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CASE REPORT

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ABL amplification in a patient with lymphoid blast crisis of chronic myelogenous leukaemia

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Abstract Although chronic phase myelogenous leukaemia (CML) is characterised by the Philadelphia (Ph) chromosome leading to a fusion of the *BCR* and *ABL* genes, additional genetic alterations involved in blast crisis are poorly understood. We report an at least 15-fold amplification of the *ABL* oncogene in a 29-year-old male patient with a variant Ph-positive t(19;22)(p13;q11.2) CML who presented in lymphoid blast crisis. Our finding suggests that an amplification of the *ABL* oncogene might play a part in the appearance of an aggressive phenotype in some cases of CML.

Key words *BCR-ABL* · Amplification · CML · Blast crisis

Introduction

Development and progression of malignancies is a multistep event including both activation of oncogenes and inactivation of tumour suppressor genes. The *ABL* gene was one of the first oncogenes to be described and has been implicated in the pathogenesis of CML, where formation of the *BCR-ABL* hybrid gene leads to an increase in its tyrosine kinase activity. However, amplification of the *ABL* gene as a mechanism of pathologic activation has been described only in the cell line K562 [2].

Case report

A 29-year-old male Caucasian was referred to the hospital for evaluation of fatigue, fever and weight loss. Physical examination revealed splenomegaly but no lymphadenopathy. Laboratory findings on admission were: WBC 106×10^9/l, RBC 4.0×10^{12}/l, Hb 118 g/l, PLT 221×10^9/l; the differential count showed blast cells (24%), promyelocytes (4%), myelocytes (19%), metamyelocytes (11%), band (17%), and segmented neutrophils (21%). Leucocyte alkaline phosphatase score was 21 U/l (normal range 10–100), LDH 876 U/l (120–240), AST 33 U/l, ALT 21 U/l, gamma-GT 49 U/l, ASP 274 U/l; other routine variables were normal. Bone marrow trephine biopsy revealed a hypercellular bone marrow with an increased myeloid-to-erythroid ratio and also an increased number of mainly micromegakaryocytes and some pseudo-Gaucher cells. Moreover, there was approximately 80% diffuse infiltration by atypical blasts (Fig. 1a). Immunohistochemistry showed the blasts to be of B-phenotype, being positive for CD10, CD19, CD22, CD38, TdT and HLA-DR but negative for CD2, CD3, CD4, CD5, CD11c, CD13, CD14, CD15, CD33, CD34, CD66 and myeloperoxidase. These results were in agreement with a FACS analysis of the peripheral blood. Cytogenetic examination of the bone marrow and peripheral blood revealed 46,XY,t(19;22)(p13;q11.2), which was interpreted as a variant translocation. RT-PCR analysis, performed according to standard protocols [6, 8], revealed the presence of a chimerical *BCR-ABL* mRNA. The size of the PCR product indicated a fusion of *BCR* exon 3 and *ABL* exon 2. On the basis of morphological and immunohistochemical findings lymphoid blast crisis of CML was diagnosed and cytoreductive therapy with daunoblastin, vincristine, cytosine-arabinoside and corticosteroids was initiated, achieving a partial remission with 20% residual infiltration (Fig. 1b). Months after diagnosis, the patient received a transplant of non-T-cell-depleted allogeneic bone marrow taken from his HLA-identical sister. Chromosomal analysis of bone marrow aspirated on day +62 revealed only female cells (46,XX). Unfortunately, the patient had to be readmitted for interstitial pneumonia on day +70. Despite intense treatment including mechanical ventilation he died of respiratory failure on day +81. Post-mortem examination revealed characteristic features of “shock lung”. Bone marrow histology did not reveal any signs of CML or lymphoid blast crisis, however.

Southern-blot analysis

Genomic DNA was prepared from leucocytes isolated from blood or bone marrow aspirates by standard procedures [5]. DNA (10 µg) was digested with 50 U of restriction enzymes, as indicated in Fig. 2, and separated in 1% agarose gel. DNA was probed with randomly primed ^{32}P-labelled DNA for the *v-ABL* gene (plasmid generous gift of H. Herbst, Berlin, Germany) or the *BCR* gene (Oncor, Gaithersburg, Md.). The final wash was performed at 50°C for 30 min using 0.2× SSC/0.1% SDS, and the membranes

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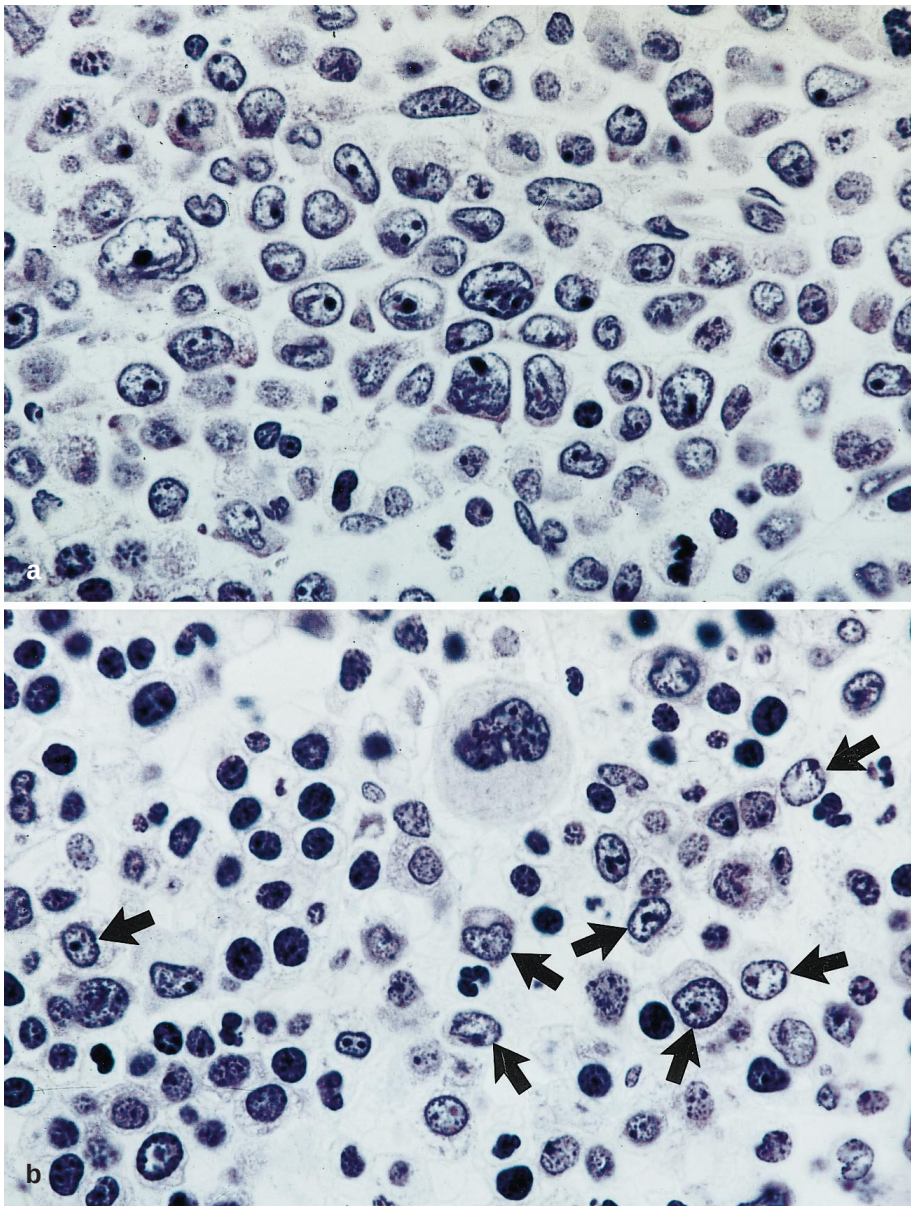

Fig. 1a Bone marrow biopsy showing diffuse infiltration by atypical blasts with round or slightly cleaved nuclei and prominent nucleoli. Occasional myeloid cells at various maturation stages are interspersed. Toluidin Blue, ×1000. **b** Bone marrow biopsy after chemotherapy, revealing cells of erythropoetic, myeloid as well as megakaryocytic lineage. Interspersed are a few atypical blasts (arrows). Toluidin Blue, ×1000

were subjected to autoradiography for 1–5 days. Densitometric analysis was performed using Docu Gel V (MWG-Biotech, Munich, Germany) video densitometer and Rflp-Scan or ONE-Dscan software (Scanalytics, Billerica, Mass.).

Results

A mixture of probes for the *BCR* gene (Oncor, Gaithersburg, Md.) revealed no normal germline-derived bands but two prominent bands of about 4.1 and 3.7 kb and three weaker bands of 8–11 kb in the patient's sample (Fig. 2). The expected normal and extra bands were found when DNA from a control cell line (K562) or DNA from a normal control or a patient with t(9;22) was used. The bands from the patient were not markedly different from normal controls in intensity. When a probe derived from the *v-ABL* gene was used, however, several bands of variable intensities were detected. With HindIII as restriction enzyme, the most prominent band showed an increase by at least 15-fold in intensity compared with normal DNA, and it comigrated with the normal-germline-derived band of 3.1 kb. Additional bands of 16 kb, 14.5 kb, 4.2 kb and 2.5 kb with 2- to 5-fold intensities were observed. With BamHI as restriction enzyme the most prominent band, again comigrating with the normal-germline-derived band, was 4 kb. Four bands of lesser intensity were detected at 14.5 kb 11.5 kb, 2.9 kb and 2.5 kb.

Although the blasts displayed a B-phenotype, no clonal rearrangement of the *IgH* gene was detected using DNA from carnoy-fixed acrylamide-embedded trephine biopsies and a seminested PCR method [10]. This is found in 10–15% of *IgH* gene rearrangements.

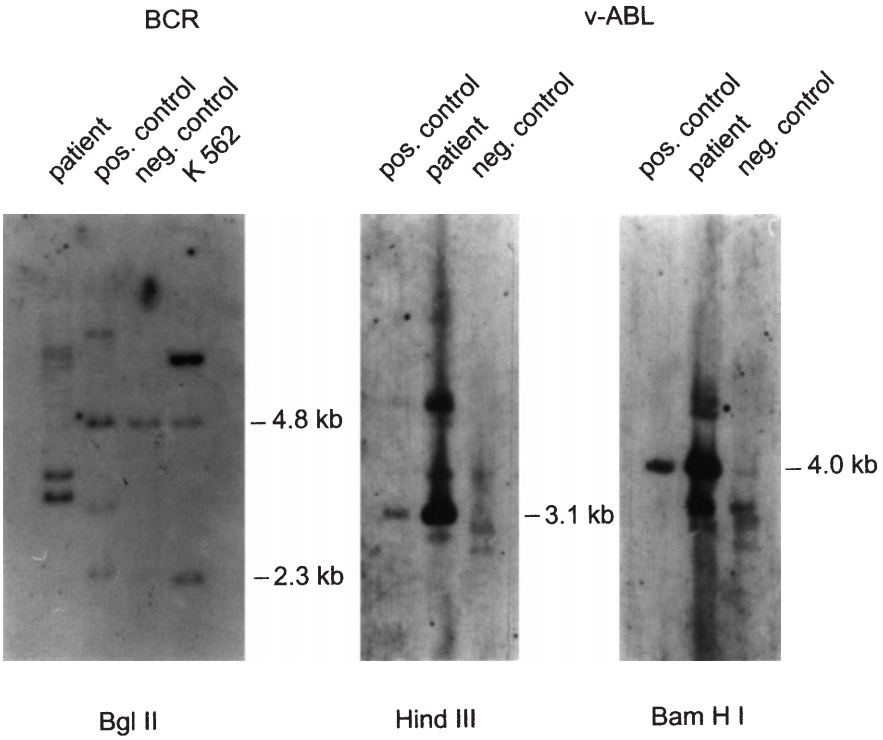

Fig. 2 Southern blot analysis of genomic DNA from the patient and positive and negative controls using probes against BCR and *v-abl* gene as described in methods. Normal germline-derived bands are indicated by their sizes

Discussion

CML usually presents in a chronic phase but eventually progresses to a blast crisis, characterised by the acquisition of further nonrandom cytogenetic abnormalities in addition to the Philadelphia chromosome [1]. However, in rare cases such as the one described in this report, blast crisis is the first manifestation. The genetic mechanisms leading to disease progression are poorly understood. We investigated the *BCR-ABL* rearrangement by Southern blot analysis using probes for both the *BCR* and the *ABL* gene. As shown in Fig. 2, at least 15-fold amplification of the *ABL* gene was detected, whereas no major changes in the amount of DNA of the *BCR* gene were found. Unfortunately, our patient did not achieve a second chronic phase after induction of chemotherapy, and assessment of the *ABL* gene in remission was not possible.

In contrast to several other oncogenes, such as *N-MYC*, amplification of the *ABL* gene has been documented in only two instances. Approximately 6-fold amplification has been described in the Ph-positive cell line K562 [2] and an 8- to16-fold higher rate of the *BCR-ABL* transcript has been observed in a CML patient who presented with lymphoid blast crisis with three Ph chromosomes [3]. Events so far associated with transformation of CML include inactivation of the tumour suppressor genes *p53* [4] and *p16* [9]. Although it has been shown that the *ABL* gene is not activated by point mutations in CML blast crisis [7], we nevertheless speculate that amplification of the *ABL* gene may contribute to the appearance of an aggressive lymphoid phenotype in some cases of CML. Based on these findings, we would like to propose that further studies, including FISH analysis, should be performed in order to clarify the role of *ABL* amplification both in lymphoid blast crisis of CML and in Ph-positive acute lymphoblastic leukaemia.

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