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CASE REPORT

Chronic myelogenous leukemia with acquired c-kit activating mutation and transient bone marrow mastocytosis

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Mutations of the c-kit gene have been reported in myeloproliferative disorders. We describe here a case of Ph+ (b2a2) chronic myelogenous leukemia that, during the course of disease, showed an unusual bone marrow mast-cell infiltration. A mutational screening for the c-kit gene, performed on DNA routinely cryopreserved during the follow-up, evidenced the D816Y-activating mutation as an additional genetic abnormality. Treatment with imatinib mesylate resulted in a substantial decrease of the BCR-ABL/ABL ratio and in the absence of c-kit mutation. It is likely that the superimposed c-kit mutation, in this case, may account for the transient bone marrow mastocytosis.
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Introduction

Mutations in the proto-oncogene c-kit (the receptor for c-kit ligand, also known as stem cell factor and mast-cell growth factor) at the level of a pluripotential hemopoietic progenitor cell have been implicated in the pathogenesis of mast-cell disorders.[1] Furthermore, it is well recognized that activating mutations of the c-kit gene on chromosome 4 are also found in myeloproliferative disorders (MPD).[2–4] In a study of 25 patients with MPD, Kimura and Nakata found point mutations in exon 2 of the c-kit extracellular domain, leading to D52N substitution in one patient with chronic myelogenous leukemia (CML) and two patients with primary myelofibrosis (PMF). The authors postulated that this mutation might affect the tertiary SCF-binding site or the efficiency of ligand-induced dimerization of the receptor leading to the enhancement of the receptor kinase activity of the intracellular domain. In addition, the D52N substitution resulted in apparently higher sensitivity of erythroid progenitor to SCF.[2,3] Using RT-PCR Inokuchi *et al.*[4] studied 80 patients with CML in various clinical phases and identified sequence alterations in the c-kit juxtamembrane domain in seven cases. The authors concluded that these mutations led to leukocytosis ($P<0.05$) and to shorter survival ($P=0.04$) of CML patients.[4] Furthermore, it is well known that there is a significant increase of c-kit protein and c-kit m-RNA level in CML when compared with healthy volunteers, and this expression correlates with the phase of disease, being highest in the blast crisis of CML.[5]

Case report

In the following, we describe the case of a Philadelphia-positive CML patient who, during the course of the disease, showed an unusual bone marrow MC infiltration and, as an additional genetic change, a D816Y mutation in exon 17 of the c-kit gene. The patient, a 36-year-old male, was referred to our department 5 years after the diagnosis of CML. Upon physical examination, an enlarged liver (4 cm below the costal margin) and a palpable spleen (1 cm below the costal margin) was noted. His white blood cell (WBC) count was 24×10^9/l with 62% neutrophils, 19% lymphocytes, 12% monocytes, 1% myelocytes, 1% metamyelocytes and 1% promyelocytes; the hemoglobin concentration was 16 g/dl and platelet count was 2540×10^9/l. A bone marrow aspirate was consistent with a CML in chronic phase and cytogenetic analysis revealed a 46 XY, t(9;22)(q34;q11) [20] karyotype. We initially treated the patient with ARA-C 400 mg/day PO and α-IFN 5 MU/day and then with α-IFN alone. The treatment with α-IFN resulted only in a minimal cytogenetic

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response as the number of Ph-negative metaphases reached only 8%. After 12 months of α-IFN treatment, the WBC count was 18×10^9/l, the bone marrow aspirate and biopsy were still consistent with a CP of CML and, for the first time, an unusual MC infiltration was noted; cytogenetics showed a t(9;22)(q34;q11)[35] karyotype and the real-time quantitative PCR evidenced a b2a2 chimeric transcript with a BCR-ABL/ABL ratio of 0.13.[6] It is worth noting that clinical signs consistent with an hystamine excess (ie urticarian rashes, flushing, gastrointestinal disorders evocative of peptic ulcer, diarrhea or hypotension) were absent, ruling out the association of hematological disease to a pre-existing condition of sporadic mastocytosis. Once the α-IFN resistance was documented, a treatment with imatinib mesylate at a dose of 400 mg/day was started.[7] At evaluation 6 and 12 months after imatinib mesylate initiation, cytogenetics showed a 46 XY[20] karyotype with a BCR-ABL/ABL ratio of 0.003 and 0.001, respectively.

The presence of the bone marrow mastocytosis led us to perform a screening for the c-kit gene as previously described.[8,9] The mutational analysis was performed on DNA extracted from viable bone marrow cell routinely cryopreserved at regular intervals during the follow-up. Four samples were studied: 4 months before the starting of imatinib mesylate therapy, at the time of the imatinib initiation and 6–12 months thereafter. The screening showed the D816Y mutation only in the bone marrow collected just before the start of imatinib mesylate administration when cytogenetics evidenced t(9;22)(q34;q11)[35] karyotype, and the BCR-ABL/ABL ratio was 0.13. A careful examination of the bone marrow aspirate and biopsy, specifically addressed to detect marrow mastocytosis, was carried out, allowing us to assess a direct relationship between the presence of the D816Y mutation and the presence of MC in the bone marrow (Figure 1). Furthermore, no marrow hypereosinophilia was detected. In conclusion, our PCR data, carried out sequentially during the follow-up, clearly demonstrate that the D816Y mutation can occur in a long-lasting CML clone as an additional genetic change. The fact that the BCR/ABL rearrangement and the kit mutation may coexist within the same cell is indirectly demostrated by the findings that following imatinib mesylate treatment, a substantial decrease of BCR-ABL/ABL ratio and the absence of kit mutation could be evidenced. It is likely that the mutated c-kit may add a further growth advantage to the CML progenitor cells driven to a sustained proliferation by the high TK activity of the BCR-ABL gene product. Finally, the superimposed kit mutation may account for the transient bone marrow mastocytosis, which indicates the capability of the leukemic progenitor cells to differentiate into the MC lineage.

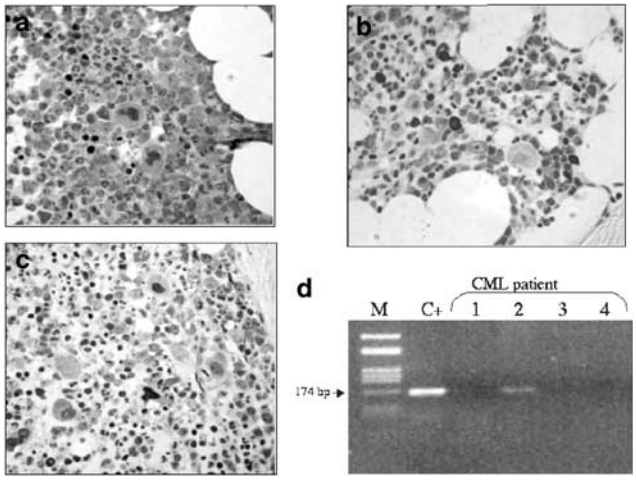

Figure 1 (a–c) Bone marrow biopsies consistent with CML in chronic phase: 4 months before starting of imatinib therapy (a), at the time of imatinib initiation (b) and 6 months thereafter (c). The photograph (b) shows an infiltration of mast cells which are mature and morphologically normal (Giemsa ×20, ×40). (d) PCR-based ARMS (Amplification Refractory Mutation System) assay for Asp816Tyr c-kit mutation was performed on BM-cells from the patient four months before starting of imatinib therapy,[1] at the time of imatinib initiation,[2] and twice thereafter.[3,4] M: molecular weight marker; C+: positive control. The arrow shows the Asp816Tyr PCR-products.

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