Letter to the Editor
Diagnostic Genetics

Ann Lab Med 2017;37:177-179
https://doi.org/10.3343/alm.2017.37.2.177
ISSN 2234-3806 • eISSN 2234-3814

ANNALS OF LABORATORY MEDICINE

Concomitant *AID* Expression and *BCL7A* Loss Associates With Accelerated Phase Progression and Imatinib Resistance in Chronic Myeloid Leukemia

Nae Yu, M.D.[1,*], Saeam Shin, M.D.[2,*], Jong Rak Choi, M.D.[1], Yoonjung Kim, M.D.[3], and Kyung-A Lee, M.D.[1]

Department of Laboratory Medicine[1], Yonsei University College of Medicine, Seoul; Department of Laboratory Medicine[2], Hallym University College of Medicine, Kangnam Sacred Heart Hospital, Seoul; Department of Laboratory Medicine[3], Yonsei University Wonju College of Medicine, Wonju, Korea

Dear Editor,

The mechanisms involved in chronic myeloid leukemia (CML) progression from the chronic phase (CP) to an accelerated phase (AP) or blast crisis (BC) remain largely undetermined, but generally involve additional molecular changes besides the Philadelphia chromosome [1]. Evidence suggests that secondary *BCR* (breakpoint cluster region)-*ABL1* (Abelson murine leukemia viral oncogene homolog 1)-dependent/independent genetic events play a critical role in blast transformation [2]. Besides *BCR-ABL1* point mutations, deletions and/or rearrangements of other cancer-related genes have been associated with CML-BC [2]. Recent studies have demonstrated that aberrant activation-induced cytidine deaminase (*AID*) expression promotes genetic instability, eventually leading to progression and drug resistance in CML [3].

We report a patient with a rare chromosomal translocation 46,XY,t(5;12)(p13;p13) that disrupts *AID* and causes a concomitant deletion of the tumor suppressor gene *BCL7A* (B-cell CLL/lymphoma 7A). This report presents evidence of a mechanism underlying CML progression and tyrosine kinase inhibitor (TKI) resistance occurring through an interaction between *AID* expression and *BCL7A* loss in a *BCR-ABL1* kinase domain mutation-independent manner.

A 57-yr-old man presented in December 2010 with generalized weakness and chest pain. Bone marrow (BM) demonstrated nearly packed marrow owing to myeloid and megakaryocyte hyperplasia (blasts [0.4%]). Written informed consent for genetic analysis was obtained from the patient according to the ethical guidance of the institutional review board. The patient exhibited the b3a2 type *BCR-ABL1* fusion transcript, and cytogenetic analysis revealed a normal karyotype. The patient was diagnosed as having CML-CP and treated with imatinib (Table 1).

Sixteen months following diagnosis, treatment was replaced by dasatinib since *BCR-ABL1* transcripts had not declined. Simultaneously, one of 25 metaphase cells exhibited an abnormal karyotype of 46,XY,t(5;12)(p13;p13) (Fig. 1A). FISH analysis using a *TEL/AML1* translocation probe presented a normal result; therefore, another gene on chromosome 12p13 was suspected to be involved. Subsequent FISH analysis revealed a break in the *AID* signal on one allele in 12.2% of interphase cells using a custom-labeled probe (Empire Genomics LLC, Buffalo, NY, USA) (Fig. 1B) compared with 0.2% of normal control cells. Quantitative analysis showed that *AID* RNA expression increased, whereas normal cells showed no *AID* expression.

Received: July 10, 2016
Revision received: August 8, 2016
Accepted: December 2, 2016

Corresponding author: Kyung-A Lee
Department of Laboratory Medicine, Yonsei University College of Medicine
211 Eonju-ro, Gangnam-gu, Seoul 06273, Korea
Tel: +82-2-2019-3531, Fax: +82-2-2019-4822, E-mail: KAL1119@yuhs.ac

*N.Y. and S.S. contributed equally to this study.

Table 1. The clinical course of the patient

Time (months after diagnosis)	0	3	12	16	23	28	29	32	37	38	46
Diagnosis	CML-CP	CHR	-	CHR	-	CML-AP	CHR	-	CML-AP	Mixed donor chimerism	Complete donor chimerism
Karyotype	-	-	-	46,XY,t(5;12)(p13;p13)[1]/46,XY[24]	-	46,XY,t(5;12)(p13;p13)[5]/46,XY[15]	-	-	46,XY,t(5;12)(p13;p13)[6]/46,XY[14]	-	-
BCR-ABL1 transcript (relative ratio of *BCR-ABL1* to *ABL1*)	0.00352	0.08	0.03	0.03	0.3	-	0.09*	0.089	-	0.000223	Negative
Interphase FISH (% of *AID* rearrangement)	-	-	-	12.2	-	-	9.5	-	9.8	-	-
AID mRNA expression (relative ratio of *AID* to *ACTB*)	-	-	-	3.53	-	-	3.23	-	5.32	-	-
BCL7A copy number changes	Normal	-	-	Deletion	-	-	-	-	-	-	-
Treatment	Imatinib	-	-	Dasatinib	-	Nilotinib	Imatinib	-	Allogeneic PBSCT	-	-

*The real-time PCR method changed from LightCycler t(9;22) Quantification (Roche Molecular Systems, Branchburg, NJ, USA) to Real-Q *BCR-ABL1* Quantification (BioSewoom , Seoul, Korea) at the six month follow-up.
Abbreviations: BM, bone marrow; CHR, complete hematologic response; AP, accelerated phase; CP, chronic phase; AID, activation-induced cytidine deaminase; PBSCT, peripheral blood stem cell transplantation.

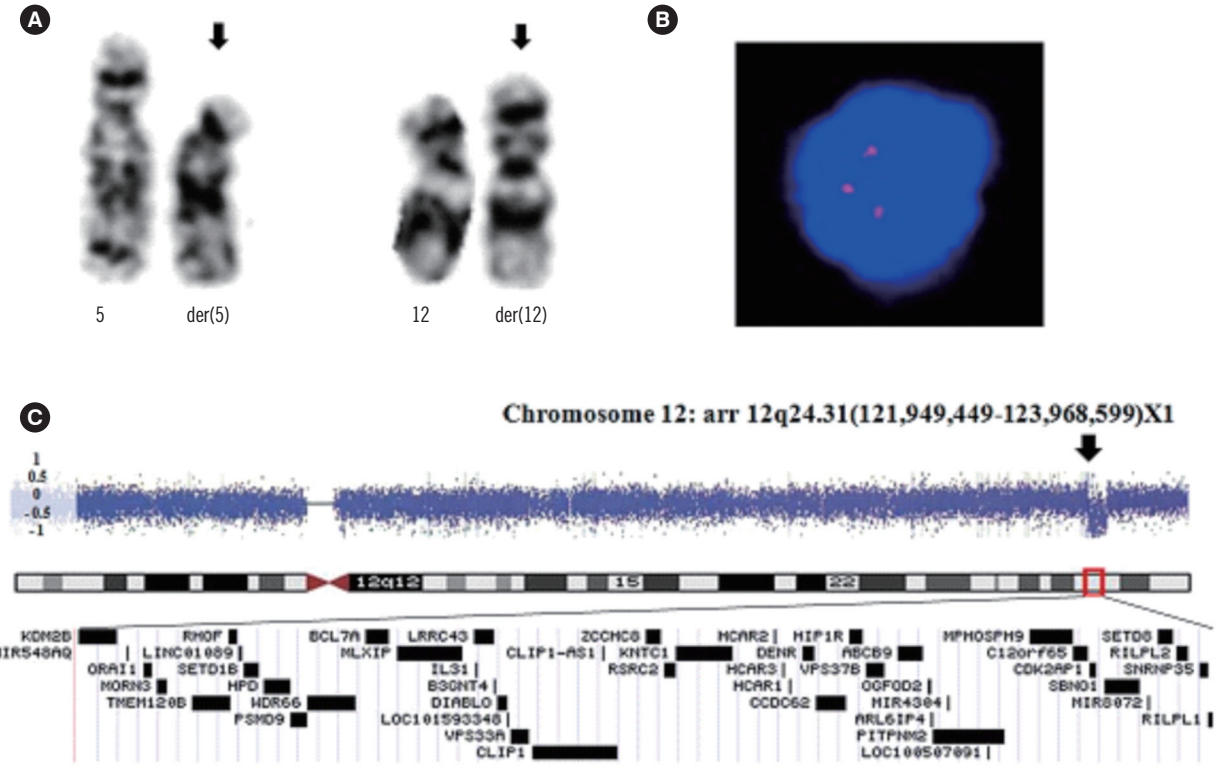

Fig. 1. Genetic analysis of CML-AP BM samples. (A) Translocation site in G-banded karyotyping of the 46,XY,t(5;12)(p13;p13) (arrows). (B) Interphase FISH confirming three signals of the *AID* probe (red), resulting from *AID* rearrangement at the 12p13 locus. (4,6-diamidino-2-phenylindole stain, ×1,000). (C) Chromosomal microarray revealing copy number loss in the chromosome 12q24.31 region (arrow). Blue dots with a log2 transformed value of -1 represent a 1:2 copy number ratio to the reference genomic DNA, indicating a heterozygous deletion. The expansion view of the 12q24 region reveals a 1.9-Mb heterozygous interstitial deletion in chromosome 12 (121,949,449-123,968,599; hg19) that includes the *BCL7A* gene.
Abbreviations: AP, accelerated phase; BM, bone marrow; *AID*, activation-induced cytidine deaminase; *BCL7A*, B-cell CLL/lymphoma 7A.

https://doi.org/10.3343/alm.2017.37.2.177

Chromosomal microarray using a CytoScan 750K Array (Affymetrix Inc., Santa Clara, CA, USA) revealed a 1.9-Mb deletion mostly containing *BCL7A* on chromosome 12q24.31 (Fig. 1C).

After 28 months, the patient progressed to CML-AP, and the CBC showed leukocyte counts of 116.8×10^9/L and 13% blasts. *BCR-ABL1* kinase domain sequencing revealed no mutation. Conversely, chromosomal abnormality of t(5;12)(p13;p13) exhibited clonal expansion. Thus, the patient was returned to imatinib therapy and subsequently demonstrated a complete hematologic response; however, *AID* breakage and *BCR-ABL1* transcript levels remained detectable.

After 37 months, the patient presented again with CML-AP, with 13.5% blasts in peripheral blood, and karyotyping showed 46,XY,t(5;12)(p13;p13)[6]/46,XY[14]. The patient received allogeneic stem cell transplantation and achieved complete donor hematopoietic chimerism.

The *AID* gene, located on chromosome 12p13, is associated with genomic instability and ensuing oncogenesis in various human malignancies including CML, AML, and ALL [3-5]. *AID* has several non-immunoglobulin targets, including *PAX5* and *BCL7A* [3, 6]; *PAX5* can induce TKI resistance and CML progression via *AID* upregulation [3].

Recently, it was shown that *AID* activation can induce mutations and translocations at the *BCL7A* locus [6]. *BCL7A* belongs to the BCL7 family, which includes *BCL7B* and *BCL7C*, and is a known pro-apoptotic tumor suppressor in B-cell oncogenesis. Specifically, BCL7B suppresses β-catenin expression, causing Wnt signaling inhibition [7]. Importantly, β-catenin deregulation in Wnt signaling is associated with genomic instability that can induce oncogenic translocations in T-cell lymphoma and AML [5, 8]. In CML, β-catenin activation is observed in the AP or BC, and is believed to promote leukemic stem cell generation that facilitates disease progression [9].

We suggest a novel pathway for disease progression and drug resistance in CML via *AID* expression and the concomitant loss of *BCL7A*. Our case supports the hypothesis that *BCL7A* deletion induces abnormal Wnt/β-catenin signaling, enhancing the potential for a chromosomal aberration and its persistence in our patient [5, 10]. Loss of the tumor suppressor *BCL7A* may cooperate with *AID* activity in disease progression by causing aberrant cell cycle regulation [1]. Moreover, *AID*-induced DNA damage checkpoint activation causes initiation of tumor suppressor genes involved in DNA repair or cell cycle control [1]. A previous study showed that decreased tumor suppressor gene expression can cause *AID*-induced translocation, eventually leading to cancer [10].

In conclusion, our report identifies that *AID*, in combination with *BCL7A*, likely confers TKI resistance in a *BCR-ABL1* kinase domain mutation-independent manner and may be a putative functional target in CML therapy.

Authors' Disclosures of Potential Conflicts of Interest

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

Acknowledgments

This work was supported by a grant from the Korean Health Technology Research and Development Project (A120030), Ministry of Health and Welfare, Republic of Korea.

REFERENCES

1. Strout MP and Schatz DG. Imatinib resistance and progression of CML to blast crisis: somatic hypermutation AIDing the way. Cancer Cell 2009;16:174-6.
2. Neviani P. Genetic events other than BCR-ABL1. Curr Hematol Malig Rep 2014;9:24-32.
3. Klemm L, Duy C, Iacobucci I, Kuchen S, von Levetzow G, Feldhahn N, et al. The B cell mutator AID promotes B lymphoid blast crisis and drug resistance in chronic myeloid leukemia. Cancer Cell 2009;16:232-45.
4. Feldhahn N, Henke N, Melchior K, Duy C, Soh BN, Klein F, et al. Activation-induced cytidine deaminase acts as a mutator in BCR-ABL1-transformed acute lymphoblastic leukemia cells. J Exp Med 2007;204:1157-66.
5. Ugarte GD, Vargas MF, Medina MA, León P, Necuñir D, Elorza AA, et al. Wnt signaling induces transcription, spatial proximity, and translocation of fusion gene partners in human hematopoietic cells. Blood 2015;126:1785-9.
6. Kato L, Begum NA, Burroughs AM, Doi T, Kawai J, Daub CO, et al. Non-immunoglobulin target loci of activation-induced cytidine deaminase (AID) share unique features with immunoglobulin genes. Proc Natl Acad Sci U S A 2012;109:2479-84.
7. Uehara T, Kage-Nakadai E, Yoshina S, Imae R, Mitani S. The tumor suppressor BCL7B functions in the Wnt signaling pathway. PLoS Genet 2015;11:e1004921.
8. Dose M, Emmanuel AO, Chaumeil J, Zhang J, Sun T, Germar K, et al. β-Catenin induces T-cell transformation by promoting genomic instability. Proc Natl Acad Sci U S A 2014;111:391-6.
9. Jamieson CH, Ailles LE, Dylla SJ, Muijtjens M, Jones C, Zehnder JL, et al. Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. N Engl J Med 2004;351:657-67.
10. Ramiro AR, Jankovic M, Callen E, Difilippantonio S, Chen HT, McBride KM, et al. Role of genomic instability and p53 in AID-induced c-myc-Igh translocations. Nature 2006;440:105-9.