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 the 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 librairies 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) 1.2×10^9/l with 42% neutrophils and 14% blasts, and platelets 109×10^9/l. The bone marrow aspirate showed normal cellularity, with 61%

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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 4.1×10^9/l with 41% neutrophils and 35% blasts, and platelets 257×10^9/l. The bone marrow aspirate was consistent with AML with multilineage dysplasia (dysgranulopoiesis and dysmegakaryocytopoiesis). 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 *EVI1* Breakapart probe (AmpliTech, Compiegne, France) was carried out on metaphase preparations from both patients, as recommended by the manufacturer. The *EVI1* 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 (*EVI1*) gene, a probe (encompassing D3S1282) labeled in Spectrum Green of 181 kb covering EVI1 and its flanking regions and a probe (encompassing D3S3523) labeled in Spectrum Orange of 124 kb telomeric to the *EVI1* gene [telomeric 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 3q21and 3q26 was performed, as described previously (13). The base pair positions (bp) of the BAC clones were predicted on Build 39 National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) and assembly February 2009 by the UCSC Genome Browser Database (http://genome.ucsc.edu/index.html).

Results of FISH. For patient 1, at diagnosis, R-banding showed an inversion of the long arm on both chromosome 3s and a monosomy of chromosome 7 in the majority of cells examined; the karyotype was written as 45,XY,inv(3)(q21q26)x2,-7[20]/46,XY[2]. A normal 46,XY karyotype was found at remission in November 2011 (25 metaphases were analyzed). The same abnormal clone was identified at relapse in February 2012. At diagnosis, metaphase FISH with the *EVI1* Breakapart probe was compatible with a break having occurred between D3S3364/D3S1614 and D3S1282/D3S3523 on both chromosome 3s, ish inv(3)(q21) (D3S3364/D3S1614+)(q26)(D3S1282+,D3S3523+)x2. In December 2011, while the patient was in remission and had a normal karyotype in banding cytogenetics, interphase FISH showed 8% of the cells to have a double inv(3).

For patient 2, at diagnosis, R-banding identified three abnormal clones, one clone showing an inversion on one chromosome 3 and another clone an inversion on both chromosome 3s. Monosomy of chromosome 7 was found in two clones. The karyotype was written as 46,XX,inv(3)(q21q26),add(5)(q1?3)[3]/45,idem,-7[7]/45,idem,inv(3)(q21q26),-7[12]. Interphase FISH with the EVI1 Breakapart probe showed two populations with inv(3), one with a sole inv(3), the other with two inv(3), nuc ish(EVI1x2)(D3S3364sepD3S1282/D3S3523)[29/100],(EVI1x2)(D3S3364sepD3S1282/D3S3523x2)[23/100].

For each patient, sequential analyses with BAC clones showed the breakpoint in band q26 on both chromosome 3s to have occurred in a region centromeric of the *MDS1* and *EVI1* complex locus (*MECOM*), which was compatible with the results obtained with the Cytocell Aquarius EVI1 Breakapart probe.

Although the area in which the breakpoints on 3q21 and 3q26 occurred was different in both patients, those were located in the same restricted regions in each patient. The breakpoints in 3q26 took place at a 58-kb interval, between base pair positions 168704881 and 168762910, and at a 12-kb interval, between 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

Table I. *Cytogenetic results of patients with double inv(3)(q21q26) reported in the literature and in the present study.*

Gender	Age (years)	Diagnosis	Karyotype	Reference
F	55	MDS	46,XX,inv(3)(q21q26)x2	(16)
F	NA	BP-CML	46,XX,inv(3)(q21q26)x2,t(9;17;22)	(9)
M	80	AML-M1	46,XY,inv(3)(q21q26)x2	(17)
M	39	AML-M4	45,XY,inv(3)(q21q26)x2,-7	(17)
F	83	AML	45,XX,inv(3)(q21q26)x2,-7/46,XX	(18)
M	65	AML-M4	46,XY,inv(3)(q21q26)x2[30]	(19)
M	36	CML *	46,XY,inv(3)(q21q26)x2,del(7)(q22q34)[20]	(15)
F	NA	AML	47,XX,inv(3)(q21q26),+inv(3)(q21q26)	(6)
M	62	AML-M1	45,XY,inv(3)(q21q26)x2,-7[20]/46,XY[2]	Patient 1
F	67	AML	46,XX,inv(3)(q21q26),add(5)(q1?3)[3]/45,idem,-7[7]/ 45,idem,inv(3)(q21q26),-7[12]	Patient 2

MDS: Myelodysplastic syndrome; BP: blastic phase; CML: chronic myeloid leukemia; AML: acute myeloid leukemia; NA: not available. *Cryptic *BCR-ABL1* rearrangement.

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

1 Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, Harris NL, Le Beau MM, Hellstrom-Lindberg E, Tefferi A and Bloomfield CD: The 2008 revision of the World Health Organization (WHO) Classification of Myeloid Neoplasms and Acute Leukemia: Rationale and important changes. Blood *114*: 937-951, 2009.

2 Bitter MA, Neilly ME, Le Beau MM, Pearson MG and Rowley JD: Rearrangements of chromosome 3 involving bands 3q21 and 3q26 are associated with normal or elevated platelet counts in acute nonlymphocytic leukemia. Blood *66*: 1362-1370, 1985.

3 Fonatsch C, Gudat H, Lengfelder E, Wandt H, Silling-Engelhardt G, Ludwig WD, Thiel E, Freund M, Bodenstein H and Schwieder G: Correlation of cytogenetic findings with clinical features in 18 patients with inv(3)(q21q26) or t(3;3)(q21;q26). Leukemia *8*: 1318-1326, 1994.

4 Horsman DE, Gascoyne RD and Barnett MJ: Acute leukemia with structural rearrangements of chromosome 3. Leuk Lymphoma *16*: 369-377, 1995.

5 Wieser R: Rearrangements of chromosome band 3q21 in myeloid leukemia. Leuk Lymphoma *43*: 59-65, 2002.

6 Lugthart S, Groschel S, Beverloo HB, Kayser S, Valk PJ, Zelderen-Bhola SL, Jan OG, Vellenga E, van den Berg-de Ruiter, Schanz U, Verhoef G, Vandenberghe P, Ferrant A, Kohne CH, Pfreundschuh M, Horst HA, Koller E, Lilienfeld-Toal M, Bentz M, Ganser A, Schlegelberger B, Jotterand M, Krauter J, Pabst T, Theobald M, Schlenk RF, Delwel R, Dohner K, Lowenberg B and Dohner H: Clinical, molecular, and prognostic significance of WHO type inv(3)(q21q26.2)/t(3;3)(q21;q26.2) and various other 3q abnormalities in acute myeloid leukemia. J Clin Oncol *28*: 3890-3898, 2010.

7 Rubin CM, Larson RA, Anastasi J, Winter JN, Thangavelu M, Vardiman JW, Rowley JD and Le Beau MM: t(3;21)(q26;q22): A recurring chromosomal abnormality in therapy-related myelodysplastic syndrome and acute myeloid leukemia. Blood *76*: 2594-2598, 1990.

8 Bernstein R, Bagg A, Pinto M, Lewis D and Mendelow B: Chromosome 3q21 abnormalities associated with hyperactive thrombopoiesis in acute blastic transformation of chronic myeloid leukemia. Blood *68*: 652-657, 1986.

9 Levy ER, Parganas E, Morishita K, Fichelson S, James L, Oscier D, Gisselbrecht S, Ihle JN and Buckle VJ: DNA rearrangements proximal to the *EVI1* locus associated with the 3q21q26 syndrome. Blood *83*: 1348-1354, 1994.

10 Fears S, Mathieu C, Zeleznik-Le N, Huang S, Rowley JD and Nucifora G: Intergenic splicing of *MDS1* and *EVI1* occurs in normal tissues, as well as in myeloid leukemia, and produces a new member of the PR domain family. Proc Natl Acad Sci USA *93*: 1642-1647, 1996.

11 Suzukawa K, Parganas E, Gajjar A, Abe T, Takahashi S, Tani K, Asano S, Asou H, Kamada N, Yokota J, Morishita K and lhle JN: Identification of a breakpoint cluster region 3' of the ribophorin I gene at 3q21 associated with the transcriptional activation of the *EVI1* gene in acute myelogenous leukemias with inv(3)(q21q26). Blood *84*: 2681-2688, 1994.

12 Wieser R, Volz A, Vinatzer U, Gardiner K, Jager U, Mitterbauer M, Ziegler A and Fonatsch C: Transcription factor *GATA-2* gene is located near 3q21 breakpoints in myeloid leukemia. Biochem Biophys Res Commun *273*: 239-245, 2000.

13 De Braekeleer E, Douet-Guilbert N, Basinko A, Bovo C, Gueganic N, Le Bris MJ, Morel F and De Braekeleer M: Conventional cytogenetics and breakpoint distribution by fluorescent *in situ* hybridization in patients with malignant hemopathies associated with inv(3)(q21;q26) and t(3;3)(q21;q26). Anticancer Res *31*: 3441-3448, 2011.

14 ISCN (2009): An International System for Human Cytogenetic Nomenclature. Basel, S. Karger, 2009.

15 Toydemir R, Rowe L, Hibbard M, Salama M and Shetty S: Cytogenetic and molecular characterization of double inversion 3 associated with a cryptic *BCR-ABL1* rearrangement and additional genetic changes. Cancer Genet Cytogenet *201*: 81-87, 2010.

16 Walter TA, Morgan R, Ondreyco S and Sandberg AA: Apparent duplication of inv(3)(q21q26) in one of five cases with inv (3) in myelodysplastic syndromes and acute leukemia. Am J Hematol *33*: 210-214, 1990.

17 Secker-Walker LM, Mehta A, Bain B and on behalf of UKCCG: Abnormalities of 3q21 and 3q26 in myeloid malignancy: A United Kingdom Cancer Cytogenetic Group study. Br J Haematol *91*: 490-501, 1995.

18 Lee CL: Double inversion (3)(q21q26) and monosomy 7 in a case of acute myeloid leukemia. Cancer Genet Cytogenet *111*: 99-101, 1999.

19 Lahortiga I, Vazquez I, Agirre X, Larrayoz MJ, Vizmanos JL, Gozzetti A, Calasanz MJ and Odero MD: Molecular heterogeneity in AML/MDS patients with 3q21q26 rearrangements. Genes Chrom Cancer *40*: 179-189, 2004.

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