Novel der(1)t(1;19) in two patients with myeloid neoplasias

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Abstract

Cytogenetic studies can be useful in the clinical management of patients with leukemia. They may also give a clue to leukemogenesis and/or pathogenesis. Numerous disease-specific chromosomal aberrations have been and continue to be identified. Translocation (1;19)(q21~q23;p13.3) involving the long arm of chromosome 1 and the short arm of chromosome 19 is usually associated with acute lymphoblastic leukemia. We found a new translocation involving one virtually identical breakpoint 19p13 and one distinct 1p13 in two cases of myeloid neoplasms. Studies of bone marrow and peripheral blood specimens specified in one of our patients acute myeloid leukemia and in another myelodysplastic syndrome. Conventional cytogenetics was supplemented by spectral karyotyping (SKY), microdissection, and fluorescence in situ hybridization. Our first case showed a der(1)t(1;19)(p13;p13.1) as the sole chromosomal change. In addition to this translocation, a pericentric inversion within chromosome 10 and with a cryptic t(10;11) were detected by SKY in the second case. Translocation (1;19)(p13;p13.1) may play a role in the leukemogenesis of myeloid diseases. © 2002 Elsevier Science Inc. All rights reserved.

1. Introduction

Malignant lymphomas and most types of malignant solid tumors are characterized predominantly by complex karyotypes [1]. In contrast, a significant proportion of patients with myelodysplastic syndromes (MDS) or acute myeloid leukemias (AML) display only one chromosome abnormality in the leukemic karyotype [2]. Many structural and numerical chromosome aberrations were found recurrently [3]. Defined chromosome changes—especially translocations and inversions—in the leukemic cells have been demonstrated to be associated with acute leukemias, both lymphoblastic and myeloid lineages [4]. Because these changes generally have not been detected in nonhematologic neoplasms, they are thought to represent primary chromosome changes. The primary chromosomal change is the basis for a new classification proposal of the World Health Organization [5]. It plays a decisive role in affecting the biology and may yield clues to possible molecular events that underlie leukemia. It is also an index of the prognosis and nature of the disease.

Translocation (1;19) was first described by three independent groups of investigators [6–8], and is one of the most common acute lymphoblastic leukemia (ALL)-associated rearrangements [9]. Translocation (1;19) is generally associated with a pre-B-ALL phenotype, where it leads to a fusion of the E2A gene (from 19p13.3; this gene normally encodes immunoglobulin enhancer binding proteins e12 and e47) with the PBX1 homeobox gene (from 1q23). The E2A/PBX1 fusion mRNAs code for chimeric proteins that consist of the N-terminal, transcription-activating motif of e12/e47 and the DNA-binding homeodomain of PBX1 [10–12]. In contrast to PBX1, the fusion protein is a more powerful activator of the transcription. This evidence indicates that target genes of the PBX1, whose transcriptions are normally obstructed by PBX1, are activated by the fusion protein.

Another important translocation, t(11;19)(q23;p13), involves the short arm of chromosome 19. In cases of ALL, the MLL gene (from 11q23) fuses with the ENL gene (from 19p13.3), and in AML cases with the ELL gene (from 19p13.1) [13]. The MLL gene fuses with many other partners. This variability underlines the functional importance of the MLL part in the fusion protein. It is probably responsible for an unspecific DNA bond and subsequently for a transcriptional dysregulation of the DNA methylation.
Here we report two cases of myeloid neoplasias with a new translocation involving breakpoints at 1p13 and 19p13.1.

2. Materials and methods

A peripheral blood sample of a 13-month-old child with the diagnosis of acute monocytic leukemia (AML M5a), and a bone marrow sample of a 21-year-old woman with MDS, subtype refractory anemia with ringed sideroblasts (RARS) probably associated with a Fanconi anemia (FA), were analyzed. After 11 years, the latter patient is suspected of having FA. Cytogenetic analysis of lymphocytes after incubation with DNA cross-linking agents could not confirm the diagnosis of FA 11 years ago or now. Mosaicism is still discussed. A full blood count and differential white cell count were performed at presentation of the two patients. The hematologic data are listed in Table 1.

Immunophenotyping of the substitution blood of patient 2 showed expression of CD34, CD4, CD56, CD11b, CDw65, CD33, CD15, and HLA-DR. Ninety-nine percent of the cells were in good shape.

Bone marrow aspirates and peripheral blood were obtained as heparinized samples. Leukocytes were isolated using the “buffy coat” technique as previously described [14]. Samples were briefly cultured overnight and then exposed to Colcemid 30 minutes before harvest. After hypotonic treatment and fixation, GTG banding was used for karyotype analysis. At least 10 to 20 metaphases were analyzed. The karyotypes were reported according to the ISCN [15].

Spectral karyotyping (SKY) was performed as previously described [16]. Twenty-four differentially-labeled chromosome-specific painting probes (Applied Spectral Imaging, Mannheim, Germany) were hybridized onto metaphase spreads of the patient. Ten SKY images and corresponding inverted-DAPI images were acquired and analyzed.

Table 1

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age (year)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Prior therapy</th>
<th>WBC/μl</th>
<th>Hb (g/dl)</th>
<th>Blasts (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21/F</td>
<td>RARS</td>
<td>Erythrocyte transfusion</td>
<td>3200</td>
<td>50000</td>
<td>8.4</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1/F</td>
<td>AML M5a</td>
<td>None</td>
<td>265000</td>
<td>68000</td>
<td>7.0</td>
<td>85</td>
</tr>
</tbody>
</table>

Abbreviations: AML, acute monocytic leukemia; F, female; Hb, hemoglobin; Plt, platelet; RARS, refractory anemia with ringed sideroblasts; WBC, white blood cell.
Fluorescence in situ hybridization (FISH) was used to validate the results of conventional cytogenetics (CC) and SKY. FISH was performed according to the manufacturer’s instructions using commercially available probes. All FISH probes used were obtainable through Appigene-Oncor (Heidelberg, Germany).

Micro-FISH was performed with few changes as previously described by Senger et al. [17]. Three copies of the derivative chromosome 1 were briefly scratched and then amplified and labeled in two-step polymerase chain reaction (PCR) in the presence of biotin. The PCR product was denatured and hybridized onto metaphase spreads of a normal person. The detection occurred 1 day later.

Multiplex reverse transcriptase polymerase chain reaction (RT-PCR) was performed with specific primers according to the protocols from Pallisgaard et al. [18], to survey whether the $E2A/PBX1$ fusion had occurred or not. The cell line 697 from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany) was used as positive control.

3. Results

In both cases an additional chromosome consisting of material of the long arm of chromosome 1 and the short arm of chromosome 19 was detected using CC, SKY, FISH, and micro-FISH.

The der(1)t(1;19) was confirmed by FISH using whole chromosome painting probes (WCP) of chromosomes 1 and 19. FISH with a probe specific for the region 19q13.1 showed only two marks of the normal chromosome 19 in metaphase and interphase nuclei.

Micro-FISH showed hybridization marks of the scratched derivative chromosome 1 on the long arm and on a small portion of the short arm of chromosome 1 and also on the short arm of chromosome 19 (Fig. 3).

For both patients an $E2A/PBX1$ fusion transcript was not detected while using multiplex RT-PCR. Fig. 4 shows RT-PCR results using cDNA from patient 1. For patient 1, der(1)t(1;19) was detected as the sole chromosomal aberration in all 10 metaphases analyzed (Fig. 1).

In the second case, an inverted chromosome 10 was also seen by CC. Spectral karyotyping detected an additional cryptic reciprocal translocation of a portion of the long arm of the inverted chromosome 10 and the telomere of the long arm of chromosome 11 (Fig. 2). The latter translocation was confirmed by FISH using whole chromosome painting probes (WCP) of chromosomes 10 and 11 (Table 2).
The final karyotypes were described as: 47,XX, +der(1)t(1;19)(p13;p13.1) (case 1) and 47,XX, +der(1)t(1;19)(p13;p13.1), der(10)inv(10)(p2?q25)t(10;11)(q10;11)(q25;q25) (case 2).

4. Discussion

Cytogenetic studies can be useful in the clinical management of leukemias. They may also give a clue to leukemogenesis. The cytogenetic changes associated with leukemias can hint toward the genetic disorganization of malignant cells. We described two patients with myeloid neoplasias, one with AML and the other one with MDS—with a novel chromosome aberration having breakpoint regions which harbor genes such as N-ras at 1p13, ENL and E2A at 19p13.3, ELL at 19p13.1 or EEN andLYL1 at 19p13. E2A and LYL1 are involved in translocations described in ALL, whereas ENL and EEN rearrangements are involved in ALL and AML. MLL-ELL fusions are demonstrated only in AML.

To the best of our knowledge, this is the fourth report including patients with a t(1;19) in myeloid disorders, the first, however, with breakpoints at 1p13 and 19p13 respectively [19–21]. The first three reports include four cases of AML with breakpoints on chromosome 1 between 1q21 and 1q25, without rearrangement of the E2A gene in at least one case. Rearrangements of E2A were not studied in the other three cases. Translocation (1;19)(q23;p13.3) is considered as a specific aberration in ALL. It may occur in two principal forms: either as a balanced translocation in 25% of the cases or as an additional translocation chromosome in 75% of cases [22], like the translocation we described. A translocation with virtually the same breakpoint 1p13 as the one we described, t(1;22)(p13;q13), is reportedly associated with acute megakaryocytic leukemia in infant [23,24]. The role of new t(1;19)(p13;q13) in leukemogenesis and its prognostic significance can not be defined by means of only two cases.

Four years before the diagnosis of RARS, the first patient began to receive erythrocyte transfusions. This therapy is ongoing. Desferal therapy began 2 months after diagnosis. Three months later erythropoietin and G-SCF were added and as a result the search for a bone marrow donor has commenced.

Early exchange transfusion was done for the second patient to prevent hyperleukocytosis/hemostasis syndrome. The girl was then treated according to protocol AML-BFM-98 (Ritter and Creutzig, Treatment Protocol of the German Society of Pediatric Oncology and Hematology) and went into remission after the first chemotherapy block AIE. Unfortunately, a CNS relapse occurred before cranial irradiation according to protocol was instituted. Despite relapse treatment according to protocol AML-BFM-Rez-97, including CNS irradiation, bone marrow relapse occurred 5 months later and the girl died due to progressive disease 8 months after diagnosis.

The accumulation of more cytogenetic and clinical data on t(1;19)(p13;p13.3) in myeloid neoplasms, whose clinical significance is currently unknown, will hopefully enable evaluation of their prognostic significance in the future.

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References


