A der(18)t(9;18)(p13;p11) and a der(9;18)(p10;q10) in polycythemia vera associated with a hyperproliferative phenotype in transformation to postpolycythemic myelofibrosis

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Abstract

Chromosomal aberrations in polycythemia vera (PV) are heterogenous and nonrandom. A prognostic predictive value of these aberrations has not been established. The V617F mutation in the JAK2 gene on chromosome 9p24.1 was identified recently in peripheral blood leukocytes in the majority of patients with PV and in approximately half of patients with essential thrombocytopenia and idiopathic myelofibrosis. Within the JAK2 V617F-positive PV patients, however, clinical presentation and degree of myeloproliferation varies to a great extent. Here we report four cases of chronic myeloproliferative disorders [two with PV, one with PV in transformation to idiopathic myelofibrosis (IMF) and one IMF patient], with the distinct karyotypic aberrations der(18)t(9;18)(p13;p11) and der(9;18)(p10;q10). Two patients had hyperproliferative PV and two had “transitional PV” and IMF, respectively. All four patients harbored the JAK2 V617F mutation. Our data, together with previously published data, clearly indicate an association of these chromosomal abnormalities with a highly proliferative PV phenotype with a propensity to transform into postpolycythemic myelofibrosis. Cytogenetic analysis seems to identify a subgroup of patients with a distinct prognostic profile, and should be performed in conjunction with a JAK2 mutation analysis in patients suspected of a chronic myeloproliferative disease. © 2007 Elsevier Inc. All rights reserved.

1. Introduction

A major breakthrough in the understanding of the molecular pathogenesis of Philadelphia chromosome—negative chronic myeloproliferative disorders has been achieved recently with the identification of the V617F mutation in the Janus Kinase 2 (JAK2) gene. The majority of patients with polycythemia vera (PV) (65–97%) and approximately half of the patients with essential thrombocytopenia (ET) and idiopathic myelofibrosis (IMF) harbor the mutation in their clonal hematopoietic cells [1–4]. The JAK2 gene is located on chromosome 9p24.1. Trisomy 9 and gains on 9p are known as some of the most frequent cytogenetic abnormalities together with trisomy 8 and del(20q) in both PV and IMF [5–9]. The spectrum of cytogenetic abnormalities in patients with PV is nevertheless heterogeneous, and a predictive prognostic value of these chromosomal aberrations has not yet been established in PV, contrasting their prognostic impact in IMF [10]. Six cases with the rare occurrence of an unbalanced translocation involving chromosome 9p and 18 have been reported in three different papers [11–13]. At least two cases presented clinically as PV transforming into postpolycythemic myelofibrosis, a condition sometimes referred to as a “transitional” state of PV [14].

We here report four new cases that showed an unbalanced translocation between chromosome arms 9p and 18. Two patients had a der(18)t(9;18)(p13;p11) and a clinical presentation of classic but highly proliferative PV, and two had a der(9;18)(p10;p10) with a clinical presentation as “transitional” PV and IMF, respectively. All four patients harbored the JAK2 V617F mutation.
2. Materials and methods

G-band karyotyping was performed as described earlier [15]. Briefly, at least 25 G-banded metaphases were karyotyped after short-term culture of unstimulated bone marrow cultures. Spectral karyotyping (SKY) was performed according to the manufacturer’s protocol (Applied Spectral Imaging, Mikdal HaEmek, Israel) [16]. At least 10 metaphases were analyzed. Interphase and metaphase fluorescence in situ hybridization (FISH) was carried out according to the manufacturer’s instructions, using CEP probes (Abbott Molecular/Vysis, Des Plaines, IL) to detect centromeric regions of chromosomes 9 and 18 in one hybridization. FISH was performed on chromosome spreads from diagnosis. Interphase FISH was analyzed by two independent observers and at least 2 × 100 nuclei were evaluated and compared to the findings in metaphases in the same preparation. Results were recorded as percentages of nuclei with a given FISH signal. DNA extracted from peripheral blood leukocytes after red cell lysis was used for the JAK2 V617F mutation analysis, which was done by allele-specific polymerase chain reaction with a three-primer design, as described by Baxter and co-workers [1].

3. Case reports

All four patients were diagnosed according to World Health Organization (WHO) criteria [17].

Patient 1, a 46-year-old female, presented with a hemoglobin (Hb) concentration of 17.9 g/dL (reference interval females: 11.3–16.1 g/dL) and a hematocrit (Hct) of 0.58 (females: 0.34–0.44). The platelet count was 498 × 10^9/L (120–400 × 10^9/L), and the leukocyte count was 10.4 × 10^9/L (3–10 × 10^9/L). A bone marrow biopsy displayed features characteristic of PV with panmyelosis, which included an increased number of large dysmorphic megakaryocytes and an increased reticulin network. The serum erythropoietin was suppressed at 1.9 IU/L (0–30 IU/L), and the weight-adjusted red cell mass was increased at 39 mL/kg (22–28 mL/kg). The patient had a transient ischemic attack (TIA) at the time of diagnosis. The spleen was not enlarged at the time of clinical examination. Since the time of diagnosis, the patient has been treated with phlebotomies and cytoreductive therapy with hydroxyurea. The follow-up time is 16 months.

Patient 2, a 55-year-old female, presented with Hb concentration of 15.6 g/dL and Hct of 0.49. The leukocyte count was 12.9 × 10^9/L, and the platelet count was initially 992 × 10^9/L. Upon clinical examination, the spleen was moderately enlarged and a large spontaneous muscular hematoma in the femoral region was found. The bone marrow findings were characteristic of PV, with pronounced hyperplasia and large dysmorphic megakaryocytes with clustering. No fibrosis was recorded. The patient has been treated with regular phlebotomies; the high platelet count was initially treated with hydroxyurea and anagrelide was added later on. Due to increasing platelet counts, the therapy was changed to sequential busulfan treatments. The patient has a history of TIA. The follow-up time is 137 months.

Patient 3, a 49-year-old male, presented with Hb concentration of 20.3 g/dL (reference intervals males: 12.9–16.9 g/dL) corresponding to Hct of 0.66 (males 0.41–0.51). The leukocyte count was 8.6 × 10^9/L, and the platelet count was 400 × 10^9/L. The spleen was slightly enlarged upon clinical examination. Bone marrow findings were characteristic of PV with trilineage hyperplasia. In particular, an increased number of large dysmorphic megakaryocytes was prominent. During the next four years, the disease became increasingly hyperproliferative, with a clinical phenotype equivalent to “transitional” PV. Accordingly, the patient displayed features of accelerated leuko- and thrombocytosis, increasing splenomegaly, and the appearance of immature myeloid cells in the peripheral blood, together with prominent constitutional symptoms and pruritus. The patient was treated with phlebotomy and required combination cytoreductive therapy with pegylated interferon and hydroxyurea. The patient had a TIA before diagnosis. The follow-up time is 87 months.

Patient 4, a 66-year-old male, presented with pronounced splenomegaly. His Hb concentration was 11.9 × 10^9/L g/dL. The leukocyte count was 18 × 10^9/L, and the platelet count was 280 × 10^9/L. The patient had a leukoerythroblastoid blood smear and teardrop erythrocytes. The bone marrow findings were characteristic of myelofibrosis, with hypercellularity, large dysmorphic megakaryocytes, and dense reticulin fibrosis. Severe constitutional symptoms and a pronounced splenomegaly necessitated treatment with hydroxyurea. The patient had suffered an acute myocardial infarction 23 years before diagnosis. The follow-up is 16 months.

The clinical, molecular, and cytogenetic data on patients in the present and previous reports are summarized in Table 1.

4. Results

The karyotype by chromosome band analysis showed an unbalanced translocation described as add(18)(p11) in all four cases (Fig. 1). SKY revealed that the additional material on chromosome 18 was originally from chromosome 9, and comparison of the G-banded karyotypes and SKY was interpreted as der(18)t(9;18)(p13;p11), leading to trisomy for 9p because all cases showed two normal chromosomes 9 (Fig. 2). Furthermore, FISH results with probes for the centromere regions of 9 and 18, respectively, in two patients showed two green (CEP9), 1 red (CEP18), and one yellow (CEP9 con CEP18) fusion spot, indicative of a der(9;18) (p10;q10), whereas the two other patients showed two green and two red spots, indicative of a der(18)t(9;18) (Fig. 3, a and b).
The four patients all harbored the JAK2 V617F mutation, which was identified by allele-specific polymerase chain reaction.

5. Discussion

The identification of the JAK2 V617F mutation in the majority of patients with PV, the fact that this mutation is sufficient to cause proliferation of hematopoietic precursor cells in the absence of erythropoietin in vitro, and the fact that it gives rise to a PV-like myeloproliferative disorder in murine models [2,18], have established its role as a primary pathogenetic mechanism in PV and a reliable molecular disease marker. Nevertheless, there are still questions to be answered. A minority of PV cases are apparently caused by a molecular mechanism different from the JAK2 V617F mutation that might involve other key elements in JAK-STAT signalling. Alternatively, the V617F

<table>
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<th>Patient no.</th>
<th>Age</th>
<th>Gender</th>
<th>Diagnosis</th>
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<th>Splenomegaly</th>
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<tr>
<td>3</td>
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<td>M</td>
<td>Conversion of PV to myelofibrosis</td>
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<td>Yes</td>
<td>46,XY;der(9;18)(p10;10)</td>
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<tr>
<td>4</td>
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<td>M</td>
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<tr>
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<td>9</td>
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Patients 1–4, present report; patients 5–8, Bacher et al. [12]; patient 9 Chen et al. [13]; patient 10, Andrieux et al. [11].

Fig. 1. The G-band karyogram showing an add(18)(p11). These findings, together with the SKY analysis (Fig. 2), were interpreted as an unbalanced translocation der(18)t(9;18)(p13;p11) leading to trisomy of 9p and monosomy of 18p.
JAK2 V617F-negative PV cases are simply falsely classified as PV because the current diagnostic criteria (Polycythemia Vera Study Group or WHO) are from the pre-JAK2 era. Furthermore, within the group of JAK2 V617F-positive PV patients, the phenotype is extremely heterogeneous and includes a subgroup of patients with aggressive disease and transformation into postpolycythemic myelofibrosis and acute leukemia after about 10 years of disease duration. Different cytogenetic abnormalities have the potential to identify characteristic subgroups of clinical phenotypes with a distinct prognostic profile within the disease category of PV. It is very interesting that a significant proportion, a total of 4/10 patients (cases 3, 4, 8, and 10 in Table 1) who were described in this report and in three previously published papers [11–13], appear to be in a “transitional” stage or myelofibrotic stage of PV. Furthermore, one patient (case 7) was reported as having post-ET acute myeloid leukemia [12]. Accordingly, this subgroup of patients, all of whom have the der(9;18)(p10;q10) karyotype, may have a high intrinsic propensity of myelofibrotic or leukemic transformation. One could hypothesize that the centromere fusion between 9p and 18p might cause an unstable genetic constitution, which accounts for the transformative nature of the disease. The der(18)(t(9;18)(p13;p11) karyotype,

Fig. 2. SKY revealing that the additional material on chromosome 18 was originally from chromosome 9.

Fig. 3. Interphase FISH results with probes for the centromere regions of 9 and 18. (a) Two green (CEP9) spots, 1 red (CEP18) spot, and one yellow (CEP9 con CEP18) fusion spot indicative of a der(9;18)(p10;q10). (b) Two green and two red spots indicative of a der(18)(t(9;18).
which was found in the other two patients in this report (cases 1 and 2), has only been reported once before [referred to as der(18)(t(9;18)(p12;p11.2)] (case 9), and all three cases in the present report appeared to have PV with a highly proliferative PV phenotype. In murine models, transplantation with bone-marrow JAK2 V617F into immunodeficient mice results in a highly proliferative PV-like disease associated with myelofibrosis [18]. There has been some speculation about a “gene—dosage effect” of the JAK2 V617F mutation because homozygosity for the JAK2 V617F mutation is reported with increasing frequency in the continuum from ET to PV to IMF [3,19]. If the association between unbalanced translocations between 9p and 18p and a highly proliferative subtype of V617F JAK2—positive PV in a transformational state into post-polycythemic myelofibrosis can be confirmed in future studies, this might contribute to this “gene—dosage effect” model because these patients are likely to have one extra copy of the mutated JAK2 gene. It is logical that this should also be the case in other PV patients with chromosomal aberrations leading to functional trisomy 9p, but to our knowledge, there are no reports on that issue. In contrast, there are several papers reporting other translocations involving 9p associated with different myeloproliferative phenotypes [e.g., t(8;9)(p23;p24) with a clinical appearance as an unclassified myeloproliferative disorder with characteristics of an erythroid preclelumia [20], as well as t(8;9)(p22;p24) presenting as an atypical chronic myeloid leukemia in two cases [21]]. Both of these karyotypes are characterized in part by a translocation with chromosomal breakpoints involving the JAK2 gene at position p24, which gives rise to a fusion gene, but other structural genetic rearrangements (alterations of promoter and/or enhancer sequences and deletions of regulatory genes) could accompany the effect of the extra copy of the mutated JAK2 gene in the present cases.

In conclusion, several mechanisms at the molecular level may contribute to a more proliferative PV phenotype with a high risk of transformation to postpolycythemic myelofibrosis associated with unbalanced translocations between 9p and 18p. In particular, the subtype der(18)(p10;q10) seems to be associated with transformation into postpolycythemic myelofibrosis and potentially acute myeloid leukemia. These findings have to be confirmed in future studies, including cytogenetic analysis, as part of the diagnostic and prognostic evaluation to further elucidate this particular hyperproliferative subtype of PV.

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References


