New types of *MYST3-CBP* and *CBP-MYST3* fusion transcripts in t(8;16)(p11;p13) acute myeloid leukemias

The t(8;16)(p11;p13) translocation, associated with poor prognosis acute monocytic leukemia, fuses *MYST3* on chromosome region 8p11 to *CBP* on chromosome region 16p13. Two types of *MYST3-CBP* and *CBP-MYST3* fusion transcripts have been identified in patients. We describe two new types of *MYST3-CBP* transcripts and a new primer set.

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Translocation t(8;16)(p11;p13) is found in 6.5% of acute myeloid leukemias (AML) of the M4/M5 FAB subtype. These poor prognosis AML are associated with erythrophagocytosis in blast cells. The translocation fuses *MYST3/MOZ* on chromosome region 8p11, which encodes a histone acetyltransferase (HAT), to *CBP/CREBBP* on chromosome region 16p13, which encodes a transcriptional co-activator and acetyltransferase.[1-4] Reverse transcriptase-polymerase chain reaction (RT-PCR) identified three types of *MYST3-CBP* and *CBP-MYST3* fusion transcripts in some previous studies,[1-7] but was unsuccessful in some others suggesting the existence of translocation variants.[1,8,9] We describe here two new types of *MYST3-CBP* fusion transcripts.

Patient #1, a 55-year old female presented with AML-M5a 2 years after breast cancer. The white blood cell (WBC) count was 2.9×10⁹/L with 17% blast cells. The bone marrow aspirate was hypercellular with 90% of monocytic blast cells with features of erythrophagocytosis. The karyotype showed a t(8;16) and a t(11;19) in all mitoses, and an additional 3q deletion and 8q tetrasomy in 65% of mitoses. The patient was treated with idarubicin and cytosine arabinoside, followed by autologous stem cell transplantation. She is still alive in complete remission.

Patient #2, a 72-year old man presented with AML-M5a. The WBC count was 51.3×10⁹/L with 76% blast cells. The bone marrow aspirate was hypercellular with 68% of monocytic blast cells with features of hemophagocytosis. A complex t(8;16) was found in 65% of mitoses with insertion of 8q material between 16p and 8p on the der(16). The patient died 1 month after diagnosis.

On RNA extracted from bone marrow cells of the patients we used nested RT-PCR as described by Schmidt *et al.*[4] for the detection of type I (*MYST3* exon 16-*CBP* exon 3) (Figure 1A, B) and type II (*MYST3* exon 16-*CBP* exon 4) *MYST3-CBP* fusion transcripts, as well as type I *CBP-MYST3* fusion transcript (*CBP* exon 2-*MYST3* exon 17) (Figure 1C, D). PCR products were sequenced after purification.

MYST3-CBP fusion transcripts were not detected in

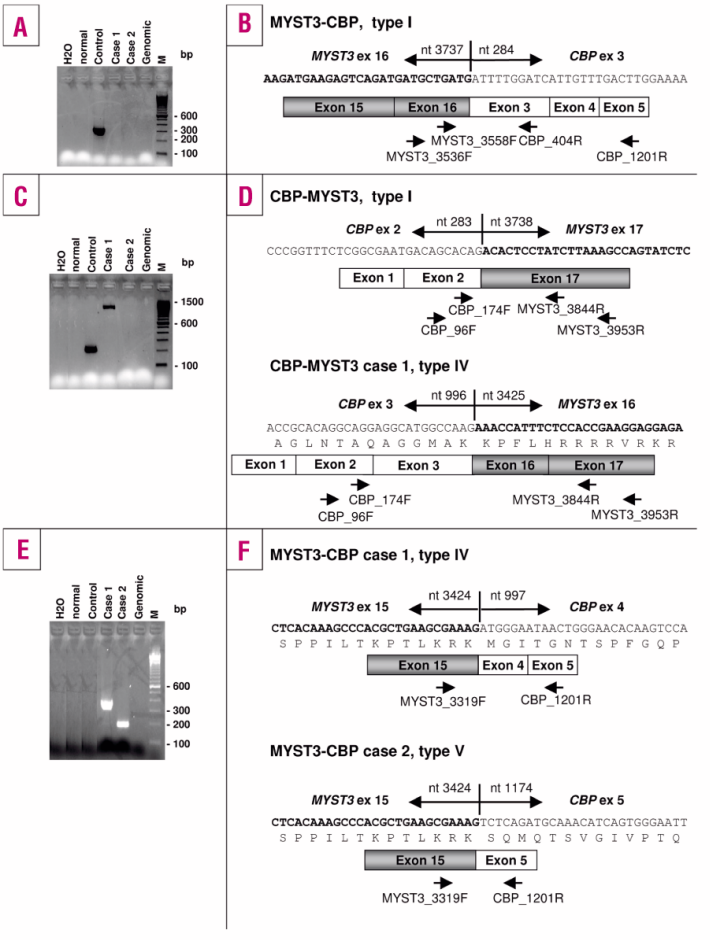

Figure 1. RT-PCR and partial sequences of *MYST3-CBP* and *CBP-MYST3* fusions. A-B. Nested PCR with the published[4] primer combination exon 16 MYST3_3536F/ exon 5 CBP_1201R and exon 16 MYST3_3558F/ exon 3 CBP_404R amplifies a ~ 330 bp fragment in the type I control. Cases 1 and 2 are not amplified. C-D. The reciprocal *CBP-MYST3* fusion transcript is observed with nested PCR and primer combination exon 2 CBP_96F/ exon 17 MYST3_3953R and exon 2 CBP_174F/ exon 17 MYST3_3844R. A 229bp fragment and a 943 bp fragment are amplified in the type I control and in case 1, respectively. E-F. PCR with primer combination exon 15 MYST3_3319F/exon 5 CBP_1201R amplifies a ~ 350 pb fragment in case 1 and a 176 bp fragment in case 2, suggesting a different breakpoint location. No fusion is amplified in the type I control. Normal: RNA lymphocytes; Control: RNA from patient with *MYST3-CBP* and *CBP-MYST3* type I transcripts; Genomic: genomic DNA; M: 100 bp DNA molecular weight ladder. The *NMA* gene was used to control the efficiency of the RT-PCR.

Table 1. Primers used for RT-PCR and sequencing.

Designation	*Sequence (from 5' to 3')*	*Position*
MYST3_3536F	CCTTTTGAAGATTCTGACTCCG	MYST3 exon 16
CBP_1201R	GTTGCAATTGCTTGTGTGGGTAC	CBP exon 5
MYST3_3558F	GAGGCCAATGCCAAGATTAGAAC	MYST3 exon 16
CBP_404R	CCTCGTAGAAGCTCCGACAGTT	CBP exon 3
MYST3_3319F	CGCTACAGTGAGGGTGACAGG	MYST3 exon 15
CBP_96F	CGCTCGCTCCTCTCCCTCGCAG	CBP exon 2
MYST3_3953R	TGGAAACGATGGGCTCAATGACGC	MYST3 exon 17
CBP_174F	GGGCTGTTTTCGCGAGCAGGTG	CBP exon 2
MYST3_3844R	GGCTCTTGCCTTTGGGCCATCC	MYST3 exon 17
CBP_643F	CAGACTGGACCTGGTATCTGC	CBP exon 3
CBP_1120F	GAGCATGGTCAACAGTTTGC	CBP exon 4
MYST3_3663R	ACATCCTGCGAAGACAAAC	MYST3 exon 16

either of the two cases. A 943bp *CBP-MYST3* product was obtained in case 1 but not in case 2. A nested PCR with two forward primers in *CBP* exons 3 and 4 and two reverse primers in *MYST3* exons 17 and 16 was also negative in case #2, as expected because of the insertion of 8q material. Sequencing of the 943 bp product showed an in-frame fusion between *CBP* exon 3 and *MYST3* exon 16 whereas the fusion obtained for the type I transcript used as positive control in this reaction showed the expected 229bp fragment[4] (Figure 1C, D). To detect *MYST3-CBP* transcripts we designed a forward primer (Table 1) in *MYST3* exon 15 (*MYST3*_3319F) and the RT-PCR reaction with the *CBP* exon 5 reverse primer (*CBP*_1201R) yielded a ~ 350bp for case 1 and a ~ 180bp for case 2. Sequence analysis showed an in-frame fusion between *MYST3* exon 15 and *CBP* exon 4, and between *MYST3* exon 15 and *CBP* exon 5, respectively (Figure 1E, F).

Up to now, 49 AML with t(8;16)(p11;p13) have been reported; 94% of these are monocytic leukemias (mostly of M5 subtype).[10] Sixteen cases have been characterized by RT-PCR.[1-7] Type I transcript is the most frequent fusion product (14/16 cases); breakpoints map in *MYST3* intron 16. Type II is a theoretical, out-of-frame sequence and has not been described in patients. In rare cases (2/16) the breakpoint is within *MYST3* exon 17,[1,3] which is fused to *CBP* exon 2 or 4 [1,3] defining type III. Our two cases thus define the fourth and fifth types, in which the *MYST3-CBP* fusions have lost *MYST3* exon 16, suggesting that the breakpoint occurs in intron 15. Thus, all the reported breakpoints, including those described here, are located downstream of exon 14. The intact MYST domain in the chimeras may be responsible for aberrant chromatin acetylation due to mistargeting of specific HAT activities and may interfere with gene expression. In type I fusions the breakpoint occurs in *CBP* intron 2. Our two cases involve exons 4 and 5 (cases 1 and 2, respectively) suggesting that the breakpoints occur in intron 3 or 4. As in type I cases, the MYST3-CBP proteins retain most of the CBP functional domains including the HAT domain.

We have established an RT-PCR assay able to detect *MYST3-CBP* type IV and V with *MYST3* exon 15-*CBP* exon 4 or 5 fusion. At diagnosis, type I may be screened for first and, if negative, completed by the search for types III, IV and V. An alternate strategy may be to use our set of primers followed by a nested PCR to detect type I or III. Our study may also help better monitoring of minimal residual disease of AML with t(8;16).

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