Gain of multiple copies of the \textit{CBFB} gene: a new genetic aberration in a case of granulocytic sarcoma

Mar Mallo\textsuperscript{a,b,*}, Blanca Espinet\textsuperscript{a}, Marta Salido\textsuperscript{a}, Ana Ferrer\textsuperscript{a}, Carmen Pedro\textsuperscript{c}, Carles Bessses\textsuperscript{c}, Encarna Pérez-Vilà\textsuperscript{a}, Sergi Serrano\textsuperscript{a}, Lourdes Florensa\textsuperscript{a}, Francesc Sole\textsuperscript{a,b}

\textsuperscript{a}Laboratory of Cytogenetics and Molecular Biology and Laboratory of Hematological Cytology (URNHE-IMAS/IMIM and URTTS-IMAS/IMIM), Pathology Service, Hospital del Mar, Passeig Marítim, 25-29, 08003 Barcelona, Spain

\textsuperscript{b}Department of Cell Biology, Physiology, and Immunology, Faculty of Life Sciences, Autonomous University of Barcelona, Bellaterra, Barcelona, Spain

\textsuperscript{c}Clinical Hematology Service, Hospital del Mar, Barcelona, Spain

Received 8 May 2007; received in revised form 27 July 2007; accepted 30 July 2007

Abstract

Granulocytic sarcomas (GS) are tumor masses of immature myeloid cells presenting at an extramedullary site, mainly the skin, bone, and lymph node. They are often associated with acute myeloid leukemia (AML) with monoblastic or myelomonocytic differentiation, including either AML M2 with t(8;21)(q22;q22) or AML M4Eo with inv(16)(p13q22). We present a case diagnosed with GS associated with AML M4 that presented a normal karyotype with conventional cytogenetic analysis. Although the myeloblasts did not show the inv(16)(p13q22) (\textit{CBFB}/\textit{MYH11}), a gain of multiple copies of the \textit{CBFB} gene was detected with fluorescence in situ hybridization analysis. To our knowledge, no cases with this rare genetic anomaly have been previously described. © 2007 Elsevier Inc. All rights reserved.

1. Introduction

Myeloid sarcomas, extramedullary myeloid tumors, or granulocytic sarcomas (GS) are tumor masses of immature myeloid cells in an extramedullary site. These tumors may develop de novo or concurrently with acute myeloid leukemia (AML) with monoblastic or myelomonocytic differentiation, including either AML M2 with t(8;21)(q22;q22) or AML M4Eo with inv(16)(p13q22). We present a case diagnosed with GS associated with AML M4 that presented a normal karyotype with conventional cytogenetic analysis. Although the myeloblasts did not show the inv(16)(p13q22) (\textit{CBFB}/\textit{MYH11}), a gain of multiple copies of the \textit{CBFB} gene was detected with fluorescence in situ hybridization analysis. To our knowledge, no cases with this rare genetic anomaly have been previously described. © 2007 Elsevier Inc. All rights reserved.
+8 (revealed by cytogenetic analysis of peripheral blood) [2].

Here we present the case of a patient with the diagnosis of GS, which exhibited a gain of multiple copies of the CBFB gene detected by FISH.

2. Case report

A 50-year-old woman was seen in the gynecology department of Hospital Verge Del Toro, Menorca, Spain, in July 2005 referring metrorrhagia, malaise, bone pain, and subcutaneous tumors in abdomen, limbs, and breasts. Neither lymphadenopathies nor visceromegalies were observed. A uterine mass was detected; biopsy revealed the presence of immature myeloid cells. The diagnosis of GS of the uterus was established.

The patient was referred to the hematology department of our hospital. The main laboratory findings (with normal values) were as follows: hemoglobin 111 g/L (130–170 g/dL), hematocrit 34% (40–54%), mean corpuscular volume 105 fl (82–97 fl), platelet count 167 × 10^9/L (150–450 × 10^9/L), and white blood cell count 4.3 × 10^9/L (4–11 × 10^9/L) without blast cells and lactate dehydrogenase serum level 760 IU/L (<450 IU/L). The hematocytic balance was normal.

The bone marrow aspiration was hypercellular and showed predominance of two populations: neutrophilic (35%, with 10% of blast cells showing Auer rods) and monocytic (31%, with 15% of blasts monocytoid). Cytochemistry study showed a result compatible with myeloid blasts. No eosinophilia was found.

Flow cytometry analysis of blast cells disclosed the presence of two populations. The first was positive for CD45 (weak), CD34, CD117, CD7, CD33, and CD15. The second was strongly positive for CD45, CD33, and CD15 and also expressed CD4, CD11b, and CD14; in addition, it was negative for CD34, CD7, and CD17. Both populations showed myeloperoxidase positivity. These results were in accordance with the presence of immature cells with myelomonocytic differentiation. The diagnosis of acute myelomonocytic leukemia (AML M4) subtype according to the FAB classification was established.

Conventional banding cytogenetic study was performed at diagnosis on a 24-hour bone marrow cell culture. Twenty metaphases were analyzed, and all showed a normal karyotype.

To rule out the pericentric inversion of chromosome 16, characteristic of AML M4 with eosinophilia (AML M4Eo), we performed FISH with LSI CBFB dual-color, break-apart rearrangement probe (Abbott Molecular–Vysis, Des Plaines, IL). We did not find cells displaying the inv(16), but did observe that, in 200 nuclei analyzed, 75% presented multiple copies of the CBFB gene: 49% with three copies, 15% with four copies, and 11% with five copies. A representative example of the CBFB gene gain of multiple copies is shown in Figure 1.

To confirm that the CBFB gene gain of multiple copies observed was not a gain of the whole chromosome 16, we applied FISH with an IGH/MAF dual-color, dual-fusion probe (Abbott Molecular/Vysis). This probe labels the MAF gene (16q23) in SpectrumGreen and the IGH gene (14q32) in SpectrumOrange. We observed only two copies of MAF (Fig. 2), suggesting gain of multiple copies of just the CBFB gene.

Reverse transcriptase–polymerase chain reaction (RT-PCR) analysis was performed to detect either CBFB/MYH11 or RUNX1 (previously AML1/RUNX1T1) (alias ETO) rearrangements, and was negative for both transcripts. Moreover, an analysis of the FLT3 gene duplication excluded the presence of duplication of the JM domain.

The patient received chemotherapy with IDICE (idarubicin, cytarabine, etoposide, and CSF) regimen and achieved a complete remission. At that time, new cytogenetic and FISH studies were performed. Conventional banding cytogenetic revealed a normal karyotype, and FISH analysis showed a gain of multiple copies of the CBFB gene, with a lower number (1.8% with >3 copies) of cells showing more copies of CBFB than those observed at diagnosis. These results are within normal for the FISH technique. A consolidation treatment with mitoxantrone and cytarabine was administered.

After this treatment, a new FISH analysis revealed persistence of the CBFB gene gain of multiple copies. Table 1 shows the results of conventional banding cytogenetic and FISH studies performed during the course of the disease.

Once the patient had finished the chemotherapeutic treatment, she underwent an allogeneic hematopoietic stem cell transplantation with a reduced-intensity conditioning regimen. However, she relapsed and died 7 months after diagnosis.

Fig. 1. Fluorescence in situ hybridization (FISH) interphase nucleus showing three copies (orange/green signals) of the CBFB gene (LSI CBFB dual-color, break-apart rearrangement probe; Abbott Molecular/Vysis, Des Plaines, IL) in a case diagnosed as acute myeloid leukemia M4 and granulocytic sarcoma (orange/green signals).
3. Discussion

GS has been described in association with a variety of chromosomal abnormalities [6,7]. GS occurs in ~18% of patients with t(8;21) AML [12]. Nevertheless, to our knowledge only 12 cases of inv(16) have been reported since 1988. In 11 of the cases, GS was located in the abdominal cavity (small intestine, ileum, mesentery, ovary, rectum, and jejunum); none of the 12 cases involved the uterus. Although there are several differences between lesions observed in t(8;21) and inv(16) AMLs, the pathogenesis could be related to regulation of CBFB transcription factor, involved in cell recognition and adhesion, thus resulting in leukemia infiltration [9]. Pathak et al. [13] reviewed 25 cases of GS of the cervix; three of them represented a relapse of an AML and nine of them were, after review, diagnosed as AML [13]. Recently, Garcia et al. [14] described 11 cases of GS involving the gynecologic tract; in 8 of them, the uterus was affected.

Here, we have described the case of a patient diagnosed as having a GS of the uterus and, simultaneously, with an AML M4. She did not exhibit the pericentric inversion of chromosome 16 [inv(16)(p13q22)], but FISH analysis revealed a gain of multiple copies of the CBFB gene.

It is often difficult to determine the chromosomal rearrangement of inv(16) by conventional banding cytogenetics, due to the presence of metaphase preparations of suboptimal quality in AML [15]. In this regard, molecular analysis with RT-PCR and FISH techniques are useful in allowing a precise diagnosis [16,17]. In the present case, we would not have detected the CBFB gain of multiple copies without the FISH analysis. This aberration might be present in other similar cases, but could go unnoticed unless FISH techniques are applied.

A normal karyotype in AML is suggestive of good prognosis. The reported case had no alterations revealed with conventional banding cytogenetics, but FISH analysis did reveal an aberration: a CBFB gene gain of multiple copies. This finding could help explain the prognosis and the clinical evolution of the patient. Nonetheless, any further cases with this rare cytogenetic aberration should be reported, in order to add to the understanding of the diagnosis and clinical implications.

For identifying the CBFB gene gain of multiple copies, we were not able to analyze a biopsy of the uterus tissue. Initially, the patient was diagnosed with GS and later with AML M4. The FISH study was performed on the bone marrow sample. Based on previous reports, we assume that AML and GS are the same lesion and so, in consequence, the genetics are the same for both. The literature does offer some confirmation of this idea. Lillington et al. [18] presented a cytogenetic and molecular study that showed evidence of marrow involvement in a GS. They studied tissue from a subcutaneous nodule and bone marrow and they identified the same alternation: an in-frame fusion of the MLL and MLLT10 (alias

**Table 1**

<table>
<thead>
<tr>
<th>Date (2005)</th>
<th>Clinical status</th>
<th>Karyotype</th>
<th>Nuclei with copies of CBFB, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3 copies</td>
<td>4 copies</td>
</tr>
<tr>
<td>October</td>
<td>Diagnosis</td>
<td>46,XX[20]</td>
<td>49</td>
</tr>
<tr>
<td>November</td>
<td>CR</td>
<td>46,XX[20]</td>
<td>1.6</td>
</tr>
<tr>
<td>December</td>
<td>CR</td>
<td>ND</td>
<td>0</td>
</tr>
</tbody>
</table>

All samples were bone marrow.

Abbreviations: CR, complete remission; ND, not done.
AF10) genes (interchromosomal exchanges between chromosomes 10 and 11). In this case, they also observed an intrachromosomal rearrangement of chromosome 5 [18].

Lee et al. [19] described a case of GS presenting as pneumonia in a child with t(8;21) AML (AML M2). They studied a lung biopsy specimen with FISH and identified a t(8;21)(q22;q22), which confirmed the retrospective diagnosis of a well-differentiated pulmonary GS [19].

Finally, Al-Quran et al. [20] presented a case of GS of the urinary bladder and epididymis as a primary manifestation of AML with inv(16). They performed conventional cytogenetic studies of marrow and demonstrated inv(16)(p13q22) in 4 of 20 metaphases. In addition, they studied the inv(16) by FISH in the bladder neoplasm. They confirmed the evidence of an associated myeloid neoplasm in marrow [20].

To better characterize the role of CBFB gene gain of multiple copies, we strongly recommend applying FISH techniques for patients with GS.

To our knowledge, we have presented here the first reported case of CBFB gene amplification in a patient diagnosed with AML M4 presenting with GS in the uterus, an aberration that has not been previously described, neither in GS nor in other pathologies.

Acknowledgments

This work was supported in part by grants from “Instituto de Salud Carlos III” of Spain (C03/07 and C03/10). We thank Carme Melero for her excellent technical assistance.

References