JAK2-V617F Mutation and Philadelphia Positive Chronic Myeloid Leukemia

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Abstract:
JAK2 is a tyrosine kinase that plays an important role in the signaling pathways of many hematopoietic growth factor receptors. A single acquired point mutation – V617F – in JAK2 occurs in the great majority of patients with polycythemia vera (PV) and approximately half of the patients with idiopathic myelofibrosis (IMF) or essential thrombocythemia (ET). In contrast, the JAK2-V617F mutation is only rarely found in chronic myeloid leukemia (CML) but, recently, some authors have reported the coexistence of JAK2V617F and BCR/ABL+ in CML patients expressing the p210 BCR–ABL oncoprotein. Here, we report a CML patient with the expression of p210/b2a2 type BCR–ABL transcript and JAK2V617F mutation.

Key words: JAK2V617F mutation, BCR-ABL translocation, ASO-PCR, ARMS-PCR

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Introduction

The acquired JAK2V617F mutation occurs in a spectrum of the Philadelphia chromosome (Ph)-negative chronic myeloproliferative disorders (CMPDs), which include polycythemia vera, essential thrombocythemia, and myelofibrosis. However, an increasing number of cases of Ph-positive CML with concomitant JAK2V617F mutation have recently been reported. There are few previous reports of the transformation of myeloproliferative disorders [PV(1,2), CIMF(3,4) or ET(5)] into chronic myeloid leukaemia. The causative relationship between the underlying disorders or their myelosuppressive treatment and the emergence of CML is uncertain. The Philadelphia-chromosome anomaly(6) and the JAK2-V617F mutation(7) might be secondary to yet unknown initial stem-cell defects that induce clonal haematopoiesis. The patient studied by Kramer's group(14) and the previously published findings(3,4,8) suggest that, in rare cases, two different subclones might evolve from the same clonal haematopoietic proliferation. We report here a case of Ph+ CMPD in combination with the JAK2V617F mutation.

Case report

In 2005, a 39-year-old man presented with leukocytosis and splenomegaly. The hematological parameters were as follows: white blood cells (WBC), 75×10^9/L, haemoglobin 12.8 g/dL, and platelets 230×10^9/L. At the time of the diagnosis, the peripheral blood cytogenetic analysis revealed a chromosome translocation [t([9;22)(q34;q11)]. Expression of B2A2 BCR-ABL mRNA (chimer protein 210 KD) was detected by a reverse transcriptase polymerase chain reaction (RT-PCR) in peripheral-blood leucocytes. A bone marrow biopsy revealed marked hypercellularity. A diagnosis of BCR-ABL positive chronic myeloid leukaemia (CML) was made and treatment with hydroxyurea (HU) was initiated with the goal of lowering blood cell count and decrease his splenomegaly, resulting in the normalization of peripheral-blood leucocytes (WBC: 5×10^9/L, Hb: 13.9 g/dL, Plt: 150×10^9/L). After one year, treatment was changed to Imatinib. In 2009, we

performed the JAK2 mutation test to detect this mutation on genomic DNA (ARMS-PCR) and RNA (ASO-PCR) which had been isolated from the whole peripheral blood of the patient.

ARMS PCR for the detection of JAK2-V617F mutations

Genomic DNA was extracted by phenol/chloroform after proteinase K digestion, following standard techniques. The JAK2 V617F mutation was detected by ARMS-PCR, according to the protocol of Jones, et al(9) with some modification. The ARMS-PCR technique uses 4 primers as follows; a forward outer primer, a reverse outer primer, a forward inner wild type specific primer and a reverse inner mutant specific primer. The forward primer from one set and the reverse from the other generate a control 463-bp band in all cases. The reverse inner mutant specific primer and the forward outer primer generate a 279-bp mutant fragment. In the presence of the wild-type JAK2, the reverse outer primer and the forward inner wild-type specific primer produce a fragment of 229-bp (Figure 1).

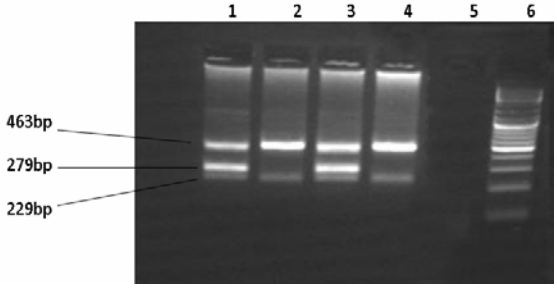

Figure 1. ARMS assay to detect the JAK2V617F mutation in genomic DNA. Lanes 6 is 100–base pair (bp) markers; lane1 show positive control with 3 bands; lane 3 is sample patient ; lanes 2 is known negative control and 5 is negative PCR control.

The PCR primers (MWG Biotech, Germany)were:
Forward Outer (FO): 5'- TCC TCA GAA CGT TGA TGG CAG-3'
Reverse Outer (RO): 5'- ATT GCT TTCCTT TTT CAC AAG AT-3'
Forward wild-type specific (FWt): 5'- GCATTTGGT TTTAAATTATGGAGTATATG -3'
Reverse mutant-specific (RMt): 5'- GTT TTA CTT ACT CTC GTC TCC ACA AAA-3'
PCR reaction was performed within a total volume of 25 µL containing approximately 25 ng DNA, 12.5 µL of TaqMan Universal PCR Master Mix 2X (Roche, Germany), 0.5 µl of each FO, RO and Fwt, and 1µl of Rmt primer. The PCR program on the thermal cycler (Eppendorf) was as follows: an initial denaturation step at 94°C for 6 min, followed by 40 cycles of 40 sec. at 94°C, 45 sec. at 56°C, 45 sec. at 72°C, and a final extension step of 10 min at 72°C.

ASO- PCR for the detection of the JAK2-V617F mutation

Total RNAs were isolated by using the TRIZOL reagent. First, strand cDNAs were prepared by reverse transcription of total RNAs with random primers. The ASO- PCR program was as follows: an initial denaturation step at 95°C for 5 min, followed by 40 cycles of 40 sec. at 94°C, 40 sec. at 56°C, 45 sec. at 72°C, and a final extension step of 10 min at 72°C.
The PCR primers (MWG Biotech, Germany) were:
Forward- 5′-GAA GAT TTG ATA TTT AAT GAA AGC CTT-3′
Reverse-5′-GTA ATA CTA ATG CCA GGA TCA CTA AGT T-3′
Mutant-5′-AGC ATT TGG TTT TAA ATT ATG GAG TAT ATT-3′
The mutant specific forward amplified a 295-bp product from the mutated allele, whereas the internal control forward primer should have given a 488-bp product from both the mutant and wild-type alleles (Figure 2). A total of 10 µl from the PCR product were electrophoresed on 3% standard agarose gels (Sigma, Germany) at 80 V for 15 min. The fragments were visualized by ethidium bromide under UV transilluminator.

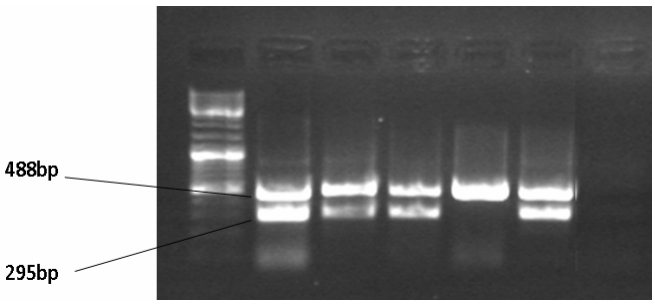

Figure- 2. ASO- PCR to detect the JAK2V617F mutation in genomic RNA. Lanes 1 is 100–base pair (bp) markers; lane 2 ,3 and 4 show positive control with 2 bands; lane 6 is sample patient ; lanes 5 is known negative controls and 10 is negative PCR control.

Discussion

After Jelinek, et al(10) reported the absence of the JAK2 mutation in chronic phase Ph positive CML (in approximately 100 patients), it was thought that the JAK2 mutation and the BCR-ABL translocation were mutually exclusive. However, an increasing number of cases of Ph-positive CML with

concomitant JAK2V617F mutation have been recently reported.(11-13) The JAK2-V617F-mutant CMPD appears to develop predominantly, after selective treatment of the Ph positive CML with the tyrosine kinase inhibitor Imatinib.(3 ,14-15) Other reports, showing that, while BCR-ABL transcripts decrease during treatment with Imatinib, the JAK2-V617F allele frequency remains constant or even increases. This certainly fits the assumption of a successive acquisition of both genomic aberrations by one subclone of progenitor cells.(3,11,14-15) On the other hand, as best evidenced by Jallades, et al,(11) at least the emergence of a Philadelphia chromosome against the background of a JAK2-V617F-mutant CMPD seems to be unrelated to prior myelosuppressive treatment. It had been speculated earlier that cytotoxic therapy of CMPD might contribute to the development of additional genomic events in CMPD (among them, a BCR-ABL translocation). In light of the paper by Jallades, et al(11) as well as by Curtin et al, who report on a patient with a JAK2-V617F-mutant ET who developed CML after treatment with aspirin only,(16,5) this scenario now has become unlikely.

Conclusion

Such rare patients with co-existing BCR-ABL translocations and JAK2V617F mutations must be identified in view of the possibility of targeted therapies. Further studies are required to determine the exact frequency and prognostic role of the JAK2V617F mutation in Ph+ CMPD patients.

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