ANTICANCER RESEARCH *39*: 4329-4332 (2019)
doi:10.21873/anticanres.13599

Unusual Karyotype in Acute Myelomonocitic Leukemia: A Case Report

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Abstract. *Background/Aim: Acute myeloid leukemia is well characterized by chromosomal aberrations that correspond to various subtypes of acute leukemias. The t(8;21)(q22;q22) is a frequent chromosomal abnormality strongly associated with acute myeloblastic leukemia with maturation (AML-M2), but is rarely associated with other subtypes. Translocation involving a third chromosome could produce new genetic rearrangements that lead to leukemogenesis. Patients and Methods: Conventional cytogenetic analysis and fluorescence in situ hybridization (FISH) were performed to identify the karyotype. Reverse transcriptase polymerase chain reaction (RT-PCR) was used to detect the AML1/ETO transcript. Results/Conclusion: We herein report a novel rearrangement with a three-way translocation involving chromosomes 8, 21 and another unknown chromosome, in an 83-year-old female patient diagnosed as AML-M4, with an ALM1/ETO negative transcript. This is an uncommon case of AML-M4 with three-way translocation in a new variant of t(8;21) acute myeloid leukaemia. The detailed mechanism of different phenotype expression is unclear. Further study is needed to identify the leukemogenetic transformation resulting from t(8;21) translocation.*

Acute myeloid leukaemia (AML) is a heterogeneous disease. Chromosome translocations are common genetic abnormalities in leukaemia and may contribute to leukemogenesis. Their definition is of paramount importance since it has been demonstrated that the karyotype is one of the most important prognostic factors. The t(8;21)(q22;q22) translocation is a frequent non-random cytogenetic abnormality in AML that is responsible for the fusion of two genes, the AML1 (RUNX1) and ETO (RUNX1T1), giving rise to the AML1/ETO fusion gene that identifies a type of AML defined as AML-M2 in the French–American–British (FAB) classification systems, a leukemia with a minimal or absent monocytic component (1). However, this translocation is not exclusively observed in M2 AML since different rearrangements of the chromosome band 21q22 can occur more rarely in other human malignant blood disorders such as M1 AML involving the *AML1* gene (2). In addition, complex variant translocations involving 8q22, 21q22, and another chromosome can occur with a frequency of 3.4% (3). AML1 may have several partners resulting in gene fusions in different chromosomal translocations (4-5), but some of these rearrangements do not result in chimeric genes and modify the functions of the truncated *AML1* gene. Herein, we report a three-way translocation involving chromosomes 8, 21 and another unknown one, in an AML1/ETO negative patient.

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Key Words: AML, variant t(8;21), three-way translocation.

Patients and Methods

An 83-year-old female was admitted in the Division of Haematology, A.O.U. Policlinico-Vittorio Emanuele (Catania) in October 2017 complaining of extensive spontaneous ecchymosis. Hematologic evaluation showed absolute leukocyte count of $7,810\times10^9$/l, platelet count of $10,000\times10^9$/l, haemoglobin concentration of 10.7 g/dl, associated to slight reduction of B12 vitamin dosage. Flow cytometry with five-colour analysis using monoclonal antibodies was performed on a bone marrow sample revealing the co-existence of 25.7% of myeloid blast cells and 20.4% of monocytes. Myeloblasts co-expressed the following markers: CD33, CD38, HLA-DR, CD45RA, MPO, CD45 and were positive for the CD34 and CD117 immaturity markers. The monocyte component was negative for CD34 and CD117 and positive for CD4, CD33, CD36, CD38, HLA-DR, Lys, MPO and CD45. The immunophenotypic investigation and the observation of the morphological preparation was compatible with the diagnosis of AML with a monocyte component (6). Molecular profiling identified a wild-type Fms related tyrosine kinase 3-Internal tandem duplication (FLT3-ITD) and Nucleophosmin (NPM). Conventional

cytogenetic analysis on bone marrow cells at the time of diagnosis showed: 46, XX, t(8;21)(q22;q22) in 95% of metaphases (19/20) (Figure 1a), and 46, XX in 5% of metaphases (1/20). The transcript AML1/ETO was checked by reverse transcriptase polymerase chain reaction (RT-PCR) and the results were negative. Fluorescence *in situ* hybridization (FISH) analysis on interphase nuclei (Figure 1b) showed three green signals for the *AML1* gene (locus 21q22) and two red signals for the *ETO* gene (locus 8q22), but no fusion signal. These data together confirmed a rearrangement of the *AML1* gene but not a rearrangement of the *ETO* gene and the involvement of a third unidentified chromosome. Due to patient's age and the presence of co-morbidities, the patient was given azacytidine, 100 mg flat dose, on days 1-5 every 28 days, but treatment was stopped earlier for supervening pneumonia. The patient died in December 2017, two months after diagnosis.

Ethics approval and informed consent. The patient provided written informed consent to diagnostic procedures. The study adheres to the declaration of Helsinki and the biological samples were collected following an institutionally approved protocol at the Azienda Ospedaliero-Universitaria "Policlinico-Vittorio Emanuele", Catania, Italy.

Results

Here, we report a case of AML-M4 with t(8;?,21)(q?;?,q22) translocation and AML1/ETO negative transcript. This case confirms that conventional cytogenetics and FISH analyses are still necessary for the determination of the genomic abnormalities that characterize AML. Evaluation of AML1-ETO by PCR as well as the search for *NPM* and *FLT3-ITD* mutations, were not informative in this case, indicating that molecular biology findings should always be considered together with all the other clinical and laboratory informations and should not be considered as substitutes of conventional cytogenetics. Most of the cases with the t(8;21)(q22;q22) translocation are classified as AML-M2. The finding of t(8;21) has been reported infrequently in other FAB sub-types of AML (M1 and M4) (7), myelodysplastic syndrome (8), and the blastic phase of chronic myelogenous leukemia (9). This translocation is responsible for the *AML1/ETO* fusion gene that is considered one of the most common genetic rearrangements that drives the development of AML. However, complex variants of t(8;21) are occasionally observed in a small percentage of AMLs (10). Most are three-way translocations involving chromosomes 8, 21 and a third chromosome such as 1, 2, 3, 5, 6, 8, 10, 12, 13, 14, 15, 16, 17, 18, 20 and X (11-14).

Discussion

We report a new translocation involving the *AML1* gene in an 83-year-old patient with AML-M4. Analysis of a conventional karyotype showed 46, XX, t(8;21)(q22;q22) [19]/46, XX[1], but FISH on interphase nuclei and metaphases showed the involvement of an unknown third chromosome. The main question arising by the detection of a novel translocation involving the *AML1* rearrangement is whether it results in a fusion gene with a gene located in the partner chromosome of the translocation different from ETO, or if the truncation of the *AML1* gene per se is sufficient to cause leukemia. The two possibilities have been reported in the literature (15). The relationship between the variations of genes like *AML1*, the genesis of the different subtypes of leukemia and their evolution is known, but the detailed mechanism of the different phenotypes is not clear and reinforces the idea that this region could be an important gene in the pathogenesis of AML. In order to understand the molecular mechanism involved in unusual rearrangements and the significance of this additional information (16, 17), evaluation of the single nucleotide polymorphism (SNP) profiling and loss of heterozygosity regions (LOH) is necessary (18, 19). It could help to more precisely define the genomic alterations that can reflect clinical features and prognosis.

Conflicts of Interest

The Authors declare that they have no competing interests regarding this study.

Author's Contributions

MLC drafted the manuscript; MLC and AR were responsible of study concept; MLC, LT, NLP, AS and DS performed molecular, cytogenetic and immunophenotypic analyses; MLC analysed and interpreted the data; AR and MAR made a critical revision of the paper and managed the patient; FDR conceived the original idea and supervised the project.

Acknowledgements

Funding: Maria Letizia Consoli received funding from Regione Sicilia, Piano Sanitario Nazionale 2014, Linea Progettuale 6-Azione 6.13.

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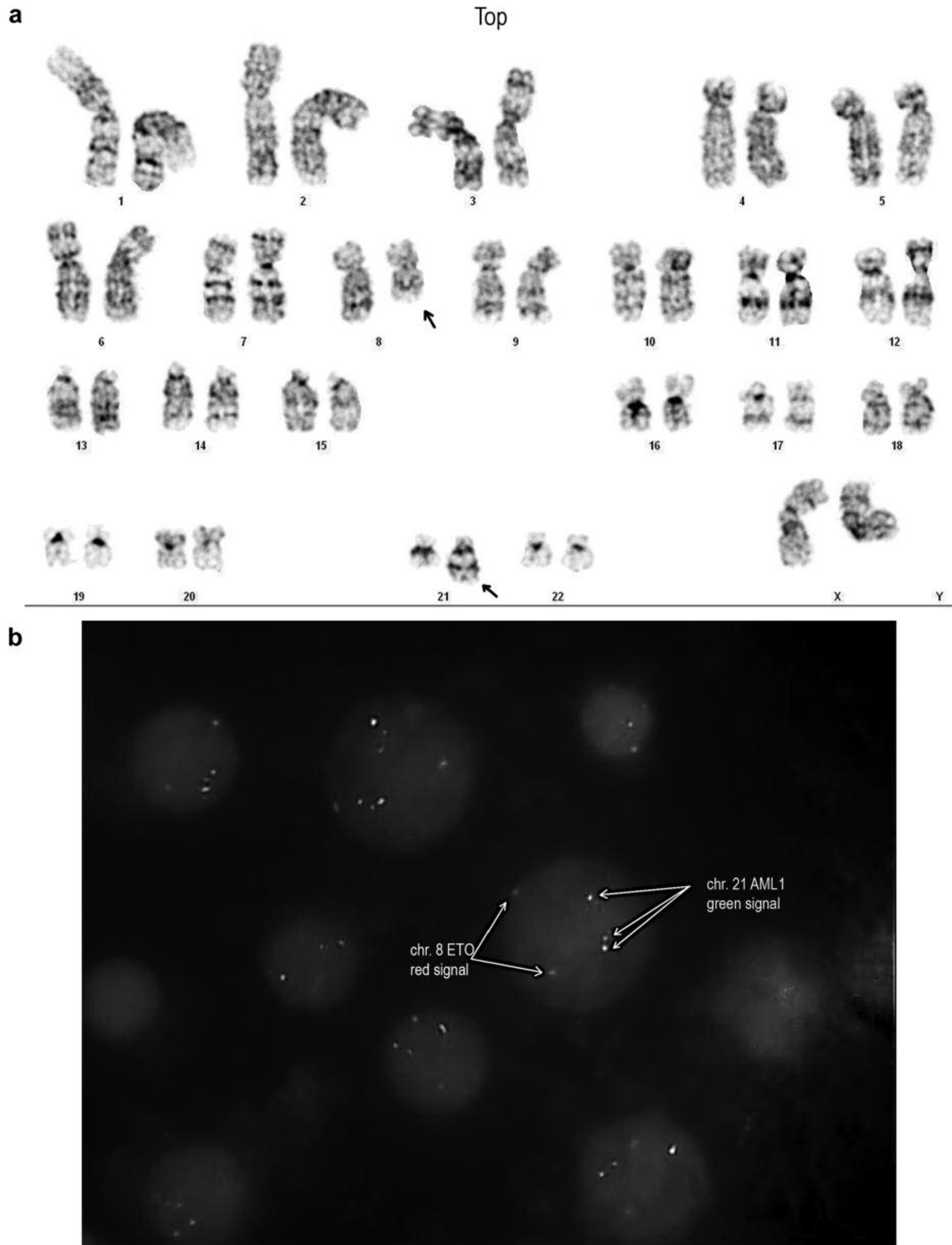

Figure 1. *Conventional cytogenetic and FISH analyses of AML-M4 with t(8;?,21)(q?;?,q22). a. G-banded karyogram of 19/20 marrow cells metaphases. Arrows indicate the t(8;21) translocation. b. FISH using AML1/ETO dual color, dual fusion probe showed three (green) signals (AML1, locus 21q22) and two (red) signals (ETO, locus 8q22) but no AML1-ETO fusion signal was detected confirming a rearrangement of AML1, but not a rearrangement of ETO and the involvement of a third unidentified chromosome.*

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Received June 25, 2019
Revised July 2, 2019
Accepted July 4, 2019