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.

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 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...
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 MYS T3 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 MYS T3 exon 16 whereas the fusion obtained for the type I transcript used as positive control in this reaction showed the expected 229bp fragment (Figure 1C, D). To detect MYS T3-CBP transcripts we designed a forward primer (Table 1) in MYS T3 exon 15 (MYS T3_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 MYS T3 exon 15 and CBP exon 4 and, between MYS T3 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.15 Type I transcript is the most frequent fusion product (14/16 cases); breakpoints map in MYS T3 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 MYS T3 exon 17,16 which is fused to CBP exon 2 or 4,16 defining type III. Our two cases thus define the fourth and fifth types, in which the MYS T3-CBP fusions have lost MYS T3 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 MYS T3 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 MYS T3-CBP proteins retain most of the CBP functional domains including the HAT domain.

We have established an RT-PCR assay able to detect MYS T3-CBP type IV and V with MYS T3 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).

References