**CBFB/MYH11 Fusion Transcripts in A Case of Acute Myelogenous Leukemia (M1) with Partial Deletion of the Long Arm of Chromosome 16**

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Pericentric inversion of chromosome 16 [inv(16)(p13q22)] is seen in patients with acute myelomonocytic leukemia with bone marrow eosinophilia. This inversion juxtaposes the MYH11 gene on p13 and the CBFB gene on q22, resulting in the formation of a chimeric mRNA transcript. We describe a patient with acute myelogenous leukemia (M1), with del(16)(q22), who expressed the chimeric transcript. Reverse transcriptase polymerase chain reaction and the sequencing of its product showed fusion of 5' CBFB at position 495 to 3' MYH11 at position 1201. To our knowledge, this is the first report of an AML (M1) case with del(16) and CBFB/MYH11 rearrangement.

*Key words:* acute myelogenous leukemia, CBFB, myosin heavy chain, partial deletion of chromosome 16, CBFB/MYH11 fusion

**Introduction**

The pericentric inversion of chromosome 16 [inv(16)(p13q22)] is a characteristic karyotypic abnormality associated with acute myelogenous leukemia (AML), most commonly of the acute myelomonocytic leukemia with bone marrow eosinophilia (M4Eo) subtype (1). This inversion has been shown to juxtapose the smooth muscle myosin heavy chain gene (MYH11) on p13 and the transcription factor gene (CBFB) on q22, resulting in the formation of a chimeric mRNA transcript of upstream CBFB coding sequences to downstream MYH11 coding sequences (2). In addition, oncogenic activities of the chimeric protein encoded by this fusion transcript were found using an NIH 3T3 transforming assay (3). Furthermore, the study of a series of 37 AML cases of inv(16) and the related translocation t(16;16) using reverse transcriptase polymerase chain reaction (RT-PCR) revealed that all but 1 of the typical inv(16) cases and all translocation cases had fusion of CBFB and MYH11 (4). On the other hand, deletion of the long arm of chromosome 16 has been reported in patients with AML (5). The breakpoint is q22, the same as that in inv(16) and in t(16;16). Of the three related chromosome 16 rearrangements observed cytogenetically in leukemia patients, the only one which has not been studied at the molecular level is the del(16)(q22). We report here an AML (M1) case of del(16)(q22) which had the fusion transcript of CBFB and MYH11.

**Case Report**

A 49-year-old man was admitted to our hospital complaining of edema on October 19, 1993. The peripheral blood examination revealed the following data: leukocytes 73,700/μl with 63.5% blasts, 2% eosinophils, and 19.5% basophils, hemoglobin 8.6 g/dl, platelets 3.5x10⁴/μl, LDH 1,784 IU/l, and serum lysozyme 38.0 μg/ml (normal range: 3.0–10.6 μg/ml).

Urine lysozyme level was under 0.1 μg/ml. Bone marrow was hypercellular with 72.6% blasts and 19.6% eosinophils (Fig. 1A). The blasts had few Azurophilic granules (Fig. 1B) and were stained positively with peroxidase stain (Fig. 1C) but not with α-naphthyl butyrate esterase nor naphthol AS-D chloroacetate esterase stains (Fig. 1D). The eosinophils were reactive to periodic acid-Schiff (Fig. 1E), in contrast to normal eosinophils (6). Immunophenotypic analysis showed that the rates of CD13, 14, and 33 positive blasts in bone marrow were 82.6%, 7.0%, and 11.1%, respectively. In the chromosomal analysis of trypsin-Giemsa-banded metaphase of marrow cells, all 12 metaphase cells investigated revealed 46, XY,
Figure 1. Morphological and cytochemical examinations of bone marrow at initial diagnosis. Wright-Giemsa stain smear showed hypercellular marrow replaced totally by leukemic blasts and eosinophilia (A; x400) and revealed leukemic blasts with few Azurophilic granules (B; x1,000). Leukemic blasts were stained positively with peroxidase stain (C) but not with α-naphthyl butylate esterase nor naphthol AS-D chloroacetate esterase stain (D). The eosinophils were reactive to periodic acid-Schiff (E).

del(16)(q22), as shown in Fig. 2. These findings led to the diagnosis of AML (M1) (6), and immediate remission induction therapy was started with a cytosine arabinoside- and daunorubicin-containing regimen. On day 30 the peripheral blood and marrow examination showed complete remission with a normal karyotype.
CBFB/MYH11 Fusion in AML with Del(16)

Bone marrow and peripheral blood samples were taken at the initial presentation and RNA was extracted following the standard protocol. RT-PCR was conducted with primers designed from the middle of the CBFB coding sequence and the 3' region of MYH11, as described by Liu et al (2); the sequences of the primers were the following: the sense primer, GCAGGCAAGGTATATTTGAGG; antisense primer, CTCTTCTCATTTCATC. In bone marrow and peripheral blood samples approximately 1.1 kb PCR-products were generated, as shown in Fig. 3. When the sample was taken during complete remission, the PCR-product was not generated. For further analyses, this 1.1 kb band of the PCR-product was excised from the gel, extracted, and sequenced using PCR primers as sequence primers, as described by Claxton et al (4). The obtained nucleotide sequence showed in-frame fusion of 5' CBFB at position 495 to 3' MYH11 at position 1201 (Fig. 4). This chimeric cDNA was corresponded to type c in the study of Claxton et al (4).

Discussion

Acute myelomonocytic leukemia with bone marrow eosinophilia is well known to be related to chromosome 16 abnormalities including inv(16), t(16;16), and del(16) (1, 5, 7). In these three rearrangements, the breakpoints on the q arms are identical, at q22 (8). In inv(16)(p13q22) and t(16;16)(p13;q22) juxtaposition of 5'CBFB on the q arm with 3'MYH11 on the p arm was found to result from joining of 16q proximal to the q22 breakpoint to 16p distal to the p13 breakpoint (4). Of these three related chromosome 16 rearrangements, only del(16) has not been studied at the molecular level. Liu et al mentioned one patient with del(16)(q22) cytogenetically having the CBGB-MYH11 fusion mRNA as unpublished results in the review article (8), but they did not show any details about it. We report here an AML case with del(16)(q22) rearrangement, and to our knowledge, this is the first report in that del(16) was studied at molecular level.

In the case presented here, although the karyotype showed deletion of 16q distal to q22 from which simple loss of 3' portion of CBFB was expected, the RT-PCR and sequencing results showed the fusion formation of 5' portion of CBFB and 3' portion of MYH11. In Philadelphia chromosome-negative/bcr-abl-positive chronic myelogenous leukemia, it is hypothe-
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esized that two-translocation model in which a second translo-
cation reconstitutes a standard t(9;22)(q34;q11) but leaves the
chromosome 9 insert, including 3' abl, on chromosome 22 (9).
It is possible that an analogous event occurs in the case pre-
sented here, but this possibility has yet to be elucidated. Another
possibility is that this case may actually be inv(16) or t(16;16)
misdiagnosed as del(16), due to technical difficulties (10, 11).
The other possibility is that this case is inv(16) associated with
loss of the distal q arm, removing the 3' end of CBFB gene.
These possibilities would be examined with FISH analysis
using both chromosome- and band-specific probes. However,
in the presented case, FISH analysis could not be done because
the patient already entered into complete remission with no
viable leukemic blasts after cytogenetic results were available.

Sequencing showed in-frame fusion of 5' CBFB at position
495 to 3' MYH11 at position 1201 in the present case. Eight
types of fusion transcripts have been identified to date (4, 8, 12,
13). In 84 of 103 patients in whom the fusion transcripts were
detected, the fusion breakpoints were at position 495 in the
CBFB gene and at position 1921 in the MYH11 gene, and 7
patients showed the fusion type identical to that in the present
case. The case with AML M1 and M2 with bone marrow
eosinophilia (designated as M1e and M2e) were reported to
show inv(16) and another breakpoint in the CBFB gene, at
position 399 (retaining 133 N-terminal amino acids compared
with 165 for the common breakpoint) (8), in contrast to the
present case who had also M1e. Further study with more
patients is necessary for clarifying correlations between the
types of fusion transcripts generated and AML subtypes.

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