Short communication

Insertion (12;9)(p13;q34q34): a cryptic rearrangement involving ABL1/ETV6 fusion in a patient with Philadelphia-negative chronic myeloid leukemia

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Abstract
We report a rare cryptic ins(12;9)(p13;q34q34), a chromosomal abnormality involving the ABL1 (9q34) and the ETV6 (alias TEL; 12p13) genes, detectable only by fluorescence in situ hybridization (FISH), in a patient with Philadelphia-negative chronic myeloid leukemia (CML). Using reverse 4',6-diamidino-2-phenylindole banding on metaphase cells, FISH analysis with BCR/ABL dual-fusion and ETV6 break-apart probes showed that a third ABL signal was inserted into 12p, splitting the ETV6 signal into two adjacent signals. CML patients with an ABL1/ETV6 fusion historically have demonstrated a variable and sometimes transient response to treatment with imatinib mesylate, which was also the case in the present patient.

1. Introduction

The t(9;22)(q34;q11.2), or Philadelphia chromosome, is usually consistent with the diagnosis of chronic myeloid leukemia (CML) and, less frequently, with the diagnosis of acute leukemia. The t(9;22) generates the BCR/ABL1 fusion gene encoding a protein with increased tyrosine kinase activity and transforming properties [1]. Although the BCR/ABL1 fusion is correlated with an initiating event in the development of CML, the underlying genetic mechanisms are less well understood. Some cytogenetic and molecular genetic changes have been associated with disease progression [2,3].

The atypical CMLs are a particular group of myeloproliferative disorders (MPDs) recently redefined as unclassified MPDs that are not characterized by the t(9;22) BCR/ABL1 fusion. Approximately a dozen patients have been reported with variant ABL1 (9q34) rearrangements involving 12p13 (ETV6), rather than 22q11.2 (BCR) [4–12]. This translocation results in an ABL1/ETV6 fusion. In various hematological disorders, ETV6 encodes for a transcriptional repressor and is known to fuse to ~20 different genes, most of which code for tyrosine kinases or transcription factors [13–15].

Here we describe the case of a 79-year-old man with atypical CML whose fluorescence in situ hybridization (FISH) studies using the BCR/ABL1 dual-fusion probe demonstrated a rare cryptic rearrangement, ins(12;9)(p13;q34q34), leading to an ABL1/ETV6 fusion. The insertion is a variant of t(9;12)(q34;p13) that has been reported in only few cases of atypical CML [1]. This case study emphasizes the importance of FISH in detecting cryptic and variant rearrangements, particularly when an abnormality is associated with a malignancy in which a specific therapy may be beneficial.

2. Patient, materials, and methods

2.1. Case report

The patient initially presented in 2005 for evaluation of increased white blood cell count after several weeks with decreased appetite, an unintentional weight loss of 7 pounds, and lower abdominal pain. His laboratory parameters showed a white blood cell count of 35.2 × 10⁹/L, hemoglobin 14.1 g/dL, platelet count 176 × 10⁹/L, with 72% neutrophils, 6% lymphocytes, 16% monocytes, and 6% eosinophils. Within 5 days, his white blood cell count increased to 41.6 × 10⁹/L; hemoglobin was 13.9 g/dL, and the platelet count was 202 × 10⁹/L. At that time, he...
had a marked left shift, with many promyelocytes and band forms, significant eosinophilia, occasional basophils, and nucleated red blood cells. The patient appeared to have a myeloproliferative disorder, likely chronic myeloid leukemia (CML). Polymerase chain reaction analysis was negative for the Philadelphia chromosome, thus excluding Philadelphia chromosome-positive CML.

In 2006, the patient experienced abdominal discomfort, respiratory problems, chills and possible fever, as well as generalized malaise and fatigue, and falling on one occasion. He also had an elevated white blood count of 205 × 10^3/μL, a hematocrit of 36%, and a low platelet count of 89 × 10^3/mL. His peripheral blood smear showed predominance of myeloid blasts. Neither lymphadenopathy nor hepatosplenomegaly was noted. After the FISH analysis, which confirmed the presence of the ABL1/ETV6 rearrangement, the patient was placed on a therapeutic regimen that included imatinib mesylate. Our patient demonstrated a transient response to imatinib, similar to what has been indicated in the literature [11]. He returned to the hospital after 1 month with respiratory failure and a white blood cell count of 205 × 10^3/μL with 39% blasts. He died within hours of arrival.

2.2. Chromosome analysis

The G-banded chromosome analysis was performed on the peripheral blood sample using standard cytogenetic protocols. Twenty metaphases were analyzed and karyograms were prepared using a computer-assisted karyotyping system (CytoVision; Applied Imaging, San Jose, CA). The karyotypes were described according to ISCN 2005 [16].

2.3. FISH

The FISH slides were processed according to the manufacturer’s guidelines. Analyses were performed on both interphase and metaphase cells. The LSI BCR/ABL dual-color, dual-fusion translocation probe was used to identify any t(9;22)(q34;q11.2) present; the LSI TEL (ETV6) dual-color, break-apart probe was used to identify any rearrangement involving the ETV6 gene; and the LSI 9q34 SpectrumAqua probe to determine the number and location of ASS signals. All probes were purchased from Abbott Molecular, Des Plaines, IL.

All FISH analyses were performed using a BX51/BX52 Olympus fluorescence microscope (Exfo America; Olympus, Richardson, TX). The images were captured using a charge-coupled device camera and the Isis imaging system (MetaSystems, Watertown, MA; Altlussheim, Germany).

3. Results

Conventional chromosome analyses performed in 2005 and 2006 yielded apparently normal results in all 20 metaphases analyzed. FISH studies were not performed in 2005. In 2006, three consecutive FISH studies were performed, which were instrumental in detecting and characterizing a rare cryptic ins(12;9)(p13;q34q34).

A FISH study using the BCR/ABL dual-fusion probe demonstrated two green signals for the BCR gene and three orange signals for the ABL1 gene in 92% of interphase cells and in the majority of metaphase cells analyzed. Additional FISH studies were performed with the 9q34 locus-specific ASS probe, proximal to the ABL1 region, mainly to determine whether the three ABL1 signals were consistent with three intact copies of ABL1 or were the result of a rearrangement of this region that split apart one of the ABL1 signals. Two ASS aqua signals were observed in all interphase cells examined. FISH on metaphase cells showed that both ASS signals still hybridized to the 9q34 region.

Translocations involving ABL1 and genes other than BCR have been reported in atypical CML. One of these translocation is the t(9;12)(q34;p13) [4,5]. Using reverse-4’,6-diamidino-2-phenylindole banding on metaphase cells, we confirmed that the third ABL1 signal was located on 12p (Fig. 1). Furthermore, FISH analysis using the ETV6 (12p13) break-apart probe demonstrated two green–orange fusion signals for the ETV6 gene in all metaphase cells examined (Fig. 2). On interphase cells, however, a split ETV6 signal was observed on the abnormal 12p, with the 3’ and the 5’ regions remaining in constant vicinity of each other (Fig. 3). Because the 3’ and 5’ regions of ETV6 were so close, no splitting was evident on metaphase cells (Fig. 2).

Fig. 1. Metaphase fluorescence in situ hybridization (FISH) with the BCR/ABL1 dual-fusion, dual-color probe shows two BCR signals (green) and three ABL1 signals (orange). The third ABL1 signal was determined to be located on 12p. Therefore, two of the ABL1 signals (small orange) are derived from splitting of the probe signal with one located on 9q34 and the other on 12p13; and one normal ABL1/ASS signal (large orange) on 9q34.
4. Discussion

The translocation (9;22)(q34;q11.2) is generally consistent with CML and, at a lower frequency, with acute leukemia [17]. Other abnormalities have been associated with atypical CML, a chronic myeloproliferative disorder mimicking typical CML but lacking the t(9;22) or the BCR/ABL1 fusion [18]. These atypical CML cases exhibit translocations involving oncogenes including, but not limited to, ABL1, PDGFR, and ETV6 [19]. The t(9;12)(q34;p13) is a recent finding and has been reported only in a few cases [4,6,7,19,20], although some cases might have been missed, given the possibly cryptic nature of the rearrangement. Fluorescence in situ hybridization should be performed when myeloproliferative disorders are highly suggestive of CML, to exclude the presence of the BCR/ABL1 fusion and then also to detect abnormalities involving either ABL1 or BCR that are more suggestive of an atypical CML.

Our case is an example of a cryptic rearrangement associated with atypical CML. The conventional chromosome studies performed in this patient yielded an apparently normal result. The FISH studies, however, were instrumental in detecting and characterizing a cryptic insertion of the ABL1 region into 12p13, splitting the ETV6 region apart. The FISH study on interphase cells revealed a consistent splitting of the ETV6 fusion signal, with the 3' and the 5' regions remaining in constant vicinity of each other. At the metaphase level, there was no significant separation of the ETV6 fusion. The FISH result was therefore interpreted as a small insertion of ABL1 into ETV6, rather than a simple split. The ETV6/ABL1 fusion gives rise to a cytoplasmic protein with elevated tyrosine kinase activity, which can transform cell lines from factor dependent to factor independent [7,20] and to induce myeloproliferative disease in mice [21]. A recent report described a patient with ETV6/ABL1-positive acute leukemia who responded transiently to imatinib [11], a tyrosine kinase inhibitor of BCR/ABL1 and ABL1 [22].

This case illustrates the importance of FISH in detecting cryptic and variant rearrangements, particularly when the abnormality in question is associated with a malignancy in which a specific therapy may be beneficial. Our patient demonstrated a transient response to imatinib mesylate. Studies of additional similar cases are warranted to better understand the biology and clinical course of these atypical CMLs.

References


