Partial duplication of the MLL oncogene in patients with aggressive acute myeloid leukemia

Background and Objectives. MLL translocations generate a fusion gene between the 5' end of MLL and the 3' end of different partner genes. Several chromosomal mechanisms including complex and cryptic changes lead to these recombinations. Our objective was to analyze the molecular composition of chromosomes in complex karyotypes with specific MLL translocations.

Design and Methods. Fluorescence in situ hybridization (FISH) was performed in two acute leukemias (AL), one acute myeloid leukemia (AML) M5a, and one treatment-related-AL (t-AL), to investigate the nature of complex changes accompanying the respective t(9;11)(p22;q23)-MLL/AF9 and t(11;16)(q23;p13.3)-MLL/CBP.

Results. In the case with the MLL/AF9 chimeric transcript, duplication of a der(1) originated from an additional unbalanced translocation between the der(9)t(9;11) and a chromosome 1. The 5'AF9/3'MLL chimeric gene was present on both der(1). In the second case, there was a t(11;16)(q23;p13.3) producing one der(11) and two der(16) which derived from both homologs. One der(16) was present in multiple copies all containing the 5'CBP/3'MLL fusion gene.

Interpretation and Conclusions. In both cases the 3' end of MLL was present in multiple copies. Mitotic recombination and non-disjunction may underlie the extra derivatives in both cases. In this genomic imbalance not only the 5'MLL but also the 3'end of MLL could play a critical role in the leukemic process.

Key words: AML, MLL gene, 3'MLL, t(11;16)(q23;p13.3), t(9;11)(p22;q23).

The MLL gene, located at 11q23 and also known as the ALL1, HTRX1, or HRX gene, is consistently rearranged in human leukemia. It may recombine with a wide variety of partner genes to express different chimeric proteins. The different MLL partners may have a specific role in determining the leukemic phenotype.1,2

The leukemic phenotype AML-M5a is usually associated with t(9;11)(p22;q23) which results in fusion of the MLL gene with the AF9 gene.3 Treatment-related acute leukemias are associated with t(11;16)(q23;p13.3)4 which leads to the MLL gene fusing to CBP at 16p13.3.5,6

We report two cases of acute leukemia: one AML-M5a with t(9;11) (p22; q23) and one biphenotypic treatment-related acute leukemic-AL with t(11;16) (q23; p13). In both cases the MLL translocation was followed by complex events leading to duplication of the fusion gene at the 3' end of MLL. In the case with t(9;11), the der(9)t(9;11) was involved in an unbalanced translocation with chromosome 1, and the derivative 1 was duplicated. The case with a t(11;16) (q23;p13.3), showed two derivative 16, i.e. der(16)t(11;16), one of which underwent further misdivisions with 2-5 replicates.

Design and Methods

Patient #1
A 9-month old girl presented with multiple skin lesions. Skin biopsy revealed blast infiltration with positivity for CD45, CD34 and CD56 antibodies. Bone marrow aspirate showed 31% blasts with weak peroxidase positivity and strong butyrate esterase positivity. A diagnosis of AML-M5a was made on morphology and cytochemistry. Reverse
transcription polymerase chain reaction showed the MLL-AF9 fusion transcript. The patient was treated with aracytine and mitoxantrone as induction therapy under the LAME 91 protocol. Complete hematologic remission was achieved after one month. Skin lesions dramatically decreased after consolidation therapy with arac-tyrine and L-asparaginase.

Eight lumbar puncture injections with aractyline, methotrexate and steroids were given as prophylaxis against central nervous system involvement. The patient underwent an autologous transplant, but two months after hematologic reconstitution she again developed cutaneous lesions. Skin biopsy revealed morphologic and molecular relapse of AML. A bone marrow biopsy was negative. After second line treatment with FLAG-IDA, the patient underwent bone marrow transplantation from an unrelated donor, but a few days after hematologic reconstitution she again showed new skin lesions. She was treated with oral etoposide, but after several months died of progressive AML involving the skin and bone marrow.

Patient #2

A 23-year-old man with severe hemophilia A had been HIV positive for fifteen years. He had been under antiretroviral treatment with nucleoside reverse transcriptase inhibitors for five years and protease inhibitors for three. In 1997 he developed a non-Hodgkin’s lymphoma (NHL) but achieved remission after six cycles of chemotherapy with cyclophos-phamide, doxorubicin, vincristine and prednisone (CHOP) and radiotherapy of the central nervous system. Thirty months later he presented with febrile cytopenia. A bone marrow aspirate showed large and small-sized blasts with high nuclear-cytoplasmic ratio and scant cytoplasm. The cells had irregular nuclei with fine chromatin and one or more prominent nucleoli. Some blasts had cytoplasmic azurophilic granulation. Positive monoclonal antibodies included: CD19 76%, CD34 73%, CD33 63%, HLA-DR 65%, MPO 36%, Tdt 59%. The patient was refractory to treatment and died three months after diagnosis.

Cytogenetics

In both patients when AL was diagnosed, chromosome studies were performed on unstimulated bone marrow cells cultured for 24 and 48 hours. Cytogenetic analysis was carried out on G- or R-banded chromosome preparations and karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN 1995).

FISH

Patient #1. The MLL gene was investigated by applying an MLL commercial probe (Oncor, Appligene, Gaithersburg, MD 20877, USA). Telomeric probes for 9p (TelVysion 9p) and 11q (TelVysion 11q) and centromeric probes for #9 (CEP 9) and #11 (CEP 11) were applied and the SpectraVysion Assay, for multicolor-FISH (M-FISH), was used according to the manufacturer’s instructions (all from Vysis, Abbot GmbH and Company, KG Diagnostika, 65205 Wiesbaden-Delkenheim, Germany).

Patient #2. FISH was done as already described using probes for the ATM (PAC 891P24) and the PLZF genes (BAC 980J15) (both kindly provided by Dr. M. Rocchi, University of Bari, Italy) at the 11q23 region. The MLL gene was investigated by applying two PAC clones that were specific for the 5’ PAC 167K13 (kindly provided by Dr. E. Schuuring, University of Leiden, The Netherlands) and for the 3’ ends PAC 217A21 (kindly provided by Dr. E. Schuuring, University of Leiden, The Netherlands).

The 16p13 breakpoint was studied with cosmids RT 53-191-203-153 for the CBP gene, and cosmids ZIT 27/29 and cosmids ZIT 14/18 for the 3’ end and the 5’ end of MYH11, respectively (all kindly provided by Dr. F. Birg, University of Marseille, France). Probes for the alphoid sequences of chromosome 11 (D11Z1) (Oncor, Appligene, Gaithersburg, MD, USA) and for the heterochromatin of chromosome 16 (pHUR195) (kindly provided by Dr. M. Stul, University of Leuven, Belgium) were applied. In order to characterize the der(14), probes for the IgH locus (cosmid a1 and cosmid U2-2, kindly provided by Dr. E. Schuuring, University of Leiden, The Netherlands) and whole chromosome paints (WCP) for #14 and #21 (Oncor, Appligene, Gaithersburg, MD, USA) were used. At least eight metaphases were analyzed using a fluorescence microscope (Provis, Olympus) equipped with a cooled CCD camera Sensys (Photometrics, Tucson, AZ, USA) run by Pathvysion software (Vysis, Stuttgart, Germany).

Results

Cyto genetic s

Patient #1. At diagnosis, cytogenetic analysis detected the following karyotype: 46, XX[4/19]47,XX, add(1)(p35), +add(1)(p35), t(9;11)(p22;q23) [15/19]. Patient #2. The karyotype of this patient was: 46-49, XY, t(11;16)(q23;p13), der(14) t(14;?) (q22-24;?), -21, +1-5 mar, in 14 out of 18 metaphases that we examined (Figure 1).

FISH

Patient #1. FISH with the MLL probe confirmed the t(9;11)(p22;q23). Interestingly, two extra signals were observed on the two identical der(1) at the ends of the p arm (1p35). M-FISH detected two clones, one with t(9;11) alone and the other with the additional abnormalities. The structure of the two identical der(1)
resulted from an unbalanced translocation between der(9)t(9;11) and chromosome 1 leading to the presence of two extra copies of 3'MLL on two der(1). FISH, using telomeric probes for 9p and 11q, confirmed this rearrangement (Figure 2).

Patient #2. The pHUR195 probe hybridized with the multiple copies of der(16) which were identified as der(16)t(11;16). The probe showed a strong heteromorphism of the pericentromeric region (Figures 1 and 3). PAC 891P24, BAC 980J15, and PAC 217A21 (5’MLL) gave two hybridization signals on normal 11 and on der(11). PAC 167K13 (3’MLL) showed hybridization signals on multiple copies of der(16). At 16p, cosmids for MYH11 hybridized with all der(16); whereas cosmids for CBP were present on all der(16) and on der(11) (Figure 3). Both WCP #14 and #21 hybridized with the der(14), whereas cosmids α1 and U2–2 gave only one fluorescence signal on normal #14.

**Discussion**

Balanced translocations contribute to malignancies by forming abnormal fusion genes that produce chimeric proteins. The MLL gene is a common target of chromosomal translocations associated with AL. MLL translocations generate chimeric proteins containing the MLL amino-terminus fused in frame with a distinct partner. Additional chromosomal rearrangements or complex changes may be observed at diagnosis or may develop during the course of the disease. These latter are probably related to disease progression rather than the genesis of the leukemia. Complex karyotypes may
involve two or more chromosomes in addition to 11q23/MLL in the so-called three-four way translocations. The karyotype may appear normal but in some cases FISH has shown 5' MLL cryptic recombinations, such as insertions.

In our first patient standard cytogenetics showed a complex karyotype with a three-way change. The t(9;11) was associated with an unbalanced translocation between the der(9) and chromosome 1 with a breakpoint on the der(9) centromeric to 9p21/AF9. The derivative 1 was subsequently duplicated (Figure 2). De novo AML-M5a was diagnosed, which is the most frequently observed FAB subtype associated with t(9;11). In the second case, with a t(11;16)(q23;p13.3), one chromosome 11 was abnormal, while both homologs 16 contained extra material and the 5' CBP/3' MLL fusion, with no normal 16. Moreover, additional copies (1~5) of one of the two der(16)s were present in the complex karyotype (Figure 3). Like the twelve previously reported cases of t(11;16),12 this patient had a therapy-related-AL after treatment for NHL. Previous chemotherapy included topoisomerase II inhibitors which are known to target both the MLL and CBP genes.13

The noteworthy finding in our cases was the karyotypic duplication of derivative chromosomes from MLL translocations. Although this phenomenon is well known,14 neither the der(16) from t(11;16), nor a derivative 1p from a complex t(9;11) had so far been reported to be duplicated. Furthermore, in our two cases, FISH proved that the 3'MLL was present in the duplicated chromosomes. In patient #1, the second, apparently identical der(1) may have arisen from two different mitotic mechanisms: duplication of the der(1) due to non-disjunction or, mitotic recombination between der(1) and the normal 1 after the onset of trisomy 1. Without clear polymorphism at the centromeric region of chromosome 1 it was impossible to understand which of the two events had given rise to this karyotype. In patient #2, two derivatives from chromosome 16 were found in the absence of normal 16. In our view mitotic recombination between normal and abnormal chromosome 16 most probably generated the two der(16) identified by a clear polymorphism of the heterochromatin (Figure 1). Mitotic recombination is a phenomenon involving other known leukemic translocations such as the t(11;21) in myelodysplastic syndrome and the BCR/ABL in chronic myeloid leukemia. Moreover, the heteromorphism of the two derivative 16 acted as a marker of the common origin of all multiple copies of one of the two der(16) through non-disjunction.

Leukemic cells of both patients gained one or more copies of the terminal portion of chromosome 11, from band q23, including the 3' end of MLL and its reciprocal chimeric gene. In particular, case 2 showed up to six copies of the derivative 16 containing the 5'CBP/3'MLL chimeric gene, which is clearly indicative of amplification. This genomic imbalance was the most likely cause of the leukemic cell proliferation and overgrowth. Indeed, both our patients were affected by very aggressive malignancies.

Over-representation of the 3'MLL was the common molecular denominator in these two cases. Although

Figure 3. Patient #2. Scheme of complex chromosomal changes. a) reciprocal translocation plus mitotic recombinations lead to one der(11) and two der(16); b) nondisjunction affects one der(16).
MLL is clearly the most representative gene of 11q23 amplifications, our present results focus, for the first time, on the amplification of the 3' end as a consequence of complex chromosomal changes. Whether amplification of 3'MLL involved in reciprocal translocations has the same biological effects as amplification of the 3'MLL duplication in AML remains to be clarified.

References