Masked inv(3)(q21q26) in a patient with minimally differentiated acute myeloid leukemia

Chromosome banding and multicolor fluorescence in situ hybridization (FISH) demonstrated monosomy 7 and a novel ins(6;3)(q23;q21q26) in a patient with minimally differentiated acute myeloid leukemia and thrombocytosis. FISH with locus-specific probes revealed the additional presence of a prognostically unfavorable inv(3) (q21q26) within the segment inserted into chromosome 6.

The inversion inv(3)(q21q26) and the translocation t(3;3)(q21;q26) are found in a small percentage of patients with acute myeloid leukemia (AML), myelodysplastic syndromes, and chronic myeloid leukemia. They are associated with normal or elevated platelet counts, micromegakaryocytes, and a particularly poor prognosis of the disease.[1-3] Here, we describe a patient with minimally differentiated AML and thrombocytosis, in whom a masked inv(3)(q21q26) was disclosed by FISH with locus-specific probes. A 37-year old woman with a six-month history of essential thrombocythemia and failure of hydroxyurea and α-interferon treatment presented with sore throat, oral candidiasis, bleeding from the gums, and hematomas. The peripheral blood showed normal leukocyte counts (9.5×10^9 leukocytes/L) with 70% blast cells and excessive thrombocytosis (903×10^9 platelets/L). Bone marrow aspirate demonstrated 50% blasts, dysplastic granulopoiesis with pseudo-Pelger cells, and hyperplastic micromegakaryocytes. The blasts stained negative for myeloperoxidase and unspecific esterase. Immunophenotyping revealed positivity for HLA-DR, CD117, CD34, CD33, and CD13.

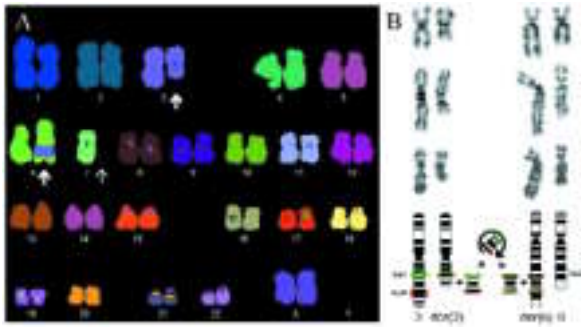

Figure 1. A) Multicolor FISH karyogram of the leukemic blasts demonstrating monosomy 7 (arrow) and an insertion of chromosome 3 material into the long arm of a chromosome 6 (bold arrows). B) Upper three rows, three partial G-banded karyograms showing the derivative chromosomes of an insertion ins(6;3)(q23;q21q26). Bottom row, schematic representation of the ins(6;3) with an inversion inv(3)(q21q26) within the inserted segment, as revealed by FISH with locus-specific probes. The chromosomal breakpoint regions 3q21 and 3q26 are depicted by green and red bars, respectively. Both regions are rearranged twice, by the ins(6;3) (arrows) and by the inv(3) (circular arrow).

A diagnosis of minimally differentiated acute myeloid leukemia, FAB M0, with thrombocytosis was made. The patient was treated according to the AML SHG protocol with one cycle of mitoxanthrone, cytosine arabinoside, and etoposide, followed by a second cycle with amsacrine and high-dose cytosine arabinoside.

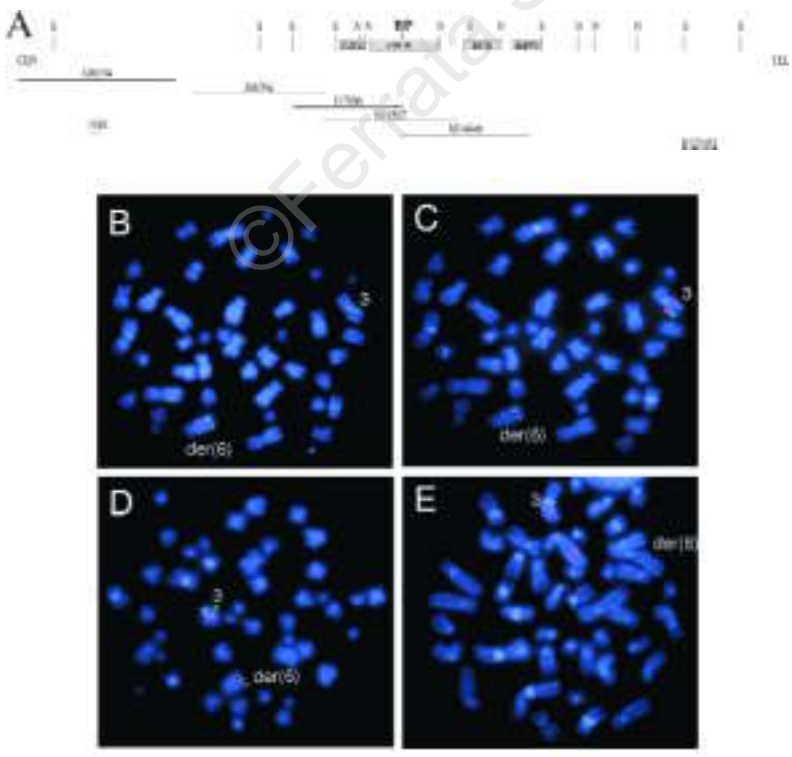

Figure 2. FISH on metaphase chromosomes with PAC clones derived from chromosome band 3q21 and YAC clones located in 3q26 confirms insertion of segment 3q21q26 into the long arm of chromosome 6 and uncovers a masked inversion inv(3)(q21q26) within the inserted segment.
A) Position of the PAC clones used for FISH analysis. BCR, originally proposed 3q21 breakpoint cluster region; cBCR, recently described, more centromeric breakpoint cluster region; BP, approximate position of the 3q21 breakpoint in the present case. GATA2, GATA-2 gene; RPN, ribophorin I gene.
Cen, centromere, tel, telomere. N, NotI restriction site; S, SalI restriction site.
B) Dual color FISH with PACs A08194 / J06704 (3q21; both labeled red for enhanced signal intensity) and PAC B121052 (3q21; green). Both signals co-localize on the normal chromosome 3. They are also found on the derivative chromosome 6, der(6), where their separation and specific locations indicate the presence of a masked inv(3)(q21q26).
C) Dual color FISH with PAC M14646 (3q21; red) and YAC 858C9 (3q26; green). On the der(6), the 3q21 PAC probe is juxtaposed to the 3q26 YAC.
D) Dual color FISH with PAC N19507 (3q21; red) and YAC 14EE12 (3q26; green). On the der(6), the signal of PAC N19507 is split, with one part remaining near the insertion breakpoint in 3q21, and the other juxtaposed to YAC 14EE12 in 3q26.
E) Single color FISH with PAC I17506 (3q21; red), whose location on the der(6) was not affected by the inv(3).

A bone marrow aspirate taken two weeks after completion of the second cycle revealed progression of the leukemia with 70-80% blasts. Allogeneic peripheral blood stem cell transplantation (PBSCT) with G-CSF mobilized unselected peripheral blood cells from an HLA identical brother was performed. A control bone marrow aspirate on day +34 after PBSCT revealed cytologic remission with <5% blasts. On day +63, the patient relapsed.

G-banding analysis of bone marrow and peripheral blood cells at diagnosis revealed monosomy 7, loss of segment 3q21q26 of a chromosome 3, and an elongation of the long arm of a chromosome 6 in all 25 metaphases analyzed. Multicolor fluorescence in situ hybridization (M-FISH) using a commercially available probe set (24×Cyte, MetaSystems, Altlussheim, Germany) and a digital image analysis system (ISIS, MetaSystems) confirmed loss of an entire chromosome 7 and showed insertion of a part of chromosome 3 into the long arm of a chromosome 6 in 5/5 metaphases. Taken together, these data suggested that segment 3q21q26 was inserted into chromosome band 6q23 (Figure 1). FISH with probes covering the genomic regions usually involved in inv(3)(q21q26) and t(3;3)(q21;q26) rearrangements was performed: several overlapping PAC clones were used to characterize the 3q21 breakpoint (Figure 2A),[4] and YAC clones 14EE12[5] and 858C9 (CEPH), which both contain the EVI-1 gene, were employed to define the 3q26 rearrangement. All of these probes hybridized to the derivative chromosome 6 (in addition to the normal chromosome 3), confirming insertion of segment 3q21q26 into chromosome 6 (Figure 2B-E). Furthermore, separation of the signals of PACs A08194/J06704 and B121052 (Figure 2B), and juxtaposition of PAC M14646 to YAC 858C9 in 3q26 (Figure 2C) indicated an inversion inv(3)(q21q26) within the inserted segment. PAC N19507 was split by the inversion breakpoint, with part of the signal remaining in 3q21 and the other part co-localizing with YAC 14EE12 in 3q26 (Figure 2D). PAC I1750, on the other hand, was not inverted (Figure 2E). In summary, the hybridization patterns suggested that the 3q21 breakpoint of the inv(3) mapped 40-60kb from the centromeric end of an originally proposed, 30 kb breakpoint cluster region (BCR),[6] in a recently described centromeric BCR.[4,7] Neither of the 3q26 YAC clones was inverted (Figure 2C,E), indicating that the inv(3) breakpoint in 3q26 was located proximal to these YACs, thus extending even further the already large genomic region over which 3q26 breakpoints have been reported to be distributed.[5,8]

Insertions of 3q21q26 into various other chromosomes have been reported in 6 cases.[9] Juxtaposition of 3q21 and 3q26 on the 3q- chromosome, as well as rearrangements of 3q21 or 3q26 with regions of the insertion acceptor chromosome have been proposed to lead to pathogenetically relevant molecular genetic alterations.[10] However, our findings suggest that the possibility of masked inversions inv(3)(q21q26) within the inserted segments should also be taken into consideration, and could represent the disease-relevant molecular event at least in some cases.

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