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Short communication

Acute myeloid leukemia (AML-M2) with t(5;11)(q35;q13) and normal expression of cyclin D1

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Abstract We report a case of acute myeloid leukemia (AML) subtype M2, with t(5;11)(q35;q13), in a 30-year-old man. Conventional cytogenetic, spectral karyotyping, and fluorescence in situ hybridization (FISH) studies on bone marrow sample obtained at diagnosis revealed an abnormal karyotype in all cells examined. FISH analysis demonstrated absence of translocations in the region of the cyclin D1 gene and real-time quantitative reverse transcriptase–polymerase chain reaction revealed normal expression of this gene. Similar to the 11q23 region, 11q13 changes can be found in both myeloid and lymphoid neoplasias with different chromosomes serving as donors in translocations.

1. Introduction

Cytogenetic analysis is considered the most important independent prognostic parameter in acute myeloid leukemia (AML) [1,2]. Chromosomal abnormalities also provide useful information for monitoring residual disease [3]. Most of the chromosomal abnormalities are detectable by classical cytogenetic analysis, and they occur in approximately 55% of de novo AML in adults [4,5]. Some chromosomal aberrations in AML are recurrent and closely associated with specific cytomorphological subtypes according to French–American–British (FAB) criteria [6–9]. However, 5–10% of AML patients do not have leukemia-specific aberrations at diagnosis and present with multiple chromosomal rearrangements involving three or more chromosomes. These patients usually have a poor prognosis, and it is likely that some of these rearrangements contribute to their disease progression [2].

The incidence of normal karyotypes in AML is as high as 42% in some investigations [3]. In a small percentage of these karyotypes, specific chromosomal rearrangements may be missed due to technical factors, such as poor chromosome morphology. Even in satisfactory cytogenetic preparations, however, G-banding analysis suffers from a limited resolution such that rearrangements <3 Mb in size may be impossible to detect. Molecular cytogenetic approaches such as fluorescence in situ hybridization (FISH) have demonstrated that a small percentage of apparently normal karyotypes may contain cryptic versions of known recurrent translocations, generated by submicroscopic insertions or more complex rearrangements [10].

Using classical cytogenetic methods, spectral karyotyping (SKY), FISH, and real-time quantitative reverse transcriptase–polymerase chain reaction (RT-PCR), we identified and here report an example of acute myeloid leukemia, subtype M2, with an 11q13 anomaly in the form of a translocation, t(5;11)(q35;q13), with normal expression of cyclin D1 (*CCND1*) and, as determined by FISH, absence of translocations involving *CCND1* in the more distal *MLL* gene. To our knowledge, this is the first occurrence of this specific translocation in association with a case of AML M2.

2. Case report

A 30-year-old man presented with dyspnea for 1 month and subsequently was admitted to the Hematology Service,

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Faculty of Medicine of Ribeirão Preto, Brazil. Peripheral blood counts were: hemoglobin 4.9 g/dL, leukocytes 29.5 × 10^3/mm^3 (with 45% of blasts), and platelets 62 × 10^3/mm^3. Bone marrow examination showed a hypercellular marrow with 92% blasts positive for myeloperoxidase and negative for esterase. Immunophenotypic studies showed HLA-DR$^+$, CD13$^+$, CD19$^-$, CD33$^+$, CD45$^+$, CD34$^+$, and CD117$^-$. A diagnosis of acute myeloid leukemia with maturation (M2) was made according to the WHO classification. The patient was submitted to induction chemotherapy with daunorubicin and cytarabine, but obtained only partial remission after two cycles of chemotherapy (10% of blasts in bone marrow after second induction). He was then submitted to allogeneic stem cell transplantation from his HLA-identical sister.

3. Materials and methods

Bone marrow aspirate withdrawn at time of diagnosis was used for classical and molecular cytogenetic analysis after a 48-hour nonstimulated culture in RPMI 1640 medium with 20% fetal calf serum. Classical cytogenetic (G-banding) and SKY analysis were performed, and the karyotypes were described according to ISCN 2005 [11].

The SKY assay was performed using the SKY probe cocktail (Applied Spectral Imaging [ASI], Migdal Ha'Emek, Israel) according to the manufacturer's instructions. Image acquisition was performed using a SD200 SpectraCube system (ASI) mounted on an Olympus BX60 microscope with a custom-designed optical filter (SKY-1; Chroma Technology, Brattleboro, VT). SKY analysis was performed with SkyView 1.6.2 software (ASI, Carlsbad, CA). FISH analyses were performed on the same cell preparations, using commercial dual-color probes for the *MLL* (LSI MLL, Abbott Vysis, Des Plaines, IL) and *CCND1* genes (LSI IGH/CCND1 XT dual color, Abbot Vysis), and also chromosome 11 painting probe (WCP 11 SpectrumOrange, Abbot Vysis).

Reverse transcription was performed with 2 μg of total RNA using the ABI high capacity cDNA archive kit (Applied Biosystems [ABI]) [12]. For real-time PCR experiments, we used an ABI Prism 5700 sequence detection system device under standard thermal cycling conditions [12]. PCR reactions were prepared in replicates at a final volume of 20 μL, as described [13]. The maximum coefficient of variation allowed between replicates was 2%; otherwise, experiments were repeated.

For quantitative analysis of the *CCND1* gene (cyclin D1), we used commercially available TaqMan probes and primers (Assays-on-Demand; Applied Biosystems), comparing experimental levels with standard curves obtained by serial dilutions of cDNA from the Granta-519 cell line, which was also used as the calibrator. The normalization factor was the geometric mean of the glyceraldehyde-3-phosphate dehydrogenase gene (*GAPDH*).

4. Results

Cytogenetic analysis in bone marrow cells at the time of diagnosis revealed the presence of a translocation involving chromosomes 5 and 11, with the karyotype established as 46,XY,t(5;11)(q35;q13) in 20 analyzed metaphases (Fig. 1). SKY study confirmed the abnormality previously seen by G-banding, and a minimum of 10 metaphases were analyzed (Fig. 2A). Dual-color FISH analysis with the *MLL* probe in metaphase cells recognized homology of two chromosomes: the normal 11, t(5;11), and confirmed that the *MLL* gene was not disrupted (data not shown). Dual-color FISH analysis with the *IGH*/*CCND1* probe showed no fused red–green signals and revealed no rearrangement of the *CCND1* locus (Fig. 2B). A translocation t(5;11)(q35;q13) was further confirmed with FISH analysis using WCP 11 probe (Fig. 2C). Real-time PCR experiments to evaluate the relationship between *CCND1* rearrangement and cyclin D1 expression, showed normal expression of

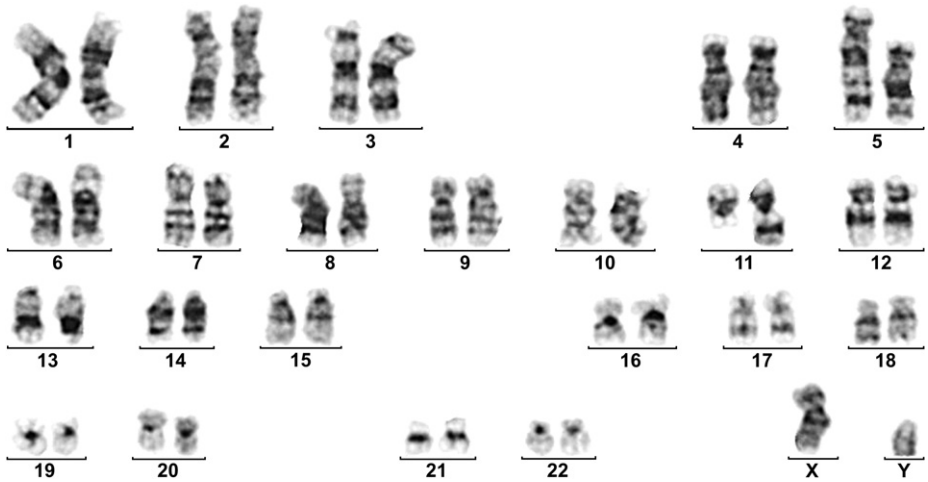

Fig. 1. Karyotype showing t(5;11)(q35;q13) (G-banding with trypsin–Giemsa).

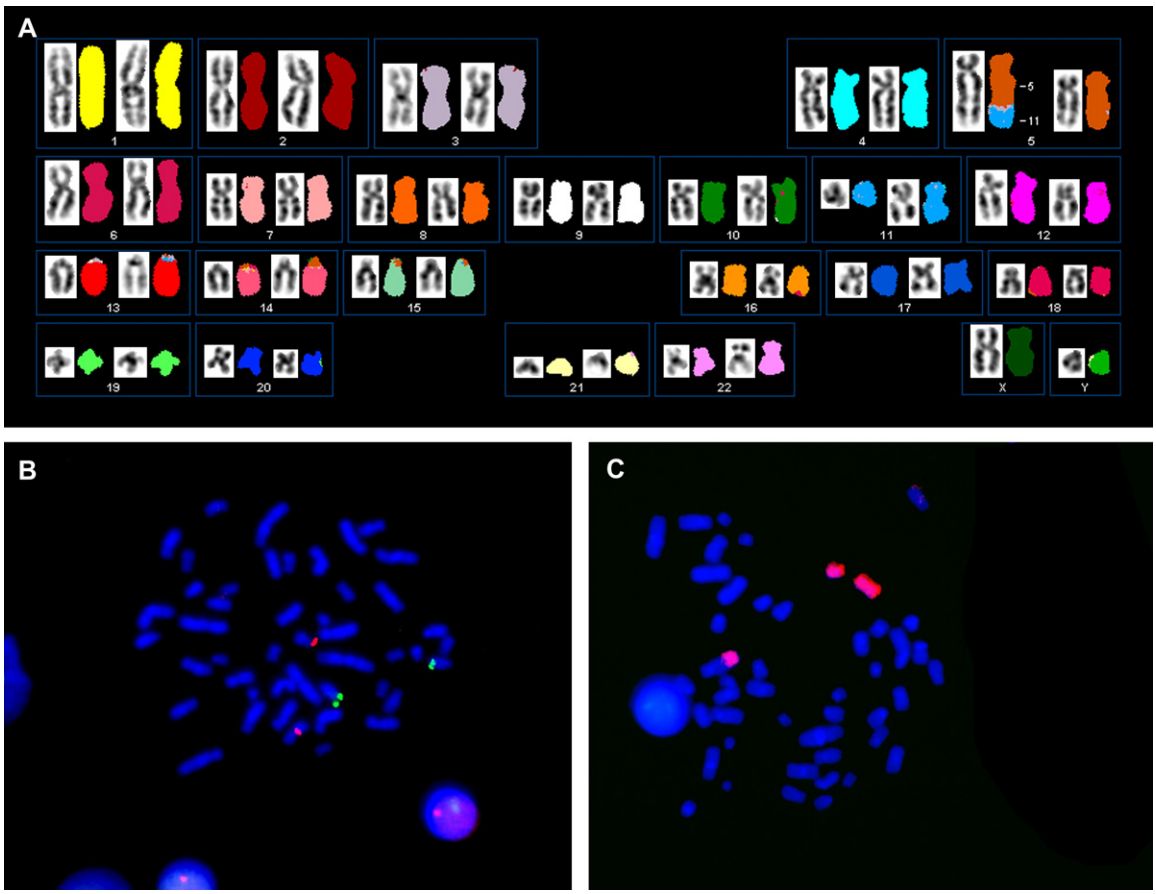

Fig. 2. (A) SKY karyotype from a leukemia cell at time of diagnosis, showing the inverted DAPI (left) and the classified (right) profiles for each chromosome. Translocation of chromosome 11 on chromosome 5, t(5;11)(q35;q13) is illustrated. (B) Metaphase chromosomes hybridized with the *IGH/CCND1* probe (LSI IGH/CCND1 XT dual color; Abbott Vysis, Downers Grove, IL). The *CCND1* segment is labeled in SpectrumOrange and the *IGH* segment in SpectrumGreen. The normal chromosome 11 and the der(11) showed a red signal, and both chromosomes 14 (14q32) showed a green signal. These findings confirm that *CCND1* gene was not disrupted by the t(5;11)(q35;q13). (C) Whole chromosome painting probe for chromosome 11 (WCP 11 SpectrumOrange; Abbott Vysis) confirmed the translocation t(5;11)(q35;q13).

cyclin D1 (data not shown). Classical cytogenetic analysis performed after chemotherapy management and bone marrow transplantation revealed karyotypes 46,XY[20] and 46,XY[15]//46,XX[15], respectively. Five months after diagnosis, cytogenetic studies performed on bone marrow cells revealed the karyotype 46,XY,t(5;11)(q35;q13)[13]//46,XX[7]. At this time the patient was considered in morphological remission.

5. Discussion

Rearrangements of 11q are known to be associated with acute leukemia, with the breakpoints clustered mainly to the 11q23 region [14]. 11q13 has been found to be frequently rearranged in AML. In fact, it has been suggested that 11q13 translocations are recurrent karyotypic aberration associated with AML of monocytic lineage [15]. *CCND1* (previously *PRAD1, BCL1*), the gene encoding cyclin D1, is located at 11q13. Cyclin D1 is a 34-kDa protein localized to the nucleus and participates in the regulation of the cell cycle. Overexpression of cyclin D1 represents one of the common genetic alterations in human neoplasia, leading to a change in G1–S transition and enhanced cell growth. Structural abnormalities involving the 11q13 locus or *CCND1* have been reported in a variety of human neoplasms [16]. Cyclin D1 overexpression analysis suggests that the gene or genes on 11q13 that could be involved in the pathogenesis of the disease are located near *CCND1* gene [17].

The t(5;11)(q35;q13) has been observed as sole abnormality in at least three independent cases of oncocytomas, and as such, should be considered as a primary chromosomal change [17]. The finding of cyclin D1 overexpression in cases with 11q13 translocations suggests that this translocation is involved in dysregulation of cyclin D1 [18]. On the other hand, no overexpression of cyclin D1, as seen in the present study suggests that this is not an inevitable outcome of this abnormality, and that other genes may be involved. Jaroslav et al. [19] demonstrated that the level of cyclin D1 negatively correlates with the proliferation

properties of leukemic cells. No significant relationship was found between cyclin D1 expression in AML patients and their clinical outcome [19].

Abnormalities associated with the long arm of chromosome 5, especially deletion of 5q, have been frequently found in hematological malignancies including acute leukemia and myelodysplastic syndrome (MDS) [20,21]. Chromosome segments or bands lost due to unbalanced structural abnormalities in at least 5% of the cases were 5q13~33 in AML, and 5q13~35 in MDS [20]. Translocations between chromosomes 11q and 5q, however, have been rarely reported. One case with t(5;11), infant acute leukemia (AMMoL, M4 subtype) reported by Sorensen et al. [22], displayed a molecular rearrangement of 11q23.

Our investigation provides additional evidence that apart from 11q23, 11q abnormalities also cluster around the more proximal region of 11q, at 11q13. Similar to the 11q23 region, the literature indicates that 11q13 changes can be found in both myeloid and lymphoid neoplasia with different chromosomes serving as donors in translocations. We have presented a case of AML M2 with t(5;11)(q35;q13). More cases with this abnormality will be required to identify causative genes and define their role in leukemogenesis.

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