Inverted ins(4;11) in a patient with Essential Thrombocythemia with Progression to Myelofibrosis

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Abstract:

We report a case of essencial thrombocythemia in a 39-year-old female patient who developed myelofibrosis at follow-up. The cytogenetic and FISH analysis revealed a pathologic clone with the following previously unreported karyotype: 46,XX,ins (4;11)(q27;q25q21). We can not conclude that the presence of this inverted insertion is associated with the overproduction of platelets but the documentation of more cases of chromosome abnormalities and ET would lead to identify different candidate genes involved in the neoplastic growth.

INTRODUCTION

Essential thrombocythemia (ET) is a chronic myeloproliferative disorder characterized by persistent thrombocytosis and an increased tendency to thrombosis and hemorrahage [1]. The transition to myelofibrosis with myeloid metaplasia, polycythemia vera and more rarely, acute leukemia is observed in some cases [1]. With regard to the myelofibrotic transformation of ET, its incidence ranges from 5% to 24% [1,2,3]. Dysmegakaryopoyesis with the subsequent production of platelets-derivated growth factor and other fibroblast-stimulating cytokines, is the factor most likely to be involved in the pathogenesis of post-ET myelofibrosis [4]. To date, karyotypic abnormalities have been described in less than 6% of cases [5] and there is no specific chromosomal abnormality associated with ET [6]. Insertions are chromosomal rearrangements characterized by a triple break, and have been rarely described in patients with hematologic neoplasias [7,8,9,10].

We describe and discuss the occurrence of an inverted insertion involving chromosomes 4 and 11, found in a routine cytogenetic study on bone marrow cells in a patient with ET who progressed to myelofibrosis. The diagnosis in these cases is not easy to establish since bone marrow fibrosis in ET may resemble the early phase of agnogenic myeloid metaplasia with myelofibrosis. A clear distinction between these two myeloproliferative disorders may some times be possible after a long follow-up [11].

CASE REPORT

In December 1991, a 39 year-old female patient had generalized hematomas and hemorrhagic blisters in the mouth with a platelet count of $1350x10^9$/L. Bone marrow (BM) biopsy showed megakaryocytic hyperplasia, normal iron stores and focal reticulin fibrosis. The cytogenetic analysis showed a chromosomal rearrangement between chromosome (chr) 4 and 11 and the BCR/ABL rearrangement was negative. A diagnosis of ET was made. In February 1992, treatment with hydroxyurea was started, which reduced the platelet count to $500x10^9$/L and resolved the hemorrhagic manifestations. However, two years later the platelet count were over $1000x10^9$/L and she presented dysesthesias in hands and feet. In August 1995 analegride treatment was started. Platelets returned to normal values with disappearance of clinical symptoms. Five months later she developed anemia with erythroblasts, anysocytosis and teardrop cells in peripheral blood smears. Ferritin, vitamin B12, folate, LDH, haptoglobin and plasmatic free hemoglobin were normal. Hemoglobin electrophoresis revealed an increased fetal hemoglobin (FHb) with normal A2Hb. In September 1996, 1% blasts were found in peripheral blood, a dry tap was obtained and the BM biopsy showed increased areas of focal fibrosis. Since August 1998 she had progressive leukocytosis, with blast up to 6%. Splenomegaly was found by ultrasonography. Peripheral mononuclear cells inmunophenotyping revealed 37% CD34+ cells with low expression of CD33, HLA-DR and CD45. Besides, CD34+ cells co-expressed CD61 and CD42a (14% and 10% respectively). At this time a new cytogenetic study revealed an ins(4;11)(q27;q25q21). In 1999, the BM biopsy showed trilineage hypercellularity, increased reticulin myelofibrosis with osteosclerosis, a diagnosis of post-ET myelofibrosis was made.

Cytogenetic studies:

Chromosome analysis was made on heparinized BM cells after short-term without stimulation cultures using standard techniques. The fixed suspension was stored at –20ºC and used for preparation of FISH slides. Chromosomes were identified by G banding technique and the karyotype classified according to the ISCN [12]. Cytogenetic studies on BM showed a der(4) with extra material from chr11 found in 80% of the cells (Fig1a). The 4 normal cells exclude a constitutional translocation, therefore, we consider the cytogenetic result represents a clonal abnormality in association with ET.

Fluorescence *in situ* hybridisation (FISH) studies:

In situ hybridisation was carried out on cytogenetic preparation from BM. The slides were pre-treated as previously described [13].

Probes: Whole-chromosome painting (wcp) probes specific to chr11 (Oncor, Gaithersburg, MD) and chr4 (Cambio, Cambridge, UK) were hybridised on the same slide according to the manufacturer's instructions.

In addition, the following clones provided by A. Hagemeijer (Center for Human Genetics, Catholic University of Leuven, Belgium) were tested: c11q-7d4 (11q14.1–14.3) [14], c11q-2c4 (11q21–22.1)[14], cosmid3.16A (11q21) [15], PAC166G16 (11q21) [16], YAC755B11 (11q22.1) [17], Cosmid19.21 (11q23.2) [18], PAC9015 (11q subtelomeric probe) [19]. Probes were biotin or digoxigenin-labeled by nick translation (Boehringer Mannheim,Germany). For these probes, 30 ng of each DNA clones was combined with 1.5 μg human Cot-1 DNA in 5μl of hybridisation mixture (50% formamide/2 x SSC). The mixtures were denatured at 75ºC for 5 min, cooled on ice and pre-annealed at 37ºC for 30 min. The hybridisation was performed over night at 37ºC in a moist chamber.

The methods for double-colour inmunodetection of hybridisation signals have been previously described [13]**.** The DNA was counter-stained with DAPI and analysed on a Leitz DMRB fluorescence microscope equipped with a cooled CCD camera (Photometrics, Tuscon, AZ) run by Smart-capture software (VYSIS, Stuttgart, Germany).

Ten to fifteen chromosome spreads were analysed for each experiment.

Double color FISH experiment with 11 and 4 wcp probes showed a red band inserted into the green painted chr4, confirming the presence of an ins(4;11) (Fig1b).

The 11q inserted region was further characterized using a panel of biotin-labeled probes. FISH with the cos7d4 probe showed that the breakpoint occurred in a more distal band from 11q14.1-14.3 (Fig1c). Using more telomeric localised probes (2c4, 3.16A, 166G16, 755B11, 19.21), the hybridisation signals were on der(4), thus all this region was inserted into chr4 and the centromeric breakpoint occurred in 11q21 band (data not shown). When 11q sub telomeric probes (PAC9015) was hybridised, the signals were on the normal chr11 and on der(11), this signal pattern showed that the 11q 21-25 region was lost such as an interstitial deletion (Fig1d). Double color FISH with 166G16 biotin and 19.21 digoxigenin labeled probes result in green-red centromeric-telomeric ordered pattern signal on the normal chr11, the der(4) showed the inverted order, demostrating that the 11q21-25 region was inserted in an inverted

orientation into chr.4 (Fig1f). Afterward, a set of six YACs biotin labeled were mapped on the long arm of chr4 and showed that the breakpoint on chr4 occurred in q27 cytogenetic band (data not shown).

With this FISH information, the new karyotype can be written as 46,XX, ins (4;11)(q27;q25q21).

DISCUSSION:

This is the first case of an ins(4;11)(q27;q25q21), found in a routine cytogenetic study in a patient with ET who developed myelofibrosis at follow-up. This patient may have ET with a progression to myelofibrosis, as it has been described [20,21]. But the reverse may also be proposed. Thrombocytosis could have been the first manifestation of myelofibrosis. We believe the existence of increased FHb values is in support of the former hypothesis [22]. This abnormality seems to be acquired based on the normal Hb electrophoresis found in her three brothers.

The inserted region was further characterized by FISH with previously mapped chr11 specific probes. These experiments demonstrated the centromeric breakpoint on 11q21and the inverted orientation with respect to the centromere of the inserted fragment 11q21-25 into chr.4 (Fig1f). The breakpoint on chr4 was mapped on q27 cytogenetic band (data not shown).

There are three genes known to map to 11q21-25 region that are likely to play some role in hematopoiesis. Two of these genes code for proteins of the Ets family. Ets consensus sequence is found in all characterized regulatory regions of megakaryocyte-specific genes [23]. The Ets-1 gene located on 11q23.3 [24] encodes a protein that is only expressed in hematopoietic tissues, might be involved in the expression of terminal megakaryocytic markers [23].

Fli-1 gene on 11q24.1-q24.3 [24] is the other Ets family member discovered in Ewing's sarcoma and peripheral neuroectodermal tumors. Recent experiments suggest that Fli-1 might play a prominent role in the regulation of lineage-specific genes during megakaryocytopoiesis [25,26]. Furthermore, promoters for the thrombopoietin receptor [27], von Willebrand factor [28] and GPIIb genes [26] have been shown to be transactivated by Fli-1.

Finally, the nuclear-factor-related-Kappa B (NFRKB) gene map to 11q24-25 [24], its protein is expressed preferentially in T and B cells and may have additional roles in hematopoietic proliferation and differentiation [29,30]. It is possible that the haploinsufficiency of one of these genes might lead to abnormal hematopoietic differentiation resulting in thrombocytopenia or pancytopenia as reported in patients with constitutional distal 11q deletions [31]. In our case, these genes are relocated to 4 chromosome and therefore, might be left under control of other regulatory sequences. In contrast, no genes involved in the hematopoiesis have been reported on 4q27. However, we can not rule out the hypothesis that the 11q24-25 inserted region might disrupt any gene with some role in the megakaryocytic regulation on 4 chromosome.

We can not postulate any relation between the inverted ins(4;11) and the myeloproliferative disorder with this single case. Nevertheless, the documentation of more cases of chromosome abnormalities and hematological neoplasia would lead to identify candidate genes involved in the neoplastic process.

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Fig1:

a: G-banded karyotype from bone marrow. Structural abnormalities are indicated by arrows.

b: Double color FISH result with 4 and 11 wcp probes showing a 11q inserted red band into green painted chr4.

c: Single color FISH result with c11q-7d4 probe showed the hybridisation signals located at the centromeric side of the 11q breakpoint.

d: FISH result with 11q sub telomeric probe (PAC9015) showing the interstitial deletion of the 11q 21-25 region.

e: Double color FISH with PAC166G16 biotin and cos19.21 digoxigenin labeled probes result in green-red centromeric-telomeric pattern signal order on the normal chr11 and inverted order on der(4), demostrating an inverted insertion of 11q21-25 region into chr.4. The centromere are indicated by the arrows.