

## Brief report

Unlike AML1, *CBFβ* gene is not deregulated by point mutations in acute myeloid leukemia and in myelodysplastic syndromes

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The core-binding factor (CBF) complex is a heterodimeric transcription factor composed of 2 subunits, *CBFα* and *CBFβ*, that play a major role in hematopoiesis. Both members of the CBF complex are frequently altered in acute myeloid leukemia (AML) by translocation, most com-

monly t(8;21), t(12;21), and t(3;21) for *CBFα*, located in 21q22, and inv<sup>16</sup>(p13;q22) for *CBFβ*, located on 16q22. Recently, a new mechanism of alteration of *CBFα*, by point mutation, has been reported in myeloid malignancies, particularly in M0 AML. In the present study, we

found no point mutation of the *CBFβ* gene in 30 myelodysplastic syndromes and 100 AMLs, suggesting a limited role, if any, of *CBFβ* point mutations in those disorders. (Blood. 2002;99:3848-3850)

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## Introduction

The core-binding factor (CBF) complex is a heterodimeric transcription factor composed of 2 subunits,  $\alpha$  and  $\beta$ . *CBFα* is encoded by a family of genes with homology to the *Drosophila* runt gene, which contains an evolutionarily conserved protein motif of 128 amino acids<sup>1</sup> that is responsible for DNA binding, transactivation, and heterodimerization with the subunit  $\beta$ .<sup>2</sup> Unlike *CBFα*, the *CBFβ* subunit does not bind DNA but enhances, by a factor of 10 to 20, the DNA binding of *CBFα*.<sup>3</sup> The CBF complex plays a major role in hematopoiesis<sup>4</sup> through regulation of the expression of various genes, including granulocyte-colony-stimulating factor (G-CSF) receptor, macrophage-colony-stimulating factor (M-CSF) receptor, myeloperoxidase, interleukin-3, and T-cell-receptor  $\beta$  gene.<sup>4-6</sup> *CBFα* (AML1), located on 21q22, and *CBFβ*, located on 16q22, are the 2 genes most frequently deregulated in leukemia, generally through translocations that produce chimeric RNA and protein.<sup>7</sup> For the *AML1* gene, the major chimeric proteins are AML1-ETO in the t(8;21)(q22;q22) translocation,<sup>8</sup> AML1-ETV6 in the t(12;21)(p13;q22) translocations, and, less often, AML1-EV11, AML1-MDS1, and AML1-EAP in the t(3;21)(q26;q22) translocations.<sup>9-11</sup> For the *CBFβ* gene, only one chimeric protein has been described—*CBFβ*-MYH11 in inv<sup>16</sup>(p13;q22) or t(16;16)(p13;q22)—present in almost all patients with the M4 E0 subtype of AML.<sup>12</sup> In most of these cases, fusion proteins act to repress transcription of the CBF target genes, which have a promoter consensus sequence for AML1.<sup>11,12</sup> In addition, homozygous knockout mice for AML1 or *CBFβ* have the same phenotype (absence of fetal hematopoiesis).<sup>5,6,13</sup> Recently, point mutations of *CBFα* (AML1) have been reported in acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS).<sup>14-16</sup> In this work, we looked for point mutations of the *CBFβ* gene that could also potentially inactivate it in AML and MDS.

## Study design

Analysis of point mutations of the *CBFβ* gene was made in 25 healthy subjects (bone marrow donors) and 130 leukemia patients—100 patients with AML (40 patients with M0 AML, 17 patients with M1 AML, 22 patients with M2 AML, 2 patients with M3 AML, 10 patients with M4 AML including 2 patients with M4 E0, and 9 patients with M5 AML according to the French-American-British [FAB] classification<sup>17</sup>) and 30 patients with MDS (5 refractory anemia, 6 refractory anemia with ring sideroblasts, 7 refractory anemia with excess of blasts, 6 refractory anemia with excess of blasts in transformation, and 6 chronic myelomonocytic leukemia according to FAB classification). A large number of patients with M0 AML were included in this study because a high frequency of AML1 mutations is observed in this type of AML.<sup>16</sup> All patients had given informed consent.

Detection of *CBFβ* gene mutations was made on DNA extracted from bone marrow cells, by single-stranded conformation polymorphism (SSCP) analysis of the 6 exons corresponding to the entire coding region of the *CBFβ* gene. Primers for polymerase chain reaction (PCR) were chosen upstream and downstream of each exon to include the detection of mutations in acceptor and donor splicing sites. PCR was performed in a total reaction volume of 50  $\mu$ L containing 50 ng DNA, 0.3  $\mu$ M each primer (primer names and sequences are given in Table 1), 1 to 3.5 mM MgCl<sub>2</sub> (Table 1), 10 mM Tris HCl, 50 mM KCl, 200  $\mu$ M each dNTP (Pharmacia, Stockholm, Sweden), 2.5 U Taq DNA polymerase (Quiagen, Courtaboeuf, France), and 0.037 MBq <sup>32</sup>P-labeled deoxycytidine triphosphate. Samples were heated for 5 minutes at 94°C and then underwent 34 cycles for 1 minute at 94°C, 1 minute at 55 to 57°C (Table 1), and 1 minute at 72°C, followed by a final elongation of 7 minutes at 72°C. After amplification, PCR products were loaded on a 2% agarose gel stained with ethidium bromide. The size of the PCR products was 163 to 276 bp (Table 1). For SSCP analysis, 4  $\mu$ L PCR product was diluted in 16  $\mu$ L solution containing 0.1% sodium dodecyl sulfate and 20 mM EDTA; 3  $\mu$ L this mixture was mixed with 3  $\mu$ L solution of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol and then was heated at 95°C

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**Table 1. Primers used for SSCP analysis**

		Primer sequence 5' 3'	Temperature (°C)	MgCl <sub>2</sub> (mM)	Size of product (bp)
Exon 1	Forward	TCA-GTC-GGT-CAG-CGC-GGA-GCC-A	57	1	228
	Reverse	ACT-TTT-CTC-CGC-CCG-GGC-CCA-CT			
Exon 2	Forward	ATT-TGG-CTC-CTG-ATT-TCG-GGC-CGT	57	1	163
	Reverse	CAA-CAA-CGT-ACC-CGC-GCG-CGC-C			
Exon 3	Forward	TTG-CAT-AAT-TTA-TGT-AAT-GTC-CTG-CT	55	3	243
	Reverse	GTA-AAT-TAC-CAG-ATA-ATC-TCT-AAC-AAA			
Exon 4	Forward	CAA-TTA-AAA-ACA-TCC-TTT-TCT-GTG-T	55	3	189
	Reverse	TAA-AGG-GAC-CAT-GCT-CAA-TAA-AGC-C			
Exon 5	Forward	TCA-AAA-TTA-AAA-TAG-GTA-TTT-CAT-GTA-T	55	2	208
	Reverse	AAA-CAT-CAC-AAA-ACA-CAG-CCT-GAG-A			
Exon 6	Forward	ACT-TTT-TGA-CCC-CAA-ATT-ATG-A	55	3.5	276
	Reverse	AAG-AGC-CAA-GAC-CCT-GTT-TTT-A			

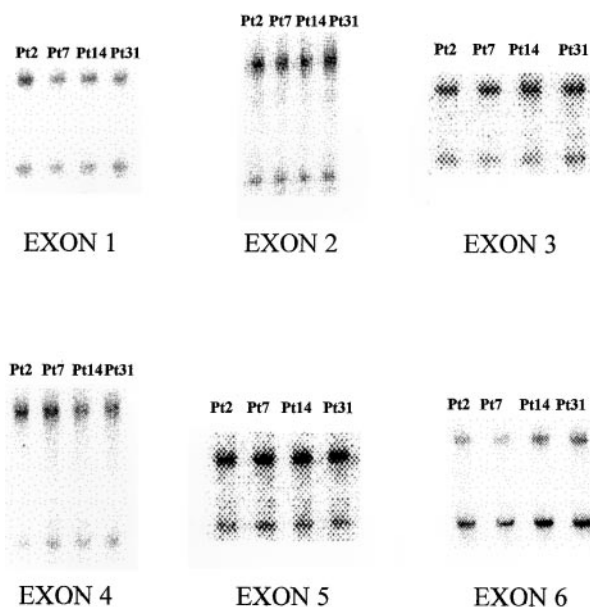
for 2 minutes and cooled in ice. Finally, 3  $\mu$ L was loaded on an MDE gel (Tebu, Le Perray en Yvelines, France). Our preliminary experiments showed the best SSCP profiles were observed with a gel containing 5% glycerol, 1 $\times$  TBE, and electrophoresis run for 12 hours at room temperature.

## Results and discussion

No abnormal SSCP profile for exons 1 to 6 of the *CBF $\beta$*  gene was observed in healthy subjects and in 130 patients with MDS and AML studied (Figure 1). These results appear to differ from results observed with the *AML1* gene. Indeed, others and we<sup>14-16</sup> reported a new mechanism of inactivation of *AML1* in hematologic malignancies, through point mutations of the gene in AML and, less frequently, in MDS and myeloproliferative disorders. All mutations of *AML1* were located in the runt domain, and most of them were observed in M0 AML or in hematologic malignancies with acquired trisomy 21. In M0 AML, most of the patients had bi-allelic alteration of the *AML1* gene either by mutation of the 2

alleles or duplication of the mutated allele with deletion of the wild-type allele or by loss of heterozygosity for *AML1*. In all patients, point mutations of *AML1* gene were acquired, and functional analysis of these abnormal proteins showed that transactivation properties of *AML1* on the promoter of the MCSF-receptor gene were abolished. Inactivation of those properties seemed to result from absent binding of the mutated protein on the DNA consensus site of *AML1* target genes. In addition, the missense mutations appeared to act in a dominant manner by higher affinity binding of CBF. Therefore, we looked for point mutations of the second partner of the CBF complex, *CBF $\beta$* , in MDS and AML.

Our negative results suggest that, unlike *AML1*, *CBF $\beta$*  is probably not inactivated by point mutations in AML and MDS. Because, except in M0 AML, the number of patients with each FAB subtype was low, we cannot completely exclude a low incidence of mutations in some specific FAB subtypes of AML or MDS. In addition, despite using optimal conditions for SSCP, including small PCR products, we could not exclude the presence of some mutations undetectable by the SSCP technique, which generally is 50% to 100% accurate. The apparent absence of *CBF $\beta$*  mutations in AML and MDS could be linked with the function of the *CBF $\beta$*  protein. Indeed, *CBF $\beta$*  acts as a cofactor, and only bi-allelic mutations or mono-allelic mutations with dominant-negative effects could inactivate the CBF complex, inducing the loss of transactivation of *HIS* target genes. This kind of alteration of *CBF $\beta$*  could have drastic effects and induce cell death. In that context, mutations with a dominant-negative effect could lead to a phenotype close to that of *CBF $\beta$*  KO<sup>-/-</sup> mice with an absence of fetal hematopoiesis. On the other hand, mutations inducing lack of the transcription of only 1 of 2 alleles could lead to a phenotype close to that of KO<sup>+/-</sup> *CBF $\beta$*  heterozygous mice, which have few abnormalities. In conclusion, unlike *AML1* mutations, *CBF $\beta$*  mutations are probably not an important event in the pathogenesis of AML or MDS.



**Figure 1. SSCP profile of exons 1 to 6 of the *CBF $\beta$*  gene.** Patient 2 was a healthy subject used as negative control. Patients 7, 14, and 31 had, respectively, M0 AML, M1 AML, and RAEB.

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