Short communication

Acute myeloid leukemia with inv(16) with CBFB–MYH11, 3'CBFB deletion, variant t(9;22) with BCR–ABL1, and del(7)(q22q32) in a pediatric patient: case report and literature review

Carlos A. Tiradoa,*, Federico Valdez a, Laura Klesse b, Nitin J. Karandikarc, Naseem Uddina, Arnaldo Arbinic, Nicholas Fustinob, Robert Collinsd, Sangeeta Patela, Ruth L. Smarta, Rolando Garciaa, Jeff Doolittlea, Weina Chen c

aClinical Cytogenetics, Department of Pathology, The University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, TX 75390-9073
bHematology/Oncology, Department of Pediatrics, The University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75390
bHematopathology Division, Department of Pathology, The University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75390
cHematology/Oncology, Department of Internal Medicine, The University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75390

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Abstract

Coexistence of inv(16) and t(9;22) is a rare chromosomal aberration, one that has been described in chronic myelogenous leukemia (CML), mainly in myeloid blast crisis, and de novo acute myeloid leukemia (AML). Approximately 14 cases have been reported, including only 1 pediatric case. Here we present the case of a 13-year-old boy with a new diagnosis of AML with some features of monocytic differentiation. Conventional cytogenetic analyses on unstimulated blood showed three related abnormal clones with inv(16) in the stemline: 46,XY,inv(16)(p13.1q22)[2]/46,idem,del(7)(q22q32)[16]/46,idem,t(9;22;19)(q34;q11.2;p13.1)[2]. Fluorescence in situ hybridization (FISH) studies on interphase nuclei and previously G-banded metaphases showed a 3'CBFB deletion and confirmed the presence of the Philadelphia chromosome in a t(9;22;19) rearrangement. Deletion 7q31 was also confirmed by interphase FISH analysis. The patient was treated with standard AML chemotherapy plus gemtuzumab as part of a clinical trial. At 10-months follow-up, he was in remission. To the best of our knowledge, this is the first description of a pediatric case of de novo AML with a stemline showing inv(16) along with 3'CBFB deletion, an abnormal clone revealing in addition a del(7)(q22q32), and another clone with a t(9;22;19)(q34;q11.2;p13.1) as an additional abnormality. © 2010 Elsevier Inc. All rights reserved.

1. Introduction

Acute myeloid leukemia (AML) is one of the most common types of leukemia in adults [1], but also accounts for 15–20% of acute leukemia cases in children [2]. One of the most recurrent cytogenetic abnormalities in AML is inv(16)(p13q22), which is usually associated with acute myelomonocytic leukemia with eosinophilia (AML M4-Eo by the French–American–British classification) [2]. This abnormality results in fusion of the myosin heavy chain 11 gene (MYH11) at 16p13 and the core-binding factor beta subunit gene (CBFB) at 16q22 [3,4]. The most common variants of this rearrangement are t(16;16)(p13;q22) and del(16)(q22). Deletion of 16q has been reported to be associated with a worse prognosis, compared with inv(16) or t(16;16), but the significance of the 3'CBFB gene deletion is not clear [5–7]. The presence of deletion 7q as an additional abnormality with inv(16) has also been described in AML [8,9].

Although the t(9;22) BCR–ABL1 rearrangement is characteristic of chronic myelogenous leukemia (CML), it is also found in a small subset (2–5%) of pediatric acute lymphoblastic leukemia (ALL), as well as in ~1% of childhood AML [10–14]. Coexistence of inv(16) and t(9;22) is rare; few cases have been described in the literature [10–14], most of which were CML in myeloid blast crisis (with a small subset of de novo AML, including one pediatric case). In the limited number of cases reported, the
prognosis is varied for AML patients with coexistence of t(9;22) and inv(16). For CML, however, such coexistence at blast phase is associated with an unfavorable prognosis.

Here we present a pediatric case of de novo AML in a 13-year-old boy with a complex karyotype containing three different but related clones. The stemline showed an inv(16) $CBFB-\text{MYH11}$ with $3'\text{CBFB}$ deletion. The second clone had a deletion q7, and the third clone had a t(9;22;19)(q34;q11.2;p13.1) as an additional abnormality.

2. Clinical presentation

The patient, a 13-year-old boy of European origin without significant past medical history, presented with a 2-month history of night sweats and weight loss (~9 kg), a 2-week history of cervical lymphadenopathy, and a 1-week history of fever up to 38.6°C. On physical examination, he was noted to have petechiae and was referred to our tertiary care center. Initial laboratory evaluation revealed a white blood cell (WBC) count of 298,000/µL with 92% abnormal myeloid cells. The patient was admitted and the diagnosis of AML was made by flow cytometric immunophenotyping and morphologic evaluation of peripheral blood and bone marrow specimens.

Over the next 24 hours, the patient began to develop opacification of his left lung and hypoxia, and was subsequently intubated. Leukapheresis was initiated, and his WBC count was reduced to 87,000/µL over another 24 hours. He was treated with standard chemotherapy (high-dose cytarabine, 100 mg/m² per dose and L-asparaginase) and antibiotics (including a 5-day course of meropenem, 50 mg/m² per dose, x 5 doses) plus gemtuzumab, as part of Children’s Oncology Group Trial AAML0531. He completed four cycles of chemotherapy. Repeat bone marrow examination at the end of the first induction demonstrated no evidence of leukemia. His initial cerebrospinal fluid was positive for leukemic blasts and was cleared with intrathecal cytarabine.

His clinical course involved various complications, including Klebsiella pneumoniae bacteremia, Bell’s palsy, significant hypotension due to Streptococcus mitis sepsis, and congestive heart failure. Given his declining heart function, a final course of high-dose cytarabine and l-asparaginase was deferred. His cardiac function improved considerably over another 24 hours. The patient remained in remission.

3. Materials and methods

3.1. Conventional cytogenetics

The unstimulated blood sample was cultivated in specific media (450 mL RPMI-1640, 100 mL fetal bovine serum, 50 mL condimed, and 10 mL L-glutamine) following conventional cytogenetic techniques. The slides were prepared by the dropping method, dried under controlled conditions (22°C, 42% humidity), and aged for 35 minutes at 90°C.

Twenty G-banded (trypsin and Wright—Giemsa) metaphases were analyzed, and the karyotypes were prepared using BandView software (Applied Spectral Imaging, Vista, CA). The karyotypes were described according to ISCN 2009 [15].

3.2. Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) analyses were performed using the following DNA probes from Abbott Molecular (Des Plaines, IL): (i) Vysis LSI CBFB dual-color, break-apart rearrangement probe for the CBFB on chromosome 16; (ii) a subtelomere probe for chromosome 16 (TelVysion 16p SpectrumGreen; TelVysion 16q SpectrumOrange); (iii) the Vysis Williams region probe LSI ELM SpectrumOrange—LSI D7S486, D7S522 SpectrumGreen specific for chromosome subband 7q11.23 (ELN, LIMK1, D7S613) and chromosome band 7q31 (D7S486, D7S522); and (iv) the Vysis LSI BCR/ABL dual-color, dual-fusion translocation probe specific for the ABL1 and BCR genes. These tests were performed on interphase nuclei as well as on previously G-banded slides.

The samples were analyzed on a Zeiss Axiosphot microscope equipped with a Chroma 83000 filter set and using Quips Pathvision software (Applied Imaging, Santa Clara, CA). The selected images were captured using a SenSys charge-coupled device CCD camera (Photometrics, Tucson, AZ).

3.3. Bone marrow examination and flow cytometry

Bone marrow and peripheral blood smears were prepared and stained with Wright—Giemsa stain. The specimens were immunophenotyped using a four-color FACSCalibur flow cytometer (BD Biosciences, San Jose, CA); data were collected with CELLQuest software and analyzed using cluster analysis with Paint-a-Gate Software (BD Biosciences). Specimen processing and antibody staining were performed as previously described [16].

4. Results

4.1. Conventional cytogenetics

A male chromosome complement with an inv(16) was observed in all 20 metaphases analyzed (Fig. 1A). Sixteen of these cells (80%) also demonstrated an apparently interstitial deletion on the long arm of chromosome 7 from 7q22 to 7q32 (Fig. 1B). Two cells (10%) had a t(9;22;19)(q34;q11.2;q13.1) rearrangement, in addition to inv(16) (Fig. 1C). The inverted 16 seen in all cells appeared to be associated with a deletion in the 3′ end of CBFB, as demonstrated by FISH. The karyotype was described as 46,XY,inv(16)(p13.1q22)[2]/46,idem, del(7)(q22q32)[16]/46,idem,t(9;22;19)(q34;q11.2;p13.1)[2].
4.2. FISH

The FISH analysis showed an abnormal CBFB pattern with a deletion of the 3' end of CBFB on the inverted 16 in 95.5% of the 200 nuclei examined: nuc ish (5'CBFB×2,3'CBFB×1)(5'CBFB con 3'CBFB×1)[191/200] (Fig. 2A). This finding was also confirmed on previously G-banded metaphases (Figs. 2C and 2D).

Fig. 1. Conventional cytogenetic karyotyping in a pediatric case of acute myeloid leukemia with complex chromosomal aberrations. (A) Karyogram showing the stemline with inv(16). (B) Karyogram showing an abnormal clone with inv(16) as well as del(7q). (C) Karyogram with inv(16) as well as the variant t(9;22;19)(q34;q11;q13.1).
DNA probes specific for chromosome subband 7q11.23 (ELN, LIMK1, D7S613) and chromosome band 7q31 (D7S486, D7S522) showed two signals for the proximal region but one signal for the distal region in 63% of the 200 nuclei examined, consistent with a deletion in the long arm of chromosome 7 in those cells: nuc ish (ELN,LIMK1,D7S613)×2,(D7S486,D7S522)×1[126/200] (Fig. 2B).

DNA probes specific for the ABL1 gene at chromosome band 9q34 and the BCR gene at chromosome subband 22q11.2 showed a pattern of fusion signals consistent with a variant BCR–ABL1 rearrangement involving three chromosomes in 13.5% of the 200 nuclei examined: nuc ish (ABL1,BCR)×3(ABL1 con BCR×1)[27/200] (Fig. 2E).

FISH studies on abnormal metaphases confirmed that the third chromosome involved in the translocation was chromosome 19 (Figs. 2F and 2G).

In light of the FISH results, the karyotype was revised as 46,XY,inv(16)(p13.1q22)[2]/46,idem,del(7)(q22q32)[16]/46, idem,t(9;22;19)(q34;q11.2;p13.1),ishinv(16)(p13.1)(3'CBFB +)(q22)(3'CBFB–)[20],t(9;22;19)(ABL1+,BCR–;ABL1+, BCR++;ABL1–,BCR+)×2 nuc ish(ELN,LIMK1,D7S613)×
2, (D7S486, D7S522) × 1 [126/200], (ABL1, BCR) × 3 (ABL1 con BCR × 1) [27/200].

4.3. Morphology and flow cytometry

Peripheral blood and bone marrow aspirate examinations revealed a predominant population of large blasts with variably irregular nuclei, dispersed chromatin, variably prominent nucleoli, and scant to moderate amounts of cytoplasm; some of these immature cells had features of promonocytes. Eosinophilia or abnormal eosinophils were largely absent.

Immunophenotypic analysis on peripheral blood revealed a large population (83–90% of total events) of aberrant myeloid blasts with the following immunophenotype: CD34+, CD4+ (partial), CD13+, CD14−, CD15+, CD16−, CD19+ (partial), CD33+, CD36+ (small subset), CD38−, CD45+, CD56−, CD64+ (partial), CD117+, HLA-DR+, myeloperoxidase+, and TdT−, with other myeloid and lymphoid markers predominantly negative. In addition, there was a smaller population (5%) of slightly aberrant monocytic cells (Fig. 3).

These findings were consistent with an AML with some features of monocytic differentiation. The overall morphologic and immunophenotypic features thus indicated an AML with some features of monocytic differentiation but with no evidence of eosinophilia.

5. Discussion

Inverted chromosome 16 is associated with acute myelomonocytic leukemia with eosinophilia (AML-M4-Eo) and carries a favorable prognosis, with high rates of complete remission [2]. In normal myeloid and lymphoid tissues, CBFβ associates with CBFα2 (encoded by RUNX1) to form the heterodimeric protein CBF (human core-binding factor). The CBF protein is a positive transcriptional regulator for hematopoiesis. Chromosomal rearrangements involving the genes coding for CBFα2 and CBFβ are observed in ~8% and ~4%, respectively, of de novo AML cases [1]. In inv(16)(p13q22), the fusion protein CBFβ–MYH11 prevents the normal differentiation process by sequestering CBFα2 in the cytoplasm, acting as a transcriptional repressor by recruiting co-repressors, and acquiring chromatin-modifying histone deacetylase activities [1,8].

The deletion of the 3′ end of the CBFβ gene in inv(16) confirmed by FISH in the present case is a rare finding, and has been described only in AML M4; its prognostic significance is as yet undetermined [5–8]. In contrast, deletion of the 5′ region of the MYH11 (16p13) is relatively common, detected in ~20% of AML with inv(16) [6].

Although deletions in the long arm of chromosome 7 are rare in childhood AML (4–5% of pediatric cases) and are associated with a poor prognosis, when del(7q) is present together with a favorable cytogenetic abnormality, such as
the inv(16) in the present case, the deletion may be associated with a higher survival rate [8,9].

Coexistence of inv(16) and t(9;22) is rare, with few reports in the literature [10–14]. The majority of these cases were CML in myeloid blast crisis, and all tested cases carried a p210 BCR-ABL1 transcript. A small subset of cases were de novo AML, including one pediatric case. Notably, all tested AML cases carried a p190 BCR-ABL1 transcript. In addition, all AML cases had been classified as AML M4-Eo (French—American—British classification) and had both inv(16) and t(9;22) in all analyzed metaphases. The prognosis was variable. Of three patients for whom the information was available, two patients (aged 9 and 25 years) died at 7 and 15 months after diagnosis [13], respectively, and one patient (aged 23 years) had remained in remission for 3 years as of the last follow-up [13].

In the present case, the t(9;22;19)(q34;q11.2;p13.1) rearrangement was seen in only a small subset of metaphases carrying inv(16), suggesting that this rearrangement is a secondary change. Variant t(9;22) rearrangements involving different partner genes have been widely described; however, there are no previous reports of t(9;22;19)(q34;q11.2;p13.1), although the 19p13 region is known to be involved in different translocations associated with both myeloid and lymphoid neoplasms.

It is not known which BCR-ABL1 transcript was present in our study, because no material was available for further molecular characterization. Based on the literature, however, our case would be expected to have the p190 BCR-ABL1 transcript. It is intriguing that the p210 BCR-ABL1 transcript is seen mostly in CML and the p190 BCR-ABL1 transcript is seen in ALL and AML [10], implying different pathogenetic pathways in these hematological entities. Our case, it should be noted, had morphologic and immunophenotypic features of AML with some monocytic differentiation but without eosinophilia. Whether these unusual features are related to this patient’s complex cytogenetic aberrations, including the additional 3′CBFB deletion and del(7)(q22q32), is not known. Furthermore, it is difficult to predict the effect of these complex cytogenetic abnormalities on clinical outcomes. Although our patient responded well to therapy and remained in remission at 10 months of follow-up, long-term follow-up is needed for accurately assessing the prognostic significance.

References


