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Novel Mutations in *CEBPA* in Korean Patients with Acute Myeloid Leukemia with a Normal Karyotype

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Mutations in the transcription factor CCAAT/enhancer binding protein α gene *(CEBPA)* are found in 5-14% of the patients with AML and have been associated with a favorable clinical outcome. In this study, we aimed to assess the frequencies and characteristics of mutations in *CEBPA*. Between 2006 and 2009, *CEBPA* mutations were assessed using archival DNA samples obtained from 30 consecutive adult patients diagnosed with AML with a normal karyotype at our institution. *CEBPA* mutations were detected using direct sequencing analyses. These mutations were detected and described with reference to GenBank Accession No. NM_004364.3. In our series, *CEBPA* mutations were detected in 4 patients (13.3%). These mutations occurred as double mutations in all 4 patients. Among the 8 mutant alleles, 5 were novel (c.179_180dupCG, c.50_53delGCCA, c.178_182delACGTinsTTT, c.243_244insGTCG, and c.923_924insCTC). The frequency of occurrence of *CEBPA* mutations in Korean patients with AML is comparable to that in previous reports. Long-term follow-up data from a larger series of patients with comprehensive molecular profiling are needed to delineate the prognostic implications.

Key Words: *CEBPA*, Mutation, Acute myeloid leukemia, Normal Karyotype, Korea

The karyotype at the time of diagnosis provides the most important prognostic information in AML patients, but 40-50% of patients do not have any clonal chromosomal aberrations [1-3]. All such cases of AML with normal karyotype (AML-NK) are currently categorized in the intermediate-risk group; however, this group is quite heterogeneous [4, 5]. In recent years, many studies have shown that acquired gene mutations in AML patients have prognostic relevance. In particular, the fms-related tyrosine kinase 3 gene-internal tandem duplications (*FLT3*-ITD) and myeloid-lymphoid or mixed-lineage leukemia gene-partial tandem duplications (*MLL*-PTD) have been associated with short relapse-free and overall survivals, whereas AML-NK with mutations in the nucleophosmin gene (*NPM1*), without concomitant *FLT3*-ITD, are associated with a more favorable outcome [6]. Other genes that are recurrently mutated in AML include *KIT, DN-MT3A, IDH1/IDH2*, and *TET2*. Patients with core-binding factor AML commonly carry activating mutations of *KIT* (20-45%) and show inferior outcomes [7]. *DNMT3A* mutations were detected in 22.1% of the AML-NK patients and were associated with adverse outcomes [8]. Although *IDH1* and *IDH2* mutations were detected in 6% of AML patients and 11-12.1% of AML-NK patients, the prognostic impacts of these mutations have been controversial so far [9]. *TET2* mutations were detected in 23% AML-NK patients and showed unfavorable outcomes in the favorable-risk group (AML-NK patients with mutated *CEBPA* and/or mutated *NPM1*, without *FLT3*-ITD) [10].

The CCAAT/enhancer binding protein α gene (*CEBPA*) is a member of the basic region leucine zipper family of transcription factors. It is an intronless gene located on chromosome 19q13.1. It is composed of 2 transactivation domains in the N-

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terminal region—a basic leucine zipper region (bZIP) that mediates dimerization with other CEBP family members and a DNA-binding domain in the C-terminal region [11]. The importance of *CEBPA* in hematopoiesis can be attributed to its crucial role during the development of granulocytes and its deregulation associated with myeloid transformation [11]. Mutations in *CEBPA* are found in 5-14% of AML patients and have been associated with a favorable clinical outcome [12]. Most *CEBPA* mutant AML patients simultaneously exhibit 2 mutations (*CEBPA*double-mut), which most frequently involve a combination of an N-terminal frame-shift mutation and a C-terminal insertion mutation, that are usually biallelic [12]. In a recent study involving a large cohort of AML-NK patients, only *CEBPA*double-mut was associated with a unique gene expression profile and favorable overall and event-free survivals on multivariate analyses including factors such as age, white blood cell count, cytogenetic information, and *FLT3*-ITD and *NPM1* mutation status. Therefore, *CEBPA*double-mut was considered a separate disease entity in the classification of AML [13-15].

In this study, we aimed to assess the frequencies and characteristics of *CEBPA* mutations in Korean patients with AML-NK. Between 2006 and 2009, *CEBPA* mutations were assessed in 30 consecutive adult patients diagnosed with AML-NK at our institution. Of these patients, 18 were men and 12 women, with a median age of 53 yr (range, 24-88 yr) at diagnosis. According to the WHO classification [16], 13 patients were diagnosed with AML with myelodysplasia-related changes, 8 with AML with maturation, 3 with AML without maturation, 3 with acute monoblastic/monocytic leukemia, and 1 each with AML with minimal differentiation, acute myelomonocytic leukemia, and acute erythroid leukemia. The patients were shown to have no recurrent molecular abnormalities on multiplex reverse-transcriptase PCR using the Hemavision-Full Kit (DNA technology A/S, Aarhus C, Denmark) and a panel of FISH assays (LSI p53 on 17p13.1, LSI D20S108 on 20q12, LSI D7S522 on 7q31/CEP 7, CEP 8, LSI EGR1 on 5q31/D5S23, D5S721 on 5p15.2, and LSI MLL dual color, LSI CBFB dual color, or LSI RUNX1/RUNX1T1 on 8q22/21q22 probes [Abbott Molecular/Vysis, Des Plaines, IL, USA]). All patients, except 3 who had refused treatment, received conventional induction chemotherapy with idarubicin and cytosine arabinoside. Data about complete blood counts and the proportion of blasts on peripheral blood (PB) and bone marrow (BM)-aspiration smear were obtained by reviewing electronic medical records. We also reviewed the data on immunophenotyping obtained by flow cytometry; *FLT3*/ITD, *FLT3*/TKD, and *NPM1* mutations; and clinical outcomes after induction chemotherapy.

Genomic DNA extracted from the BM aspirates was analyzed using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. Written informed consent was obtained from the patients. The study protocol was approved by the Institutional Review Board of our institution. The coding sequences and flanking intronic regions of the *CEBPA* genes were amplified using the previously described primer sets [13, 17]. PCR was performed using a thermal cycler (model 9700; Applied Biosystems, Foster City, CA, USA). Cycle sequencing was performed using the ABI Prism 3100 Genetic Analyzer and the BigDye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems). When variations were observed in the sequences obtained using these methods and the reference sequences, we performed follow-up tests by using BM or PB specimens collected at complete remission; 2 PB specimens from healthy volunteers and 2 BM specimens showing no involvement of lymphoma during staging workup were used as controls. *CEBPA* mutations were described according to the guidelines of the Human Genome Variation Society by using the reference sequences NM_004364.3 and NP_004355.2. Only an insertion polymorphism or variation(s) not leading to amino acid changes was considered wild type [13, 18, 19].

In our study, *CEBPA* mutations were detected in 4 patients (13.3%), and all had double mutations (Fig. 1). *CEBPA* double mutations in these 4 patients were c.179_180dupCG at the N-terminal region [N] and c.929_930insTCT at the C-terminal region [C]; c.50_53delGCCA [N] and c.912_913insTTG [C]; c.178_182delACGTinsTTT [N] and c.923_924insCTC [C]; and c.243_244insGTCG [N] and c.912_913insTTG [C]. Among these mutations, c.179_180dupCG, c.50_53delGCCA, c.178_182delACGTins TTT, c.243_244insGTCG, and c.923_924insCTC were novel mutations. All patients (4/4, 100%) with *CEBPA* mutations showed a CR after induction chemotherapy, whereas 84.6% (22/26) of patients without *CEBPA* mutations showed a CR after induction chemotherapy. The *CEBPA* mutations were not observed in the follow-up samples at complete remission. *FLT3*-ITD was detected in 10% (3/30) of the patients, and the *NPM1* mutation in 37.5% (9/24). Among the 4 patients with *CEBPA*double-mut, 1 also had the *FLT3*-ITD mutation, whereas none of them had the *NPM1* mutation. Detailed clinical and molecular characteristics of these 4 patients are shown in Table 1. Six-nucleotide in-frame insertion polymorphisms of *CEBPA* were observed in 30% (9/30) of the patients. This polymorphism was also observed in the follow-up samples at complete remission and in control BM specimens.

Our results showed that the frequency of *CEBPA* mutations in Korean patients with AML-NK is 13.3% (4/30), which is compa-
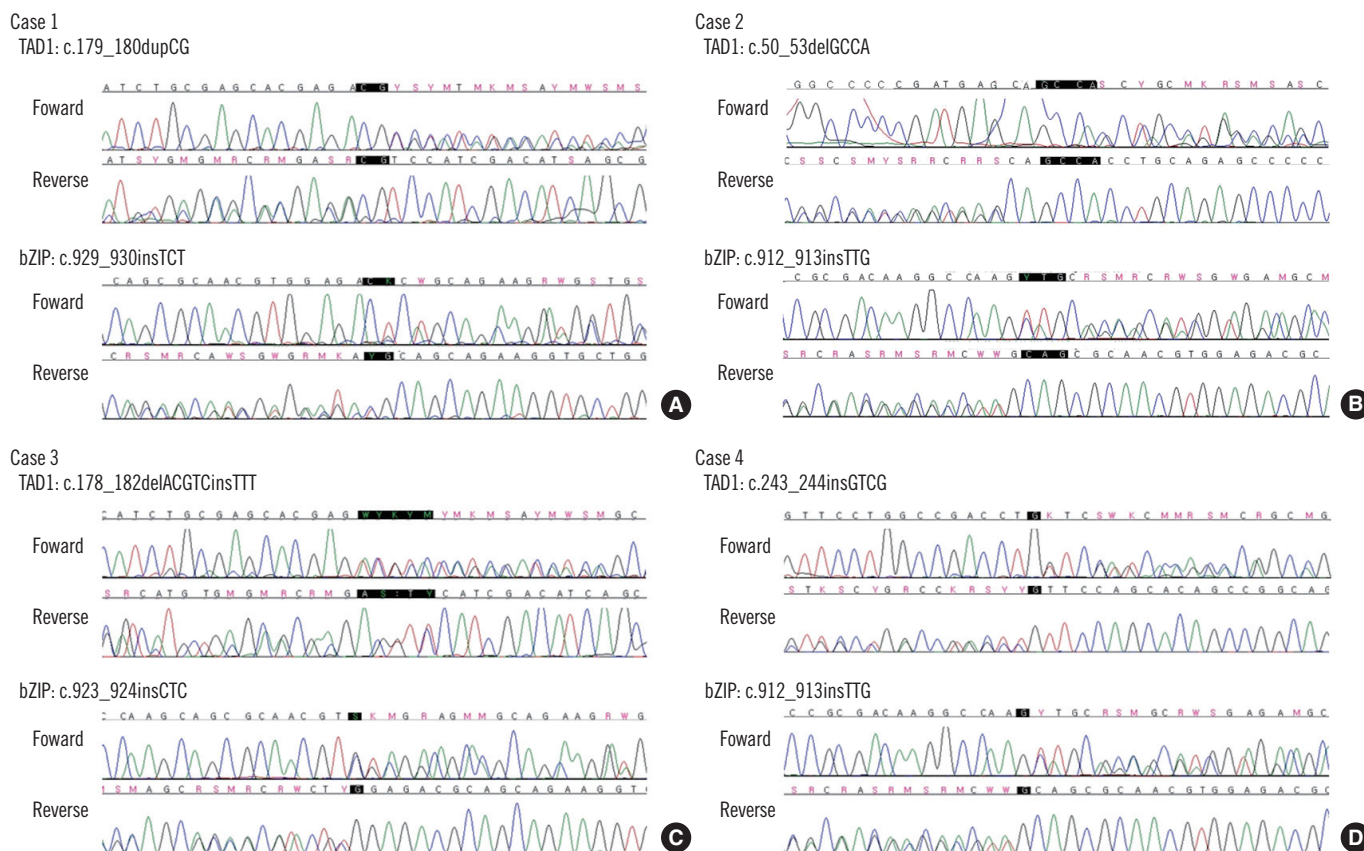
Case 1
TAD1: c.179_180dupCG

Foward

Reverse

bZIP: c.929_930insTCT

Foward

Reverse

Case 2
TAD1: c.50_53delGCCA

Foward

Reverse

bZIP: c.912_913insTTG

Foward

Reverse

Case 3
TAD1: c.178_182delACGTinsTTT

Foward

Reverse

bZIP: c.923_924insCTC

Foward

Reverse

Case 4
TAD1: c.243_244insGTCG

Foward

Reverse

bZIP: c.912_913insTTG

Foward

Reverse

Fig. 1. *CEBPA* mutations detected in 4 patients.
CEBPA mutations occurred as double mutations in all 4 patients. *CEBPA* double mutations in these patients were c.179_180dupCG [N] and c.929_930insTCT [C], c.50_53delGCCA [N] and c.912_913insTTG [C], c.178_182delACGTinsTTT [N] and c.923_924insCTC [C], and c.243_244insGTCG [N] and c.912_ 913insTTG [C]. Among these mutations, c.179_180dupCG, c.50_53delGCCA, c.178_182delACGTinsTTT, c.243_244insGTCG, and c.923_924insCTC were novel mutations.

Table 1. Clinical and molecular characteristics of 4 Korean patients with AML-NK harboring *CEBPA* double mutations

Patient No.	Age (yr)/ Sex	BM diagnosis	Immunophenotype	*CEBPA* gene mutation*		*FLT3*/ITD	*NPM1*
				N-terminal	C-terminal		
1	51/F	AML with MRC	CD34+, HLA-DR+, CD13+, CD33+, CD117+, cMPO+, CD64+, ectopic CD7+	**c.179_180dupCG** (p.S61Afs)	c.929_930insTCT (p.310_311insL)	Neg	Neg
2	41/F	AML with maturation	CD34+, HLA-DR+, CD13+, CD33+, CD117+, MPO+, CD64w+, ectopic CD7+	**c.50_53delGCCA** (p.H18Qfs)	c.912_913insTTG (p.K304_Q305insL)	Pos	Neg
3	48/F	AML with maturation	CD34+, HLA-DR+, CD13+, CD33+, CD117+, MPO+, CD64+, ectopic CD7w+	**c.178_182delACGTinsTTT** (T60Ffs)	**c.923_924insCTC** (p.K304_Q305insL)	Neg	Neg
4	42/F	AML with maturation	CD34+, HLA-DR+, CD13+, CD33+, CD117+, MPO+, CD64w+, ectopic CD7+	**c.243_244insGTCG** (p.F82Vfs)	c.912_913insTTG (p.K304_Q305insL)	Neg	Neg

*Description at the cDNA level (reference sequence: NM_004364.3) and that at the protein level (NP_004355.2) in parentheses, according to the guidelines of the Human Genome Variation Society.
Novel mutations are in bold.
Abbreviations: AML, acute myeloid leukemia; NK, normal karyotype; BM, bone marrow; ITD, internal tandem duplication mutations; MRC, multilineage-related dysplasia; Neg, negative; Pos, positive.

rable to the frequency reported in previous studies on other ethnic groups [20-24]. All 4 patients showed double mutations (one each in the N- and C-terminal regions). We also found 5 novel mutations: c.179_180dupCG [p.S61Afs], c.50_53delGCCA [p.H18Qfs], c.178_182delACGTinsTTT [T60Ffs], c.243_244insGTCG [p.F82Vfs], and c.923_924insCTC [p.K304_Q305insL]. Moreover,

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we also confirmed the disappearance of *CEBPA*double-mut in the follow-up samples at complete remission (leukemia-specific genetic changes). N-terminal mutations are located between the major translational start codon and the second ATG in the same open reading frame [13]. These mutations introduce a premature termination of translation of the p42 *CEBPA* protein, while preserving the translation of a p30 isoform that has been reported to inhibit the function of the full-length protein. In contrast, mutations in the C-terminal bZIP region are in-frame mutations, and they may impair DNA binding and/or homodimerization and heterodimerization [13].

We found that c.584_589dupACCCGC (9/30, 30%) and c.690G>T (2/30, 6.7%) were the most common types of *CEBPA* polymorphisms. Although c.584_589dupACCCGC was first reported as a mutation [25], this 6-nucleotide in-frame duplication has recently been shown to represent a germline polymorphism (P194_H195dup) [18, 26, 27]. We detected this variation in the follow-up samples of patients at CR and in control samples.

Of the 4 patients with *CEBPA*double-mut, 1 also had the *FLT3*-ITD mutation, whereas none of them had the *NPM1* mutation. Acquired gene mutations in AML fall into either of the 2 broadly defined complementation groups (Class I and II) [1]. Class I comprises mutations that activate signal-transduction pathways and thereby increase the proliferation or survival, or both, of hematopoietic progenitor cells [1]. Mutations that activate members of the receptor tyrosine kinase FLT3 or RAS families are considered Class I mutations. Class II comprises mutations that affect transcription factors or components of the transcriptional coactivator complex and cause impaired differentiation. On the basis of their known physiological functions, mutations in *CEBPA*, *MLL*, and possibly *NPM* fall into this group [6]. A recent study that comprehensively analyzed gene mutations in AML showed that 103 of 165 patients had multiple gene mutations, which most frequently occurred as a combination of Class I and Class II mutations [20]. In addition to gene mutations, hypermethylation of the distal promoter region of *CEBPA* has been reported to have a prognostic implication in a significant proportion of AML patients [28, 29]. Notably, hypermethylation and gene mutation were mutually exclusive in AML [28]. These evidences suggest that methylation testing may be needed along with gene mutation studies to detect *CEBPA* mutations in AML.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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