

BRIEF COMMUNICATION

t(10;16)(q22;p13) and MORF-CREBBP Fusion Is a Recurrent Event in Acute Myeloid Leukemia

José L. Vizmanos,^{1*} María J. Larráyoiz,¹ Idoia Lahortiga,¹ Filomena Floristán,² Carmen Álvarez,² María D. Odero,¹ Francisco J. Novo,¹ and María J. Calasanz¹

¹Department of Genetics, School of Science, University of Navarra, Pamplona, Spain

²Hematology Service, Hospital de Cruces, Servicio Vasco de Salud-Osakidetza, Baracaldo, Spain

Recently, it was shown that t(10;16)(q22;p13) fuses the *MORF* and *CREBBP* genes in a case of childhood acute myeloid leukemia (AML) M5a, with a complex karyotype containing other rearrangements. Here, we report a new case with the *MORF-CREBBP* fusion in an 84-year-old patient diagnosed with AML M5b, in which the t(10;16)(q22;p13) was the only cytogenetic aberration. This supports that this is a recurrent pathogenic translocation in AML. © 2003 Wiley-Liss, Inc.

Some acquired chromosomal abnormalities resulting in fusion genes characterize acute myeloid leukemias (AMLs) and their detection is important for the clinical management and to uncover the molecular basis of the disease. Recently, it was shown that the t(10;16)(q22;p13) fuses *MORF* and *CREBBP* genes in a case of childhood AML M5a (Panagopoulos et al., 2001). *CREBBP* is a large nuclear protein and a transcriptional coactivator, implicated in hematopoiesis, which physically interacts with various sequence-specific DNA-binding factors through conserved domains (McManus and Hendzel, 2001; Blobel, 2002; Cantor and Orkin, 2002). Mutations and constitutional translocations affecting the *CREBBP* gene, located at 16p13, are responsible for Rubinstein–Taybi syndrome (RTS), a complex developmental disorder that leads to an increased incidence of neoplasia (Petrij et al., 1995, 2000). *CREBBP* is also rearranged with *MOZ* in leukemias with the t(8;16)(p11;p13) (Borrow et al., 1996; Chaffanet et al., 1999; Panagopoulos et al., 2000a) and with *MLL* in hematologic malignancies with t(11;16)(q23;p13) (Rowley et al., 1997; Satake et al., 1997; Sobulo et al., 1997; Taki et al., 1997; Sugita et al., 2000). *MORF* (monocytic leukemia zinc finger protein-related factor) is a ubiquitously expressed gene, located at 10q22, which displays a high degree of similarity with *MOZ* and which also contains zinc fingers, a histone acetyltransferase (HAT) domain, an acidic region, and a COOH-terminal Ser/Met-rich domain (Champagne et al., 1999).

We report here a t(10;16)(q22;p13), with a *MORF-CREBBP* fusion, as the only cytogenetic abnormality in an AML M5b. This is the second case of such a translocation reported in the litera-

ture, suggesting that this translocation is a recurrent event in AML.

An 84-year-old patient was admitted to the hospital because of pain in the left upper limb irradiating to the flank and a flulike syndrome. The peripheral blood values were hemoglobin 13.6 g/100 ml, platelets $10.9 \times 10^9/L$, and WBC count $24.5 \times 10^9/L$ with 33% blasts. The bone marrow aspirate was almost completely infiltrated with atypical cells (89% monocytes). Bone marrow cytogenetics showed a t(10;16)(q22;p13) in 100% of the metaphases (38/38) as the only aberration (Fig. 1A). A diagnosis of AML M5b was made. The patient received no curative intended treatment, and died one month later.

One patient with AML (M4) with a similar translocation t(10;16)(q21;p13), showing also an 11q23 rearrangement, was previously reported (Pebusque et al., 1988), although molecular studies were not performed. Recently, a 4-year-old girl with AML M5a was reported (Panagopoulos et al., 2001) with a complex karyotype including a t(10;16)(q22;p13). Fluorescence in situ hybridization (FISH) and molecular analyses showed that the t(10;16)(q22;p13) resulted in a fusion between *MORF* and *CREBBP*. This finding prompted us to investigate whether the same fusion gene could be present also in our patient.

*Correspondence to: Dr. José L. Vizmanos, Department of Genetics, University of Navarra, C/Irunlarrea s/n, 31080 Pamplona, Spain. E-mail: jlvizmanos@unav.es

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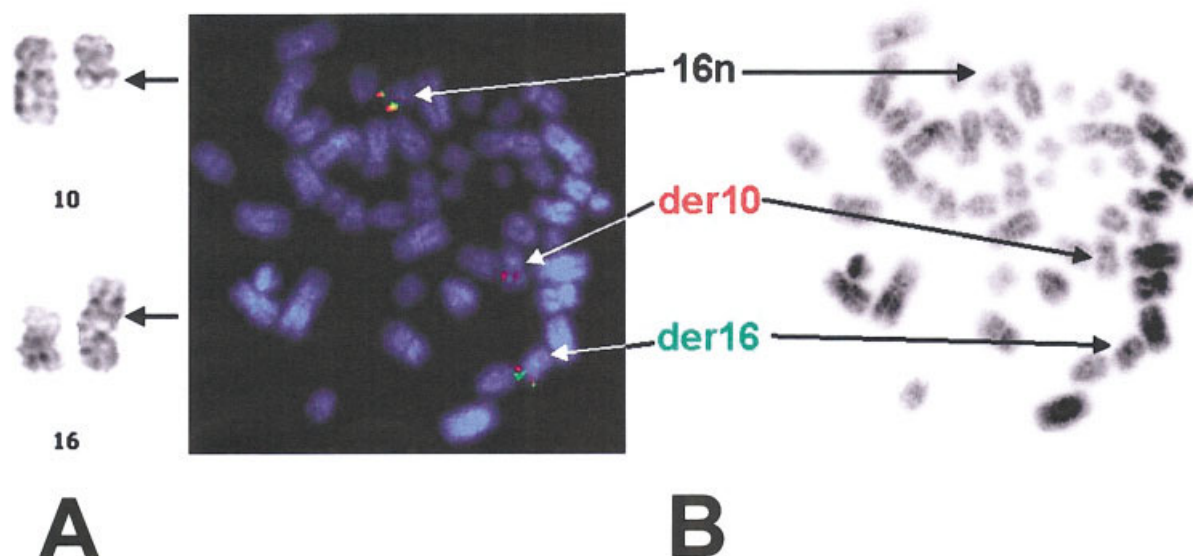


Figure 1. G-Band and FISH analyses. **A:** Partial karyotype showing the $t(10;16)(q22;p13)$. Arrows indicate breakpoints. **B:** FISH analysis by use of BACs RPCI-11 461A8 (green) and RPCI-11 95J11 (red) showing that the signal of 95J11, which covers the initial part of the *CREBBP* gene, is split between *der(10)* and *der(16)*. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Reverse transcriptase–polymerase chain reaction (RT-PCR) analysis was performed as reported by Panagopoulos et al. (2001). One-step RT-PCR with primers MORF2843F and CBP425R was done as described and yielded the expected 0.4-kb fragment, showing the presence of the *MORF-CREBBP* fusion gene (Fig. 2A). Nested RT-PCR with primers CBP96F/MORF3383R and CBP174F/MORF3277R was necessary (Panagopoulos et al., 2001) to amplify the expected 0.3-kb fragment corresponding to the reciprocal *CREBBP-MORF* transcript (Fig. 2A). Sequence analysis of the *MORF-CREBBP* amplification product showed that the translocation fused *MORF* exon 15 with *CREBBP* exon 3 (Fig. 2B). Thus, the breakpoints in our case led to the fusion of the same exons as described in the previously reported patient. FISH analysis of bone marrow cells with bacterial artificial chromosomes (BACs) RPCI-11 461A8 and RPCI-11 95J11 (Fig. 1B) was consistent with the disruption of the *CREBBP* gene in this region because the split affected 95J11, which covers the initial part of the gene.

The present case displays some features in common with the one published by Panagopoulos et al. (2001). Both were diagnosed as AML M5 and the breakpoints led to fusion of the same exons of *MORF* and *CREBBP*. However, the case published previously was a 4-year-old girl who was alive at the time of publication. We report an 84-year-old male

who died one month later. Furthermore, the $t(10;16)(q22;p13)$ was not the only cytogenetic aberration in the previous case. Hence, it was unclear whether the *MORF-CREBBP* fusion was responsible for the development of leukemia. In our patient, the $t(10;16)$ was the sole cytogenetic event in leukemic cells, suggesting that the fusion gene could contribute directly to the development of the AML.

MORF is very similar to *MOZ*, a gene fused to *CREBBP* in $t(8;16)(p11;p13)$ (Borrow et al., 1996; Chaffanet et al., 1999; Panagopoulos et al., 2000a), *TIF2* in $inv(8)(p11q13)$ (Carapeti et al., 1998, 1999; Liang et al., 1998; Panagopoulos et al., 2000b; Billio et al., 2002), and to *EP300* in $t(8;22)(p11;q13)$ (Chaffanet et al., 2000; Kitabayashi et al., 2001). Fusion genes involving *MOZ* are mainly seen in young patients with AML M4/M5, in which the leukemic blasts frequently display erythrophagocytosis. Thus, it is frequently associated with certain morphological and clinical features irrespective of the partner gene. However, despite the similarities between *MOZ* and *MORF*, the morphological analysis of bone marrow aspirate from our patient did not reveal any signs of erythrophagocytosis. Unlike the case with $inv(8)(p11q13)$ and *MOZ-TIF2* fusion recently published (Billio et al., 2002), the case reported here was negative for the *FLT3-ITD* (Nakao et al., 1996) and D835 mutation (Yamamoto et al., 2001) (data not shown).

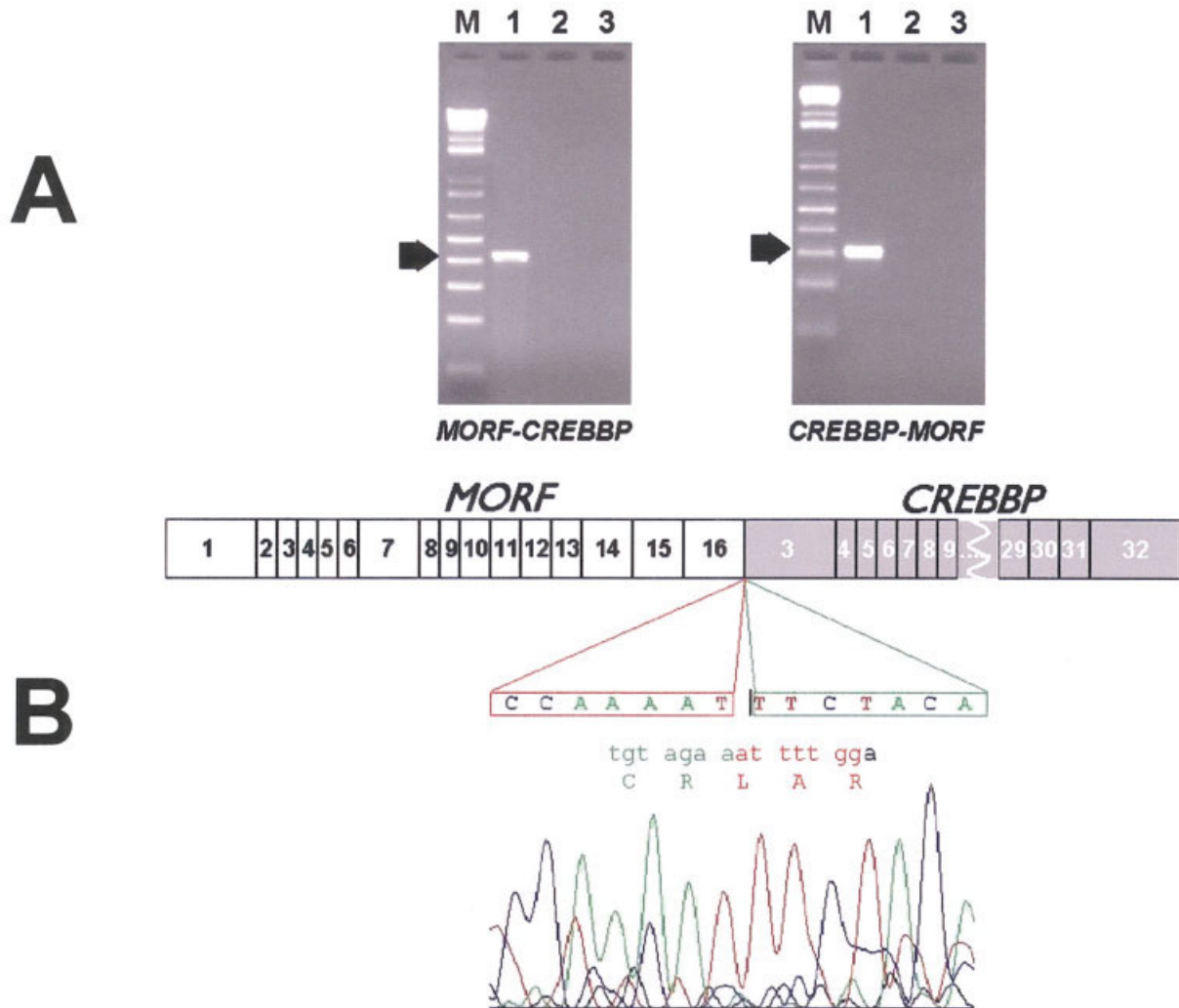


Figure 2. Molecular analyses. **A:** One-step RT-PCR amplification of the *MORF-CREBBP* chimeric transcript (left, 0.4 kb) and nested RT-PCR amplification of the *CREBBP-MORF* chimeric transcript (right, 0.3 kb). In both cases: lane M, molecular weight marker 1 Kb Plus DNA Ladder (Invitrogen Life Technologies, Paisley, UK); lane 1, cDNA from the patient; lane 2, cDNA from a normal donor; lane 3, water. **B:** Sequence

analysis of the *MORF-CREBBP* amplification product showing the fusion between *MORF* exon 16 and *CREBBP* exon 3. The sequence profile shown (capital letters) corresponds to the reverse complement strand of the fusion (lowercase letters) between *MORF* and *CREBBP*. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

In both patients with the $t(10;16)(q22;p13)$ described to date, both *MORF-CREBBP* and *CREBBP-MORF* transcripts were detected. It is not yet clear which of them is mainly responsible for the leukemogenic effect, but it has been suggested that this results from the substitution of the COOH-terminal part of *MORF* or *MOZ* by the COOH-terminal part of *CREBBP*, P300 or TIF2. As in the previously reported patient, a one-step RT-PCR was sufficient to detect the *MORF-CREBBP* transcript, whereas a nested RT-PCR was necessary to detect *CREBBP-MORF*. This could indicate that the transcription level of the former transcript is higher.

In summary, we report an 84-year-old patient with AML M5b showing a $t(10;16)(q22;p13)$ as the sole cytogenetic aberration, with the presence of *MORF-CREBBP* and *CREBBP-MORF* fusion transcripts. This case, the second one reported thus far, proves that this is a recurrent pathogenic event in AML.

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