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BRIEF COMMUNICATION

Type I *MOZ/CBP (MYST3/CREBBP)* Is the Most Common Chimeric Transcript in Acute Myeloid Leukemia with t(8;16)(p11;p13) Translocation

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The t(8;16)(p11;p13) fuses the *MOZ* (*MYST3*) gene at 8p11 with *CBP* (*CREBBP*) at 16p13 and is associated with an infrequent but well-defined type of acute myeloid leukemia (AML) that has unique morphocytochemical findings (monocytoid blast morphology with erythrophagocytosis and simultaneously positive for myeloperoxidase and nonspecific esterases). RT-PCR amplification of *MOZ/CBP* (*MYST3/CREBBP*) chimera has proved difficult, with four different transcripts found in four reported cases. We studied 7 AML-t(8;16) patients, 5 with cytogenetically demonstrated t(8;16) and 2 with similar morphocytochemical and immunophenotypical characteristics. Clinically, 3 cases presented as therapy-related leukemia. Extramedullar involvement was observed at presentation in 2 patients and coagulopathy in 4. The clinicobiological findings confirmed the distinctiveness of this entity. Of note is the erythrophagocytosis in 5 of 7 cases and the immunological negativity for CD34 and CD117 and positivity for CD56. Using a new RT-PCR strategy, we were able to amplify a specific band of 212 bp in six cases in which sequence analysis confirmed the presence of the previously described *MOZ/CBP* fusion transcript type I. This is the largest molecularly studied AML-t(8;16) series, which demonstrates that *MOZ/CBP* breakpoints are usually clustered in intron 16 of *MOZ* and intron 2 of *CBP*. The newly designed single-round PCR provides a simple tool for the molecular confirmation of *MOZ/CBP* rearrangement.

Recurrent chromosomal translocations resulting in expression of fusion gene products are frequently observed in acute myeloid leukemia (AML). Most of these cytogenetic abnormalities characterize disease entities with specific clinical and biological features. AML with t(8;16)(p11;p13) [AML-t(8;16)] is an infrequent type of leukemia reported in approximately 50 de novo AML and therapy-related AML (t-AML) cases with distinct clinical and hematological characteristics (Sun and Wu, 2001). AML-t(8;16) patients have frequent extramedullar involvement and coagulation disorders at diagnosis. The prognosis is usually extremely poor, with a median survival of only two months (Hanslip et al., 1992; Stark et al., 1995; Velloso et al., 1996; Sun and Wu, 2001). The proliferating cells are of myelomonocytic lineage, exhibit prominent erythrophagocytosis, and show dual myeloperoxidase (MPO) and nonspecific esterase cytochemical staining. At the molecular level, the t(8;16) translocation fuses *MOZ* (*MYST* histone acetyltransferase–monocytic leukemia–3) gene, located at 8p11, with *CBP* (CREB-binding protein), at 16p13 (Borrow et al., 1996; Aguiar et al., 1997). Although genomic rearrangements of the *MOZ* and *CBP* genes have been identified by fluorescence in situ hybridization and Southern blot (Borrow et al., 1996; Giles et al., 1997), amplification of the *MOZ/CBP* transcript and its reverse, the *CBP/MOZ* transcript, by RT-PCR has proved difficult (Giles et al., 1997; Bernasconi et al., 2000). Thus, only 4 AML-t(8;16) cases analyzed by RT-PCR have been published so far, with recognition of four different *MOZ/CBP* fusion transcripts and

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TABLE 1. Main Clinical and Hematological Characteristics of Patients with AML-t(8;16)

Patient	1	2	3[a]	4	5	6	7
Age/gender	28/M	51/F	19/M	51/F	53/F	79/M	30/F
Onset	De novo	De novo	De novo	Therapy-related	Therapy-related	Previous MDS	Therapy-related
Extramedullar	No	No	Skin and lymph nodes	No	No	Skin, liver, and spleen	No
WBC (10^9/L)	8	40	14	21	12	16	6
DIC	No	No	No	Yes	Yes	No	Yes
BM blasts (%)	92	89	70	NA[b]	96	63	56
Hemophagocytosis	No	Yes	Yes	NA[b]	Yes	Yes	Yes
MPO/NSE	+/+	+/+	+/+	+/+	+/+	+/+	−/+
CD34	−	−	−	−	−	±	−
CD117	−	−	NA	−	−	±	−
HLA-DR	−	+	±	+	+	+	±
CD13	+	+	NA	+	−	+	+
CD33	+	+	NA	+	+	+	+
CD15	+	+	+	+	+	+	+
i-MPO	+	NA	+	+	+	+	+
CD4	+	+	+	+	+	+	±
CD11b	−	+	NA	+	±	+	+
CD11c	+	NA	NA	+	NA	NA	+
CD56	+	+	±	±	−	−	+
Karyotype	46,XY,t(8;16) (p11;p13) [20]	46,XX,t(8;16) (p11;p13) [8]	46,XY,t(8;16) (p11;p13)[20]	NA[b]	46,XX,t(8;16) (p11;p13) [20]	NA	46,XX,t(8;16) (p11;p13) [3]
MOZ/CBP type I	+	+	NA	+	+	+	+
Outcome	CCR (34+ mos)	CR but relapse at +13 mos	CCR (27+ mos)	Early death (alveolar hemorrhage)	Early death (GI bleeding)	Early death (cerebral hemorrhage)	CR Relapse at +4 and 2nd CR, Dead at +20 (alloSCT)

MDS, myelodysplastic syndrome; DIC, disseminated intravascular coagulation; NA, not assessable.

[a]Phenotyped by immunohistochemistry.

[b]Bone marrow necrosis.

MPO, cytochemical myeloperoxidase; NSE, nonspecific esterases; i-MPO, immunological myeloperoxidase; CCR, continuous complete remission; CR, complete remission; alloSCT, allogeneic stem cell transplantation; Mos, months; GI, gastrointestinal.

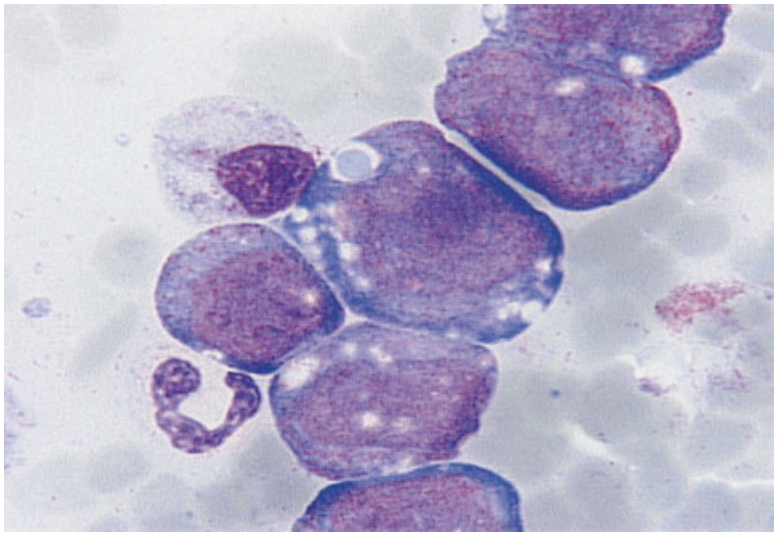

Figure 1. Large blasts with monocytic appearance, heavy granulation, and erythrophagocytosis in a patient with AML-t(8;16). Bone marrow, May-Grünwald Giemsa.

one *CBP/MOZ* isoform (Borrow et al., 1996; Panagopoulos et al., 2000, 2002).

We have identified 7 patients with AML-t(8;16), 5 showing a t(8;16) and the other 2 having similar morphocytochemical and immunophenotypical characteristics but in which the cytogenetic study had failed. These cases were screened for *MOZ/CBP*, together with 11 FAB M4/M5 acute myeloid leukemias. The clinical, immunophenotypical, and cytogenetic findings were collected from the medical records, and the morphological characteristics of the bone marrow, peripheral blood, and tissues were reviewed.

For the study of *MOZ/CBP* rearrangement, we performed RT-PCR using RNA extracted from peripheral blood and/or bone marrow samples in 17 cases and from a lymph node in 1 case. Total RNA was isolated by a modified one-step guanidium thiocyanate-phenol-chloroform method using Ultraspec RNA (Biotecx Laboratories, Houston, TX) as previously reported (Chomczynski and Sacchi, 1987). In 1 of the 5 cases with cytogenetically demonstrated t(8;16), the RNA obtained was not of good quality for RT-PCR analysis. One microgram of total RNA was denatured at 65°C for 5 min, and then reverse transcription was performed with 0.75 U/μL Moloney–murine leukemia virus reverse transcriptase (Invitrogen, Gaithersburg, MD) in the manufacturer's buffer with 0.75 U/μL of RNase inhibitor (Promega, Madison, WI) and 2.5 mM random hexamer primers at 37°C for 1 hr in a final volume of 40 μL. First-round PCR was done in a total volume of 25 μL containing 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 μL of Expand High Fidelity *Taq* polymerase (Roche, Mannheim, Germany), 0.25 μM of each of the primers MOZ3558F (5′-GAGGCCAATGCCAAGATTAGAAC-3′) and CBP1201R (5′-GTACCCACACAAGCAATTGCAAC-3′), and 5 μL of the cDNA. After an initial denaturation at 95°C for 5 min, 40 cycles of 1 min at 95°C, 1 min at 60°C, and 1 min at 72°C were run, followed by a final extension for 10 min at 72°C. For the second-round PCR, 2.5 μL of the first PCR product was reamplified using primers MOZ3558F and CBP1047R (5′-AGCTTGACTAAAGGGCTGTC-3′) for the inner reaction. Using these primers, a product of 936 bp corresponding to *MOZ/CBP* transcript type I, and a band of 220 bp corresponding to *MOZ/CBP* transcript type II (accession numbers HSA251843 and HSA251844, respectively), should be amplified. Subsequently, we designed a single-round PCR for amplification of transcript type I using a new reverse primer, CBP335R (5′-GGTATCAGCTCATCAGGAAGATCA-3′) and an annealing temperature of 55°.

For the detection of reciprocal transcript *CBP/MOZ*, we performed nested PCR using the primers CBP96F, MOZ3953R, CBP174F, and MOZ3844R, as previously described (Panagopoulos et al., 2000).

Ten microliters of the PCR products was analyzed by electrophoresis through 2% agarose gels, stained with ethidium bromide, and visualized under UV. The PCR products were purified by gel excision with the QIAEX II agarose-gel extraction kit (Qiagen, Hilden, Germany) and directly sequenced from both strands, using the Big Dye Terminator Cycle Sequencing Ready Reaction (versions 3 and 3.1, Applied Biosystems, Foster City, CA) following the manufacturer's instructions. Sequencing analysis and alignments were

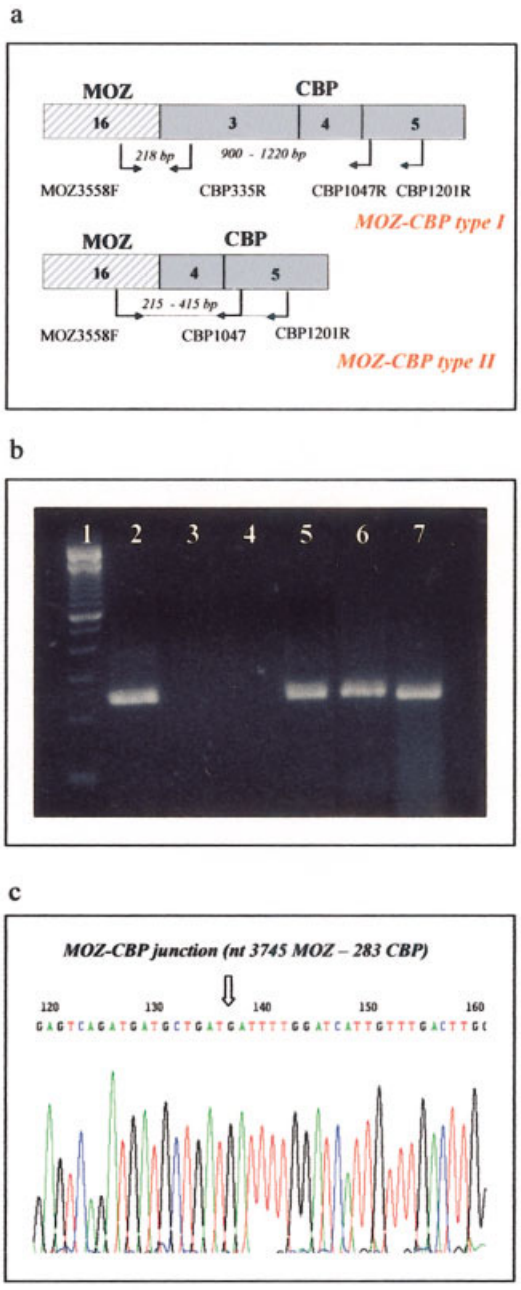

Figure 2. a. Schematic representation of types I and II *MOZ/CBP* chimeric transcripts (accession numbers AJ251843 and AJ251844). The positions of the primers used in the RT-PCR are indicated (not drawn to scale; adapted from Panagopoulos et al., 2000). b. Chimeric *MOZ/CBP* transcripts in four AML cases with t(8;16)(p11;p13). RT-PCR amplification of *MOZ/CBP* transcript type I. Lane 1: 100-bp molecular-weight DNA ladder; lanes 2, 5, 6, and 7: positive AML-t(8;16) samples; lane 3: no cDNA template; and lane 4: negative AML sample. c. Partial sequence chromatogram of the 212-bp amplified fragment, corresponding to mRNA of type I *MOZ/CBP* chimeric transcript, from one AML-t(8;16) representative case.

performed using BLAST software (www.ncbi.nlm.nih.gov/BLAST/).

The main clinical and biological characteristics of the patients are summarized in Table 1. Interestingly, AML arose as t-AML in 3 patients who had received topoisomerase-II inhibitors for a previous neoplasia, and 1 patient had previously had myelodysplastic syndrome. Although an early death from a hemorrhagic event in the context of a severe coagulation disorder was observed in 3 patients, a complete remission (CR) was achieved in 4 cases, 2 of them remaining in durable response after undergoing an allogeneic stem cell transplantation (alloSCT) in the first CR. Therefore, intensive therapy, probably including alloSCT, might cure a proportion of patients with this high-risk AML.

The blasts showed a monocytic appearance with heavy granulation, with erythrophagocytosis in 5 of the 7 and dual MPO/NSE staining in 6 of the 7 cases (Table 1, Fig. 1). Immunophenotyping by flow cytometry or immunohistochemistry disclosed a homogenous profile, with the expression of HLA-DR, the absence of CD34 and CD117, and a myelomonocytic differentiation pattern, in accord with the results in the few published reports (Stark et al., 1995; Sun and Wu, 2001). Of note, CD56 was positive in 5 of 7 cases. Although this feature is related to extramedullar involvement (Baer et al., 1997), only 1 of our 5 CD56+ patients had blastic infiltration of the skin and lymph nodes. This clinicobiological profile may be highly suggestive of AML-t(8;16), but some of their characteristics, such as coagulopathy, heavy granulation of blasts, strong positivity for MPO, and negativity for CD34, may be present in other AML subtypes such as acute promyelocytic leukemia (Sun and Wu, 2001). In this sense, the molecular studies can contribute to the differential diagnosis. Conventional cytogenetics disclosed the t(8;16)(p11;p13) in 5 cases; in the other 2 patients, an informative karyotype was not available because of bone marrow necrosis and the absence of assessable metaphase cells.

To the best of our knowledge, only 4 cases of AML with *MOZ/CBP* rearrangement analyzed by RT-PCR have been reported (Borrow et al., 1996; Panagopoulos et al., 2000, 2002). Panagopoulos et al. (2000) detected two types of *MOZ/CBP* fusion transcripts (types I and II), of 1,128 and 415 bp, respectively, in 2 patients. The sequencing of these fragments disclosed the in-frame fusion of nucleotide (nt) 3,745 of *MOZ* (accession number U47742) with nt 283 of *CBP* (NM_004380) in transcript type I, whereas the same locus of *MOZ* was fused out-of-frame with nt 997 of *CBP* in transcript type II (Fig. 2a; Panagopoulos et al., 2000). Recent genomic studies of these cases localized the breakpoint within intron 16 of *MOZ* (Panagopoulos et al.,

2003). Furthermore, two additional cases have been described; these have breakpoints within exon 17 of *MOZ* (Borrow et al., 1996; Panagopoulos et al., 2002). In our series, we initially followed a slightly modified version of a previously published RT-PCR strategy (Panagopoulos et al., 2000), obtaining 2 weak bands, of 936 bp and 220 bp, in only 2 of the studied cases. We subsequently designed a single-round RT-PCR for the amplification of in-frame transcript type I using the same forward MOZ3558F primer and an inner reverse primer (CPB335R). This strategy yielded amplification of a 212-bp band in all cases with the available RNA (Fig. 2b). Direct sequencing of the PCR product confirmed the presence of the type I *MOZ/CBP* fusion rearrangement, with breakpoints at nt 3,745 of *MOZ* and nt 283 of *CBP* (Fig. 2c). This rearrangement was not found in any of the other 11 M4/M5 AML cases tested. Of note, similar breakpoints within intron 16 at *MOZ* have been described in AML with inv(8)(p11q13) and t(8;22)(p11;q13), which juxtapose *MOZ* to *TIF2* (nuclear receptor coactivator 2) and *EP300* (E1A-binding protein p 300), respectively (Carapeti et al., 1998; Liang et al., 1998; Kitabayashi et al., 2001b). It has been suggested that this site of *MOZ* is prone to breakage, which would explain the frequency of t-AML harboring this rearrangement. An alternative explanation is there being a selective advantage to in-frame hybrids generated in this region (Panagopoulos et al., 2003). Nevertheless, as previously mentioned, alternative *MOZ* breakpoints, in exon 17 in two AML-t(8;16) and in intron 15 in one AML-t(8;22), have been reported (Borrow et al., 1996; Panagopoulos et al., 2000; Kitabayashi et al., 2001b).

The *MOZ* gene is composed of 17 exons and contains a *MYST* domain with histone acetyltransferase (HAT) activity. This domain, in exons 9–14, remains intact in all the t(8;16) translocations described to date (Kitabayashi et al., 2001a; Panagopoulos et al., 2003). *MOZ* modulates the transcription of specific target genes by coactivating the *RUNX1* (runt-related transcription factor 1) transcription factor complex (Champagne et al., 2001; Kitabayashi et al., 2001a). Possible leukemogenic mechanisms derived from the *MOZ/CBP* rearrangement are aberrant chromatin acetylation by the mistargeting of specific HAT activity and an inhibition of *RUNX1*-mediated transcription (Champagne et al., 2001; Kitabayashi et al., 2001b; Panagopoulos et al., 2003). On the other hand, the *CBP* gene is believed to coordinate the transcriptional effects of multiple signals from cell surface and nuclear receptors (Aguiar et al., 1997). *CBP* is also fused to other partners such as *MLL* in t-AML with t(11;16)(q23;p13) and *MORF* (*MYST* histone acetyltransferase–monocytic leukemia–4) in t(10;16)(q22;p13), a gene highly homologous to *MOZ* in structure and function that also breaks within intron 16 (Panagopoulos et al., 2001). All the breakpoints reported in *CBP* chimeras are in intron 2 (Borrow et al., 1996; Aguiar et al., 1997; Giles et al., 1997; Rowley et al., 1997; Panagopoulos et al., 2000, 2002, 2003).

The reciprocal *CBP/MOZ* transcript was not amplified in any of our cases. In some reports, the *CBP/MOZ* transcript was either out-of-frame (Borrow et al., 1996) or not expressed (Panagopoulos et al., 2002). Therefore, *MOZ/CBP*, but not the *CBP/MOZ* transcript, is believed to be of importance in the leukemogenic process (Panagopoulos et al., 2002).

In summary, our cases constitute the largest AML-t(8;16) series with *MOZ/CBP* rearrangement analyzed at the molecular level, showing that breakpoints are clustered in intron 16 of *MOZ* and intron 2 of *CBP* in almost all patients. Moreover, we have designed a single-round PCR that can be a simple tool for the molecular confirmation of *MOZ/CBP* rearrangement.

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