ELSEVIER

Cancer Genetics and Cytogenetics 198 (2010) 62–65

Cancer Genetics and Cytogenetics

Short communication

Segmental amplification of *MLL* gene associated with high expression of *AURKA* and *AURKB* genes in a case of acute monoblastic leukemia with complex karyotype

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Received 10 August 2009; received in revised form 28 November 2009; accepted 8 December 2009

Abstract We report a case of acute monoblastic leukemia showing a jumping translocation with the *MLL* gene in a 17-year-old male. Classic cytogenetic and spectral karyotyping revealed a complex karyotype, and fluorescence *in situ* hybridization (FISH) demonstrated amplification of the *MLL* gene followed by translocation to chromosomes 15q, 17q, and 19q. In addition, molecular analyses showed a high expression of *AURKA* and *AURKB* genes. It is already known that overexpression of Aurora kinases is associated with chromosomal instability and poor prognosis. The formation of jumping translocations is a rare cytogenetic event and there is evidence pointing toward preferential involvement of the heterochromatin region of donor chromosomes and the telomere ends of recipient chromosomes. Jumping translocation with the *MLL* gene rearrangement is an uncommon phenomenon reported in leukemia cytogenetics.

1. Introduction

Jumping translocations (JT) are chromosomal rearrangements involving one donor chromosome and several recipient chromosomes [1]. First described in a patient with Prader–Willi syndrome by Lejeune et al. [1], JT are present in patients with constitutional disease and are rare in cancer cytogenetics [2]. Acquired JT were also seen in hematologic disorders including lymphomas, myeloid disorders, and lymphoid diseases [3,4]. It has been shown that some chromosomal regions are not randomly involved in some malignancies, such as chromosome bands 1q12~q21 as donors and telomeric regions as recipients [4]. There is evidence pointing toward the preferential involvement of some heterochromatin region of donor chromosomes [1] and the telomere ends of recipient chromosomes [1]. Shortened telomeres have been linked to the generation of JT in a case of acute myelomonocytic leukemia [5].

Aurora kinases are a recently discovered family of kinases consisting of highly conserved serine/threonine protein kinases found to be involved in multiple mitotic events: regulation of spindle assembly checkpoint pathway, function of centrosomes and cytoskeleton, and cytokinesis. Aurora kinase overexpression leads to genomic instability and triggers the development of tumors. In fact, the altered expression of these genes has been implicated in the pathogenesis of various cancers, including those of the breast, pancreas, colon, ovary, and urinary bladder [6]. In hematologic malignancies, an overexpression of Aurora kinase has also been observed widely [7].

In this investigation, we identified *MLL* gene amplification consistent with segmental JT and overexpression of *AURKA* and *AURKB* in a pediatric case of acute monoblastic leukemia with a complex karyotype. The mechanism for gene amplification in leukemias with JT still remains unknown, and its association with complex karyotypes and high expression of aurora kinases suggests an important role for genome architecture in the arising and maintenance of genomic instability.

2. Materials and methods

2.1. Case report

In August 2008, a 17-year-old male was admitted to our institution to treat respiratory failure caused by extensive

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0165-4608/10/$ – see front matter
doi:10.1016/j.cancergencyto.2009.12.005

bilateral pneumonia. He had complained of fever, fatigue, and clinical manifestations of purpura for 1 week before admission. The blood analysis revealed hemoglobin 9.1 g/dL, white blood cells 14.8×10^9/liter, and platelets 14.0×10^9/L. The distribution of white cells were as follows: 18% blasts, 2% metamyelocytes, 2% myelocytes, 3% band neutrophils, 29% segmented neutrophils, 4% monocytes, and 42% lymphocytes. A bone marrow aspirate for morphologic, immunophenotypic, and cytogenetic analyses was collected. The morphologic revealed an extensive infiltration by blasts with monoblastic appearance, negative for myeloperoxidase (cytochemical stain) and positive for chloroacetate esterase (82%). The blasts were positive for CD13 and HLA-DR, CD33, CD11b, CD14, CD34 (35%), and negative for CD19, CD2, CD4, and CD117. Cytogenetic studies were performed on a nonstimulated culture of bone marrow in RPMI 1640 medium with 20% fetal calf serum. Chromosome preparations were obtained by using standard procedures, and subsequent cytogenetic analysis and interpretation were made according to the International System for Human Cytogenetic Nomenclature (ISCN) 2005 [8]. The following karyotype was established: 50,XY, t(3;10)(q27;p12),der(11)ins(11;3)(q23;?)ins(11;10)(q23;?), +4,+5,+8,+16[cp20] (Fig. 1A). Slides for spectral karyotyping (SKY) were prepared by using the same fixed chromosome preparations and stored at −20 °C, as done for G-banding analysis. Chromosome labeling was performed with the SKY fluorescent labeling kit (Applied Spectral Imaging, Migdal HaEmek, Israel) according to the manufacturer's protocol. Image acquisition was performed with a SD200 Spectracube (Applied Spectral Imaging, ASI) mounted on an Axio Imager M2 microscope using a custom-designed optical filter (SKY-1; Chroma Technology, Brattleboro, VT). Automatic identification of chromosomes was based on the measurement of the spectrum for each chromosome. At least 20 metaphases were analyzed using SkyView software (version 5.5; ASI, Carlsbad, CA). Spectral karyotyping analysis confirmed abnormalities previously seen and identified a complex rearrangement involving chromosomes 3, 10, and 11 (Fig 1B). Fluorescence *in situ* hybridization (FISH) analysis was performed on the same cell preparations using commercial dual-color probes for the *MLL* gene (LSI MLL; Abbott Molecular, Des Plains, IL) according to the manufacturer's instructions. The cells were counter-stained with DAPI and viewed on an Axio Imager M2 microscope equipped with FISHView software (version 5.5; ASI). MLL FISH analysis showed that the relocated (''jumped'') segments included the *MLL* signal (red and green) and were translocated to the distal region of chromosomes 15q, 17q, and 19q in 18/20 cells examined (Fig. 1C). For *AURKA* and *B* gene expression, total RNA from bone marrow aspirate cells was isolated using Trizol reagent (Invitrogen, Carlsbad, CA). Complementary DNA (cDNA) was synthesized from 1 μg of total RNA using a High Capacity cDNA Reverse Transcription Kit (Applied BioSystems, Foster City, CA) following the manufacturer's instructions. *AURKA* and *B* and *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH* - #4308313) were used as endogenous internal controls, and were analyzed in duplicate in the same MicroAmp optical 96-well plates using a 7500 Real-Time PCR System (Applied BioSystems). The primers and probe for Aurora kinase genes were developed by Assay on Demand (*AURKA*: Hs00269212_m1, *AURKB*: Hs00177782_m1; Applied BioSystems). All experiments were carried out in duplicate. The comparative cycle threshold (Ct) method was used to determine the relative expression level of *AURKA*/*B*. The differences of cycle threshold (ΔCt) were derived by subtracting the Ct values of each evaluated genes from the Ct value of the *GAPDH* gene. The *ARUKA*/*B* gene expression of bone marrow mononuclear cells (BMMC) from a healthy donor ($n = 9$) was also evaluated and used as reference. Relative *AURKA*/*B* expression values were calculated as a relative quantification, using the ΔCt from the patient subtracting the ΔCt from median of the BMMC (ΔΔCt) and expressed as $2^{-\Delta\Delta Ct}$. The relative expression of *AURKA* and *AURKB* was higher in the leukemic sample compared with the median of gene expression of BMMC from the healthy donor (*AURKA* [fold change]: 73.91 vs. 1.6 ± 1.24, $P = 0.015$; *AURKB*: 48.92 vs. 1.1 ± 0.47, $P = 0.005$).

3. Results and Discussion

We applied FISH analysis to determine if the *MLL* gene was involved in a complex structural abnormality involving chromosomes 3q27, 10p12, and 11q23 in a pediatric patient with diagnosis of acute monoblastic leukemia. However, the analyses showed an amplified *MLL* gene translocated to the distal region of chromosomes 15q, 17q, and 19q, as well as the absence of rearrangements involving *MLL* with the other gene. According to the literature, there are 12 reports of JT in acute myeloid leukemia, and 7 of these are in acute monocytic leukemia [1]. Our patient represents the youngest case of acute monoblastic leukemia with a jumping translocation involving the *MLL* gene.

JT are considered rare findings in hematologic cancers and almost always are associated to the long arm of chromosome 1. These abnormalities involve at least one breakpoint located at a region rich in repetitive DNA, suggesting that the mechanism may be related to recombination between regions with homologous DNA sequences [1]. The features of constitutional and acquired JT, mainly those of hematopoietic malignancies, were compared in a recently published review [1]. Despite the heterogeneity of diseases and the types of cytogenetic abnormalities, the main differences between acquired and constitutional abnormalities included the localization of chromosome breakpoints of donor chromosomes, most often located in the pericentromeric region of the long arm of chromosome 1 in acquired aberrations, 1q10~1q11 breakpoints, while being more

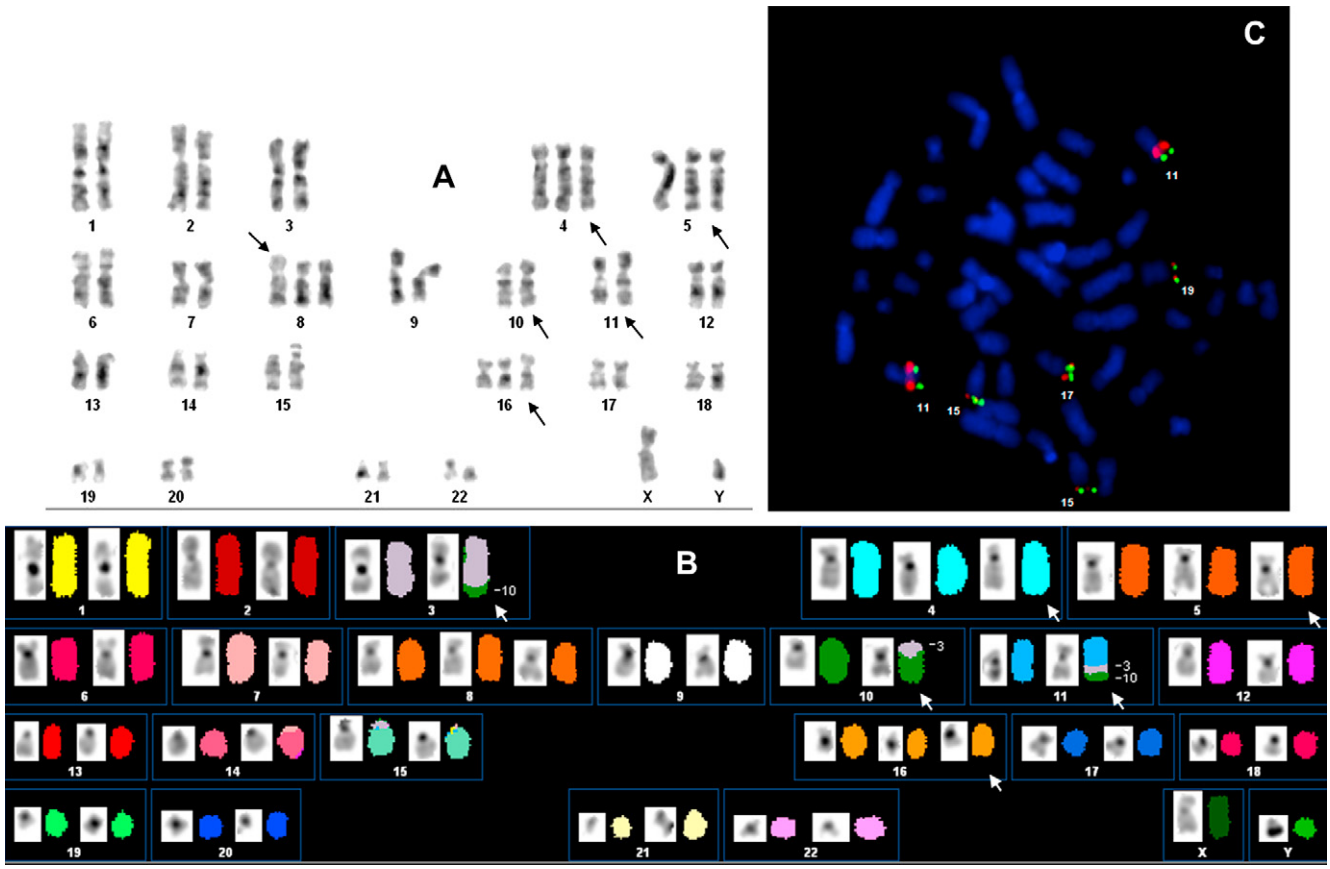

Fig. 1. (A) GTG-karyogram from a leukemia cell at the time of diagnosis showing extra copies of chromosomes 4, 5, 8, and 16, as well as a t(3;10)(q27;p12) and a der(11)ins(11;3)(q23;?)ins(11;10)(q23;?) (arrows). (B) SKY karyogram from a leukemia cell at time of diagnosis showing the inverted DAPI (left) and the classified (right) profiles for each chromosome. Translocation (3;10)(q27;p12),der(11)ins(11;3)(q13;?)ins(11;10)(q23;?) and extra copies of chromosomes 4, 5, 8, and 16 are illustrated (arrows). (C) Metaphases chromosomes hybridized with the *MLL* gene probe (LSI MLL; Abbott Molecular). The probe recognized homology of the two normal chromosomes 11 as well as translocation of part of the *MLL* gene to the distal region of chromosomes 15q, 17q, and 19q (arrows).

widely distributed among chromosome pairs in constitutional abnormalities [1].

Different conservation of telomeric DNA repeats in both constitutional and acquired chromosome abnormalities suggest different mechanisms in the two types of rearrangements. The partial conservation of such sequences in hematopoietic disorders and their loss in others may result from chromosome instability associated with progression of the malignant process. It has been shown that telomeric regions of human chromosomes show high frequency of mitotic recombination [9] and the mechanisms of DNA repair are less efficient in human chromosome 1 heterochromatin [10]. In tumor cells, the shortened telomeres may become favorable the presence of JT [11]. The epigenetic status of DNA sequences may play a role in the decondensation of heterochromatin of pericentromeric regions, particularly of chromosome 1, as has been shown in some cases [11]. It is possible that somatic telomeric recombination is a common phenomenon acting to maintain telomeric integrity, whereas somatic recombination between a normal telomere and an abnormal interstitial telomere may result in the aberrant behavior of JT. Thus, internalization of telomeric sequences by inversion or intrachromosomal insertion, for example, will lead to a jumping translocation.

In addition, we demonstrated an overexpression of *AURKA* and *AURKB*. These genes are members of the serine/threonine kinase family involved in multiple mitotic events [6] and their overexpression has been linked with centrosome amplification, aneuploidy, and chromosomal instability [7]. In fact, *AURKA* is a key regulatory component of the *TP53* pathway, as its overexpression leads to increased TP53 degradation, thus facilitating oncogenic transformation [12]. Moreover, *AURKA* expression in tumors is often associated with poor histologic differentiation and poor prognosis [13]. Recent studies have revealed that *AURKA* and *B* are frequently overexpressed in various types of leukemia, including acute myeloid leukemia (AML), acute lymphoblastic leukemia, and chronic myeloid leukemia [14]. In a recent investigation (data still not published), our group demonstrated a significant, higher-expression *AURKA* and *B* associated with unfavorable cytogenetic findings in AML samples. Interestingly, the higher expression was not

observed in intermediate/favorable cytogenetic groups, which had a similar or even lower expression compared with BMMC healthy donors. Some investigations have demonstrated a connection between aneuploidy and genomic instability, cell malignant transformation, and tumorigenesis. It is believed that aneuploidy is caused by abortive cytokinesis as a result of deregulation of mitotic regulator proteins. Previous experiments have shown that *AURKA* overexpression leads to premature anaphase entry, which subsequently results in centrosome amplification and aneuploidy, which in turn result from incomplete cytokinesis [7].

It is possible that somatic telomeric recombination is a common phenomenon acting to maintain telomeric integrity, whereas somatic recombination between a normal telomere and an abnormal interstitial telomere may result in the aberrant behavior of JT. Thus, internalization of telomeric sequences by inversion or intrachromosomal insertion, for example, will lead to a genomic instability.

Acknowledgments

This investigation was supported by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo, Proc. 07/52462-7) and the National Institute of Science and Technology in Stem Cell and Cell Therapy, Ribeirão Preto (São Paulo, Brazil).

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