blood at relapse by HLA-DQB1 genotyping

Patient disease status	Blast (%)		BM chromosome	HC in PB (recipient allele %)	HLA-DQB1 typing (SBT)		
	PB	BM			Recipient PB†	Recipient BM‡	Donor PB§
AML transformed from MDS	5	65.0	46,XY[20]		*03:01, *03:03		
One month after primary haploHSCT	0	1.0	//46,XX[20]	0			
Relapsed AML 14 months after primary haploHSCT	4	60.0	46,XY[10]//46,XX[10]	51			*02:02, *03:01
One month after secondary haploHSCT	0	0.2	//46,XX[20]	0			
Relapsed 2nd AML 4 months after secondary haploHSCT	75	76.4	46,XY[5]//46,XX[9]	100	*03:01	*02:02, *03:01	

†Recipient HLA was genotyped by the SBT method as A*11:01, A*30:01; B*13:02, B*15:01; C*03:03, C*06:02; DRB1*09:01, DRB1*12:02; ‡The discrepancy in HLA between the PB and BM results was due to remnant donor cell clones in the BM according to the BM chromosome results; §Both donor HLAs were genotyped by the PCR-SSP method as A*01, A*11; B*15(B62), B*37; C*03, C*06; DRB1*07, DRB1*12.
Abbreviations: PB, peripheral blood; BM, bone marrow; HC, hematopoietic chimerism; SBT, sequence-based typing; haploHSCT, haploidentical hematopoietic stem cell transplantation; PCR-SSP, PCR-sequence specific primer.

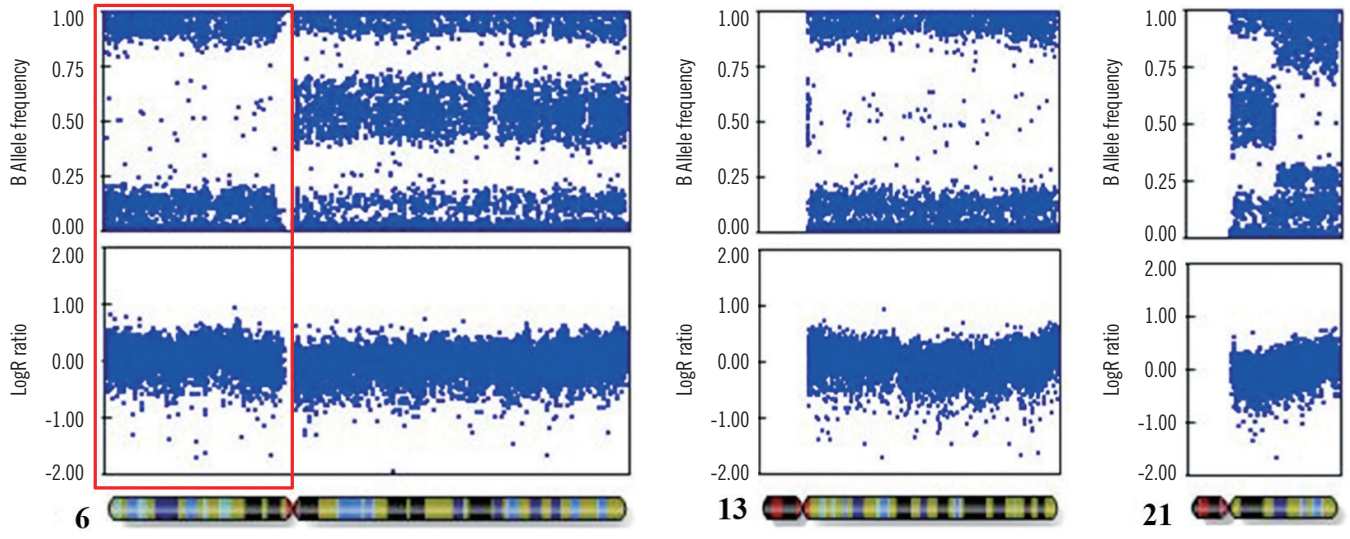

Fig. 1. Copy number variation analysis of a bone marrow specimen at relapse using the HumanCytoSNP-12 bead chip demonstrates a logR ratio of 0 and a B allele frequencies (BAF) of 85%, indicating copy number-neutral loss of heterozygosity (CN-LOH) of 6p in all clones (red box). HLA genes are located in 6p, revealing that acquired uniparental disomy was responsible for the loss of the patient-specific HLA allele. Additionally, CN-LOH of 13q12.11-q34 in all clones (a logR ratio of 0 and a BAF of 85%), and CN-LOH of 21q21.3-q22.3 in subclones (a logR ratio of 0.1 and a BAF of 75%) were detected.

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Thus, various types of CN-LOH that were identified in the patient's leukemic cells are presumed to have occurred at the time of diagnosis or relapse. The loss of patient-specific HLA could lead to disease relapse by allowing them to escape recognition by donor T cells. The HLA types of the primary and second donors were identical. Therefore, the patient's CN-LOH could have been detected at the time of primary relapse, although no data were available to confirm this.

HC analysis using the detection of individual specific STR is the most commonly used marker for residual disease after allogeneic HSCT, with a sensitivity of less than 5% [7]. Both BM and PB can be used, but the latter is more commonly used because it is easy to collect and the collection procedure is less invasive. With progressive disease relapse, the mixed cellular population can differ between BM and PB, and remnant donor cell clones can lead to discrepancies in HLA results between these samples (Table 1). The case described above demonstrates that HLA typing using PB might be sufficient to estimate the LOH when excess leukemic blasts are present in PB.

In conclusion, the present case suggests that HLA typing performed in PB, along with HC analysis, could be clinically useful for the detection of the loss of patient-specific HLA in PB. The mismatched HLA could lead to disease relapse. Additional studies might be necessary to confirm whether detecting the loss of mismatched HLA before overt disease relapse is possible.

Authors' Disclosures of Potential Conflicts of Interest

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

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