Double Inv(3)(q21q26), a Rare but Recurrent Chromosomal Abnormality in Myeloid Hemopathies

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Abstract. Inv(3)(q21q26)/t(3;3)(q21;q26) is a feature of a distinctive entity of acute myeloid leukemia (AML) associated with normal or elevated platelet count, atypical megakaryocytes and multilineage dysplasia in bone marrow, as well as minimal to no response to chemotherapy and poor clinical outcome. The presence of an inversion on both chromosome 3s is a rare event, as only eight cases have been reported in the literature. Recently, we identified two patients with AML carrying a double inv(3)(q21q26). Using libraries of bacterial artificial chromosome clones mapping to bands 3q21 and 3q26, we found that the regions in which the breakpoints occurred were different in both patients, but located in the same restricted areas in each patient. Although it cannot be excluded that inversion occurred independently on both chromosome 3s, it is more likely that the presence of a double inv(3) is the result of loss of the normal chromosome 3 followed by a duplication of the inverted chromosome, or segmental loss of heterozygosity followed by a somatic repair mechanism.

The revised 2008 WHO classification of tumors of hematopoietic and lymphoid tissues recognized acute myeloid leukemia (AML) with inv(3)(q21q26)/t(3;3)(q21;q26) as a distinctive entity of AML with recurrent genetic abnormalities of prognostic significance (1). Patients with inv(3)/t(3;3) frequently demonstrate normal or elevated platelet count, atypical megakaryocytes and multilineage dysplasia in bone marrow as well as minimal to no response to chemotherapy and poor clinical outcome (2-4). Inv(3) and t(3;3) occur in 1-2.5% of all FAB types of AML, except M3 (5, 6). They are also observed in myelodysplastic syndromes (MDS) (7) and in the blastic phase of chronic myeloid leukemia (CML) (8).

The molecular consequence of the inv(3)/t(3;3) rearrangements is the juxtaposition of the RPN1 gene (ribophosphorin 1 gene located in band 3q21) with the EVI1 gene (ecotropic viral integration site-1 gene located in band 3q26.2) (9). Two alternative forms exist, one generated from EVI1, the other MECOM (MDS1 and EVI1 complex locus) through intergenic splicing with MDS1 (myelodysplasia syndrome 1), a gene located 140 kb upstream of EVI1 (10). In contrast to most of the inversions and translocations associated with AML that lead to fusion genes, the inv(3)/t(3;3) does not generate a chimeric gene, but rather induces gene overexpression (11, 12).

Recently, two patients with acute myeloid leukemia were found to have a double inv(3)(q21q26). They are the subject of this report.

Case Report

From 1998 to 2012, 16 patients referred to the Cytogenetics Laboratory of the Brest University Hospital, were found to have an inv(3)(q21q26) (13). Among them, two patients had a double inv(3).

Patient 1, a 62-year-old male, was first seen in August 2011 for abundant bleeding of hemorrhoids and palpitations. At admission, hematological data were as follows: hemoglobin 9.5 g/dl, white blood cells (WBC) 1.2×10⁹/l with 42% neutrophils and 14% blasts, and platelets 109×10⁹/l. The bone marrow aspirate showed normal cellularity, with 61%...
blasts. Immunophenotyping showed the blast cells to be CD33-positive. A diagnosis of AML-M1 (French–American–British classification) was made. The induction therapy included cytarabine and idarubicin, which led to complete remission. Consolidation and maintenance therapy were continued until February 2012 when relapse occurred. The patient died in May 2012.

Patient 2, a 67-year-old female, was seen in May 2012 for abdominal pain. At admission, hematological data were as follows: hemoglobin 7.2 g/dl, WBC 4.1×10⁹/l with 41% neutrophils and 35% blasts, and platelets 257×10⁹/l. The bone marrow aspirate was consistent with AML with multilineage dysplasia (dysgranulopoiesis and dysmegakaryocytopenia). As no remission could be achieved under induction therapy, solely palliative care was given. The patient died four months following diagnosis.

**Conventional cytogenetics.** Conventional cytogenetic analysis was performed on bone marrow cells at the time of diagnosis and relapse. Briefly, a 24-h unstimulated bone marrow culture was synchronized with fluorodesoxyuridine (10⁻⁷ M) for 17 h followed by thymidine (10⁻⁵ M) for 6 h before colcemide exposure and standard harvesting. R-Banding chromosomal analysis was performed according to standard procedures and the karyotypes were described according to the International System for Cytogenetic Nomenclature (ISCN 2009) (14).

**Fluorescent in situ hybridization.** A fluorescent in situ hybridization (FISH) study using the Cytocell Aquarius EVII Breakapart probe (AmpliTech, Compiegn, France) was carried out on metaphase preparations from both patients, as recommended by the manufacturer. The EVII Breakapart probe contains three probes: a probe (encompassing D3S3364/D3S1614) labeled in Aqua of 562 kb in size centromeric to the ecotropic viral integration site 1 (EVII) gene, a probe (encompassing D3S1282) labeled in Spectrum Green of 181 kb covering EVII and its flanking regions and a probe (encompassing D3S3523) labeled in Spectrum Orange of 124 kb telomeric to the EVII gene [telomorphic of myoneurin (MYNN) and covering leucine rich repeat containing 34 (LRRC34)].

In order to more precisely determine the breakpoints involved in the inversion, FISH using a library of bacterial artificial chromosome (BAC) clones mapping to bands 3q21 and 3q26 was performed, as described previously (13). The base pair positions (bp) of the BAC clones were involved in the inversion, FISH using a library of bacterial containing 34 (LRRC34), MYNN myoneurin (52128203529 and 128255217, in patients 1 and 2, respectively. In band 3q21, the breaks occurred at a 22-kb interval, between base pair positions 168565551 and 168577352, in patients 1 and 2, respectively. In band 3q21, the breaks occurred at a 22-kb interval, between base pair positions 128233583 and 128255193, and at a 52-kb interval, between 128203529 and 128255217, in patients 1 and 2, respectively.

**Discussion**

Paracentric inversion of the long arm occurring on both chromosome 3s appears to be a rare event. Indeed, among the 394 patients with an inv(3)(q21q26) registered in the Mitelman database (http://cgap.nci.nih.gov/Chromosomes/Mitelman, last accessed October 2012), only eight had a double inv(3) (Table I). Unfortunately, no or few hematological and/or clinical data was available on most of the patients.

Different mechanisms could explain the presence of a double inv(3). Inversion could have occurred independently on both chromosomes. However, this is quite unlikely as in
both patients reported here, the breaks in 3q21 and 3q26 took place in the same restricted genomic region on both homologs. Loss of the normal chromosome 3 followed by duplication of the inverted chromosome appears to be more likely. Therefore, duplication would be a secondary event, as suggested by the karyotype of patient 2, in whom three abnormal clones were observed, two with a sole inv(3) and one with a double inv(3).

Based on a high-resolution single-nucleotide polymorphism (SNP) array analysis in a patient with a double inv(3), Toydemir et al. found a segmental loss of heterozygosity starting from 3q13.2 to the telomere of the long arm. They suggested that a somatic repair mechanism took place, leading to homozygosity for inv(3) (15). Unfortunately, no DNA was available from our patients to test these hypotheses.

Other chromosomal aberrations, such as monosomy of chromosome 7, 7q deletion and 5q deletion, have been associated with inv(3) (13). Five out of the 10 patients so far reported with a double inv(3), also had monosomy of chromosome 7 or 7q deletion and one had a deletion of the long arm of chromosome 5 (Table I).

Inv(3)(q21q26) is frequently associated with minimal to no response to chemotherapy and poor clinical outcome (2-4). Although the data available on remission and survival is incomplete for the patients with double inv(3) reported in the literature, the presence of two inv(3) also carries a poor prognosis, as shown by both patients reported here.

In conclusion, although rare, double inv(3)(q21q26) is a recurrent abnormality in myeloid hemopathies. The presence of a second copy of inv(3) appears to be a secondary event that carries a poor prognosis. Data regarding more patients need to be reported in unselected series to determine the prevalence of this abnormality and to better define the biological and clinical characteristics of these patients.

References


