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Identification of a new translocation that disrupts the *RUNX1* gene in a patient with de novo acute myeloid leukemia

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Abstract Translocation (8;21)(q22;q22)/*RUNX1-RUNX1T1* is a molecular marker that is usually associated with a favorable outcome in both pediatric and adult patients with acute myeloid leukemia (AML). The present report describes the results of hematologic, cytogenetic, and fluorescence in situ hybridization analysis of a case of AML with maturation in a 23-year-old woman. Cytogenetic analysis revealed a balanced translocation involving chromosomal band 21q22, which disrupts the *RUNX1* gene, and 10q22, with the following karyotype: 45,X,-X, t(10;21)(q24;q22)[cp16]/46,XX [4]. Interphase FISH showed, in 67% of the 300 interphase nuclei analyzed, three signals for *RUNX1* and two *RUNX1T1*, but no signals corresponding to *RUNX1-RUNX1T1* fusion gene. These results were corroborated by RT–PCR, which revealed negative results for the amplification of *RUNX1-RUNX1T1* fusion gene. The patient was refractory to conventional and salvage chemotherapy regimens and early relapsed after unrelated donor bone marrow transplantation (BMT), dying of pneumonia, acute respiratory failure, and sepsis on day +80 after BMT, 1 year after diagnosis.

Keywords Acute myeloid leukemia · t(10; 21) · Cytogenetics

Introduction

Recurrent chromosomal aberrations are identified in approximately 55% of adults with de novo acute myeloid leukemia (AML) by conventional cytogenetic analysis (G-banding), and these findings have been reported as the most important diagnostic and prognostic markers in this disease [1]. The World Health Organization (WHO) classification of myeloid neoplasms emphasizes the importance of cytogenetic and molecular abnormalities in AML, and large collaborative studies have demonstrated that it is possible to stratify patients in favorable, intermediate, and unfavorable risk groups based on specific cytogenetic findings at diagnosis [2]. The t(8;21)(q22;q22), along with inv(16)(p13q22)/t(16;16)(p13;q22) and t(15;17)(q22;q12), constitutes favorable molecular cytogenetic risk groups in AML [3]. The t(8;21)(q22;q22) occurs in approximately 5–12% of AML cases [4, 5], presenting good response to chemotherapy and high complete remission rates with long-term disease-free survival [5].

This subtype of AML usually correlates with specific morphological features, which include large blast cells with Auer rods, often containing numerous azurophilic granules and very large pseudo-Chediak-Higashi granules, as well as homogeneous pink-colored cytoplasm in neutrophils [6, 7]. Molecularly, the *Runt-Related Transcription Factor 1* gene (*RUNX1*) located at 21q22 is fused, in 96% of cases, with the *RUNX1T1* gene located at 8q22, generating a

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chimeric *RUNX1-RUNX1T1* fusion gene on the der(8) [6]. However, approximately 3–4% of AML cases harbor variant translocations involving the band 21q22 of chromosome 21 and other partner genes with prognostic significance not yet defined. According to de Braekeleer et al. [8], about 32 partner chromosomes involved in translocations with *RUNX1* gene have been described, but the partner gene has been identified only in 17 translocations.

Using classical cytogenetic methods, *fluorescence* in situ *hybridization* (FISH), and reverse-transcriptase-polymerase chain reaction (RT–PCR), we report a de novo AML case with a new variant translocation, t(10;21)(q24;q22), involving *RUNX1* gene. To our knowledge, this is the first occurrence of this specific translocation in association with a case of de novo AML, which presented an unfavorable outcome.

Materials and methods

Case report

A 23-year-old woman was admitted to the University Hospital of the Medical School of Ribeirão Preto, University of São Paulo, in July 2003, after a complete blood count drawn for complaints of fever, fatigue, and sore throat for 10 days. She had used oral second-generation cephalosporin for the last 7 days before admission. Family and past medical histories were negative for hematological disorders. Physical examination showed moderate pallor and oropharynx hyperemia, without other abnormalities. The peripheral blood analysis showed the following: hemoglobin = 9.7 g/dl, white blood cells (WBC) = 6.700/mm^3, with 20% of circulating blasts, and platelets = 295.000/mm^3. Bone marrow (BM) aspirate revealed dysgranulopoiesis and infiltration of 90% of blasts of large size, intermediary nuclear-to-cytoplasm ratio, nucleus with an immature chromatin and visible nucleoli, slight basophilic cytoplasm with fine apparent granules but no Auer rods, shown to be positive for myeloperoxidase and negative for non-specific esterase (Fig. 1a, b). Immunophenotyping showed positivity for CD13, CD33, CD34, CD117, CD11b, and HLA-DR and negativity for CD2, CD19, CD14, CD11c, and CD64. According to WHO criteria, the patient was morphologically diagnosed with acute myeloid leukemia with maturation. Heparin and EDTA-collected BM samples were taken to cytogenetic and molecular biology studies, respectively, as detailed below.

After diagnosis, the patient was treated with the standard daunorubicin (60 mg/m^2/day) and cytarabine (100 mg/m^2/day), so-called 3 + 7 AML induction regimen. On day +24 after chemotherapy, there was persistent pancytopenia and the BM was still infiltrated by 87% of myeloblasts. On day +30 after the first induction regimen, the patient was submitted to a second course of chemotherapy with cytarabine 3 g/m^2/day for 5 days + daunorubicin 45 mg/m^2/day for 3 days but the patient did not achieve hematological remission, with BM aspirate showing a persistent infiltration by 70% of blasts. At this point, it was decided to administer 21-day cycles of low-dose cytarabine while waiting allogeneic unrelated bone marrow transplantation (BMT), which was performed 6 months later, and resulted in graft rejection and relapse. There was leukemia progression during the following months and the patient died with pneumonia, acute respiratory failure, and sepsis on day +80 after BMT, 1 year after diagnosis.

Cytogenetic analysis and fluorescent in situ hybridization (FISH)

Chromosome analysis was performed on a non-stimulated culture of bone marrow aspirate in RPMI 1640 medium with 10% fetal calf serum. Chromosome preparations were acquired according to the standard procedures, and the subsequent cytogenetic analysis and interpretation were described according to ISCN 2009 [9]. FISH analysis was performed on interphase nuclei using *Vysis LSI AML1/ETO dual-color, dual-fusion translocation probe* (Abbott Molecular, Des Plaines, IL, USA) specific for *RUNX1* labeled with SpectrumGreen and for *RUNX1T1* labeled with SpectrumOrange, respectively. Three hundred interphase cells were counter-stained with DAPI and viewed with an Axio Imager M2 microscope equipped with the *FISHView* software, version 5.5 and appropriate filters (ASI, Carlsbad, CA, USA).

RNA extraction and reverse transcriptase-polymerase chain reaction

Total RNA from mononuclear cells isolated from bone marrow was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA). Complementary DNA (cDNA) was synthesized from 1 μg of total RNA using a high capacity cDNA reverse transcription kit (Applied BioSystems, Foster City, CA, USA), following the manufacturer's instructions. RT–PCR for the *RUNX1-RUNX1T1* fusion gene was performed according to the standard methods previously described [10]. The PCR products were analyzed by 1.5% agarose gel electrophoresis stained with ethidium bromide.

Screening for FLT3-ITD mutations

Genomic DNA was extracted using the PureGene kit (Gentra System, Minneapolis, MN, USA) according to the

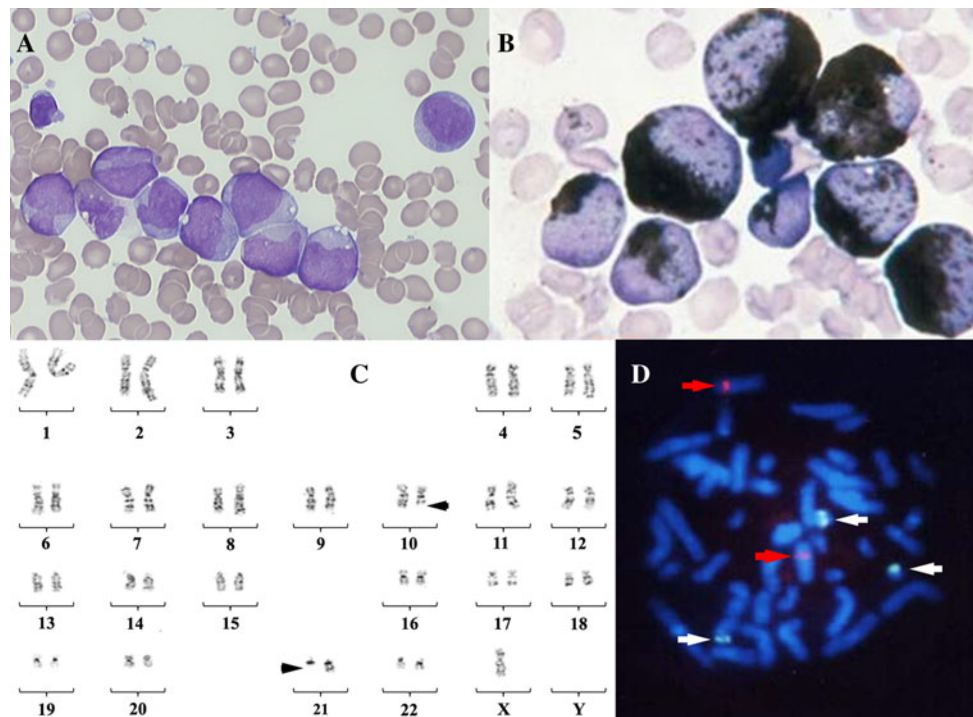

Fig. 1 Cytomorphology and cytogenetics analysis of leukemic blasts of a 23-year-old woman. **a** Bone marrow smear showing blasts of intermediary nuclear-to-cytoplasm ratio, nucleus with an immature chromatin and visible nucleoli, slight basophilic cytoplasm with fine apparent granules and no Auer rods (Leishman-stained; original magnification ×100). **b** The leukemic blasts were positive for MPO (original magnification ×100). **c** Conventional karyotyping analysis (GTG) of non-stimulated bone marrow cells. The analysis revealed an abnormal karyotype with a novel balanced translocation involving chromosomal band 21q22, 10q22 (*black arrows*), as well as loss of the X chromosome. The karyotype was described as 45,X,-X, t(10;21)(q24;q22)[cp16]/46,XX [4]. **d** Interphase fluorescence in situ hybridization (FISH) analysis on metaphase cells using *Vysis LSI AML1/ETO dual-color, dual-fusion translocation probe*, showing three signals of AML1 probe, corresponding to *RUNX1* gene (*white arrows*) and two signals of ETO probe, corresponding to *RUNX1T1* (*red arrows*). No signals corresponding to *RUNX1-RUNX1T1* gene fusion was detected. The complete signal *RUNX1* from 21q22 is visible in the center of picture. Products resulting from the break of a second gene copy (result of translocation) are the one from der(21), on the *right side*, and second one from der(10), in the *left corner* (color figure online)

manufacturer's protocol. Screening for *FLT3-ITD* was performed by PCR according to the method of Kiyoi et al. [11], followed by electrophoresis on 3% agarose gel stained with ethidium bromide.

Results

Cytogenetic analysis of non-stimulated bone marrow cells revealed an abnormal karyotype with a novel balanced translocation involving chromosomal band 21q22 that disrupts the *RUNX1* gene, and 10q22, as well as loss of the X chromosome, resulting in the following karyotype: 45,X,-X,t(10;21)(q24;q22)[cp16]/46,XX[4] (Fig. 1c). In 67% of the 300 interphase cells analyzed, FISH analysis on metaphase cells showed three signals of AML1 probe, corresponding to *RUNX1* gene and two signals of ETO probe, corresponding to *RUNX1T1*, but no signals corresponding to *RUNX1-RUNX1T1* gene fusion (Fig. 1d). Corroborating these findings, molecular analysis did not detect *RUNX1-RUNX1T1* fusion transcripts. All cytogenetic and molecular analysis performed have ruled out the involvement of RUNX1 gene in the present report. In addition, the analysis of *FLT3* gene revealed no internal tandem duplication (ITD) of juxtamembrane domain, as well as, point mutations within the activation loop of the tyrosine kinase domain (TKD).

Discussion

RUNX1 gene codes for the CBFα2 subunit of the core binding factor complex, which binds to DNA and regulate expression of many genes involved in hematopoiesis including growth factors, surface receptors, signaling molecules, and transcription activators. In AML, t(8;21)(q22;q22) is the most common translocation involving *RUNX1* gene and is associated with favorable response to chemotherapy and extended survival. Additional chromosomal abnormalities, such as loss of sex chromosome,

deletion of the long arm of chromosome 9 and trisomy of chromosome 6, have been reported to accompany t(8;21)(q22;q22), without modifying risk group stratification and prognosis [2]. Complex variants of this translocation are occasionally observed in a small percentage of AML cases, most of them presenting three-way translocations and, rarely, a four-way variant complex translocation [7]. Similarly, the majority of these variant translocations have no prognostic implications in the clinical evolution of the patients [2].

In the present study, given the small number of reported cases with translocations involving the chromosomal regions 10q24 and 21q22, it seems premature to define the hematological characteristics and its implications in prognosis of the present case, but some important issues require particular attention. Reported cases with balanced chromosome aberrations involving 21q22 are mainly associated with therapy-related AML (t-AML) and myelodysplastic syndromes (t-MDS) [12–15]. Pui et al. [16] described the first case of leukemia harboring a chromosome structural abnormality involving 21q22 locus and the chromosome 10, leading to translocation t(10;21)(p12;q22). This abnormality was found in a t-AML patient with a prior history of acute lymphoid leukemia (ALL), treated with chemotherapy including epipodophyllotoxin and radiotherapy. In our case, the aberrations involving 21q22 locus were described in a 23-year-old woman with no prior history of cancer, myelodisplasia or toxin exposure, who never achieved remission despite intensive chemotherapy regimens and BMT, contrasting with good prognosis frequently observed in t(8;21)(q22;q22) AML cases.

The morphological picture presented by the patient differed from the commonly described features in t(8;21)(q22;q22) AML, mainly regarding the expression of the antigen CD19, frequently expressed in the classical t(8;21)(q22;q22) AML subtype, and was not detected in the blasts as determined by the immunophenotypic analysis. Moreover, no mutations involving the *FLT3* gene (*FLT3-ITD* and *FLT3-TKD*) were detected. *FLT3* gene, which encodes a class 3 of receptor tyrosine kinase, plays an important role in hematopoiesis and has been reported to be an independent prognostic marker in AML patients [17]. The present case also presented loss of a sex chromosome (45,X,-X,t(10;21)(q24;q22)[cp16]/46,XX [4]), which was described as the most common aberration in t(8;21)(q22;q22) [18].

Considering the differences in morphological, immunophenotypical, and molecular features between the reported case and the common t(8;21)(q22;q22) AML cases, we believe that the poor outcome observed here may be associated with the partner gene located at 10q24 chromosomal region. Specifically at this region, about 92 genes were identified, of which the majority have already been cloned and characterized according to biological function. Unfortunately, the partner gene that disrupted the *RUNX1* gene at 10q24 locus has not been identified. There are some potential candidate partner genes, all of them located at the 10q24 and somehow associated with hematological disorders, such as *retinol binding protein 4* (*RBP4*), TNF receptor superfamily member 6 (*FAS*), *interferon-induced protein with tetratricopeptide repeats 3* (*IFIT3*), *LIM domain binding 1* (*LDB1*), and *5′-nucleotidase, cytosolic II* (*cN-II*). Indeed, Galmarini et al. [19] demonstrated that high mRNA levels of *cN-II* and low mRNA levels of *cN-III* are correlated with worse clinical outcome and suggested that these enzymes may have a role in sensitivity to cytarabine in AML patients.

To our knowledge, the present patient represents the first case of de novo AML with t(10;21)(q24;q22). Given the frequent involvement of *RUNX1* in AML pathogenesis and the uncommon association with poor prognosis observed here, attention should be called to the better understanding of this new chromosomal abnormality in AML.

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