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Acute Myeloid Leukemia Associated With Near-Tetraploid Karyotype and Mutations in the FLT3 Gene

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Abstract

Tetraploidy and near-tetraploidy are rare in acute myeloid leukemia (AML), contrary to other hematological disease. In this paper we describe a case of a 52-year-old male with AML associated with tetraploidy, mutation in tyrosine kinase receptor *FLT3*, and very short survival. At presentation maculopapular rash with crustae, lymphadenopathy, and hepatosplenomegaly was diagnosed. The blasts comprised 80% of marrow nucleated cells (POX negative and PAS finely granular positive). Immunophenotyping done on marrow cells was (CD34, HLA DR, CD14, CD64, CD33, CD11b, and CD15) and correlated with the acute monoblastic leukemia. Detection of FLT3 mutation was done by polymerase chain reaction (PCR). Cytogenetic analysis show: 85-93, XXYY,inc(cp5)/46,XY. Based on these considerations, we suggest the detection of FLT3 mutations as a diagnostic procedure for all AML patients.

Keywords: acute myeloid leukemia, tetraploidy, FLT3 mutation, survival, clinical presentation

Tetraploidy and near-tetraploidy are rare cytogenetic findings in acute myeloid leukemia (AML). It is found in approximately 1.2% of all AML patients and in about 3% of patients with acute lymphoblastic leukemia (ALL) with various prognosis.[1,2] Tetraploidy and near-tetraploidy have been described frequently in cases of erythroleukemia, myelodysplastic syndromes, and carcinomas.[3,4] Occasional reports of similar cases have been previously published, but some of them lack detailed reference to FLT3 mutation and specific clinical and pathologic associations regarding the morphological abnormalities in AML.[5-25]

The fms-like tyrosine kinase 3 (FLT3) is a member of the class III receptor tyrosine kinase group, with a structure resembling KIT, FMS, and the platelet-derived growth factor receptor.[26-28] It is predominantly expressed on hematopoietic progenitor cells including CD34 positive cells with a high expression of CD117 (c-Kit), but it is also found in a variety of hematological malignancies including AML, B-precursor cell ALL, a fraction of T cell ALL, myelodysplastic syndrome in leukemic transformation, and chronic myelogenous leukemia in blast crisis.[26-30]

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Abbreviations

AML, acute myeloid leukemia; PCR, polymerase chain reaction; ALL, acute lymphoblastic leukemia; FLT3, fms-like tyrosine kinase 3; APL, acute promyelocytic leukemia

The investigation of the simultaneous mutation status of the *FLT3* and *N-RAS* genes is very important because they may elicit their effects through a pathway not used by the *p*53 protein. This could potentially provide further insight into the molecular basis of rare near-tetraploid cases of AML.

Case Report

A 52-year-old male developed malaise and fevers in August 2004. A maculopapular rash with crustae, cervical about 1 cm, axillary about 2 cm, and lymphadenopathy was diagnosed in October 2004. The liver and spleen were just palpable. Laboratory examinations showed hemoglobin of 90 g/L, platelets 109 × 10^9/L, and leukocytes 97 × 10^9/L with 29% monoblasts, 56% mature-looking monocytes, and 16% lymphocytes in the formula. The blasts comprised 80% of marrow nucleated cells (POX negative and PAS finely granular positive). Immunophenotyping done on marrow cells was (CD34, HLA DR, CD14, CD64, CD33, CD11b, and CD15) correlated with AML, FAB subtype. Cytogenetic analysis was performed on unstimulated bone marrow cells by direct preparation, and following 24 hours in RPMI 1640 culture medium with 25% fetal calf serum at 37°C. At least 20 metaphases HG-banded by Giemsa stain were examined and described according to the ISCN nomenclature.[31] Cytogenetic analysis shows: 85-93, XXYY,inc(cp5)/46,XY(15) (**Figure 1**). Detection of the FLT3 mutation was done by PCR (**Figure 2**). Briefly, for this analysis, genomic DNA was isolated from mononuclear cell preparations using GFX genomic Blood DNA Purification Kit (Amersham Biosciences, GE Healthcare, London, U.K.) according to the manufacturer's recommendations. As the location of FLT3/ITDs is restricted to exons 14 and 15, the PCR amplification was carried out as previously described[29] and its products were resolved on a 4% agarose gel stained with ethidium bromide. Each sample displaying an additional PCR product (longer

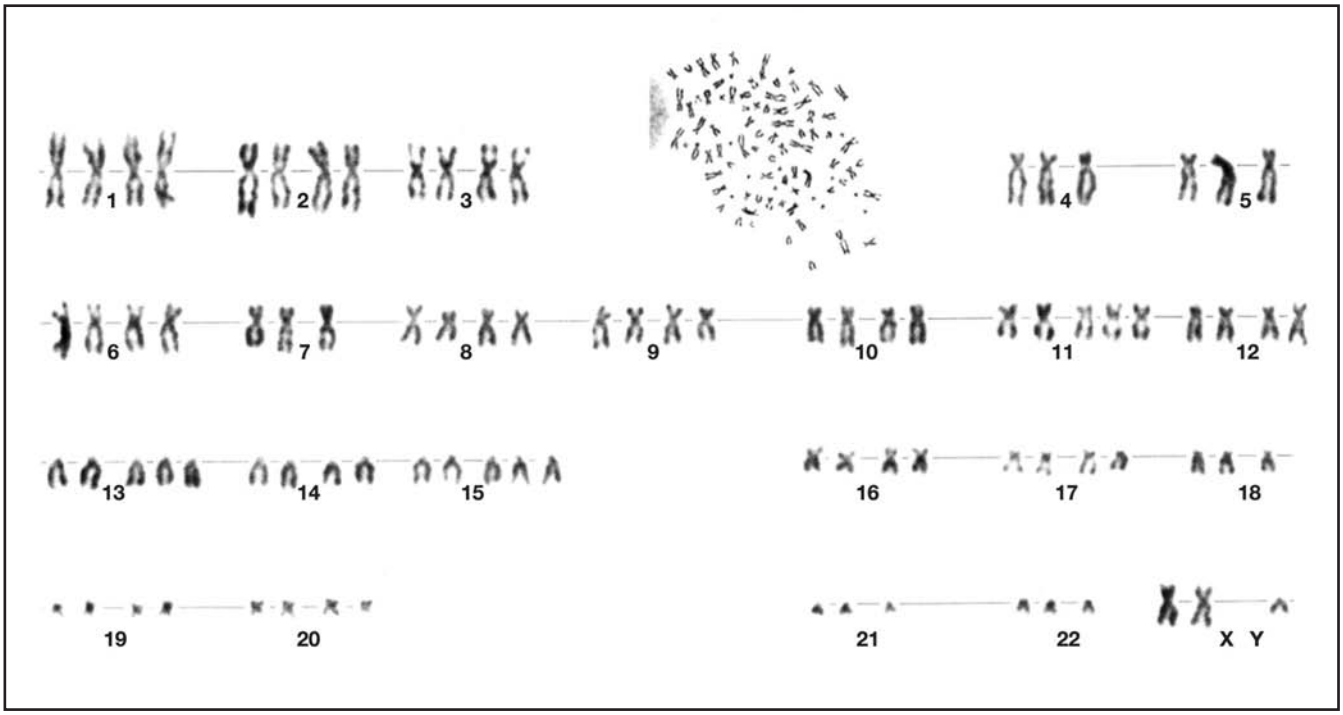

Figure 1_The patient's karyotype.

than 325 bp) was considered as the 1 containing the internal tandem duplication. Longer PCR products were directly sequenced by the same primers used for amplification. Analysis of the FLT3/D835 mutation was carried out as follows: exon 20 of the FLT3 gene was amplified by genomic DNA PCR as previously reported.[41] The PCR products digested with EcoRV (New England Biolabs, Hertfordshire, England) were resolved on the 8% polyacrylamide gel.

The patient was treated with ADE Protocol (Adriblastine 90 mg i.v. on days 1-3, Vepeside 200 mg i.v. days 1-5, and Cytosar 200 b.i.d., i.v. push, days 1-8). The patient developed profound marrow aplasia following this treatment and died on the 23rd day of therapy due to sepsis.

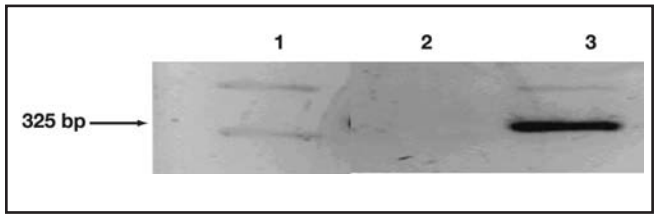

Figure 2_Detection of the FLT/ITD mutations on agarose gel electrophoresis. 1 - DNA marker 100 bp ladder; 2 - water control; 3 - patient (ITD/wt).

Discussion

In this paper we describe a patient with tetraploidy (85-93,XXYY,inc[cp5]/46,XY[15]) associated with FLT3 mutation but without N-RAS and *p53* mutation. Literature data indicated that polyploidy was found in 10%-100% of blast cells in patients with acute leukemia and myelodysplastic syndromes. Acute myeloid leukemia with tetraploidy can show large, bizarre blasts,[23] but in some cases a large series of ALL with near-tetraploidy and complete tetraploidy had not been reported as related to blast morphology.[32] The correlation between blast size, morphology, and DNA content in AML was described by Kwongs who found that increased DNA content is directly related to blast size.[25] As seen in routine karyotyping, polyploid cells are present in small numbers; the increased fraction of such cells in the marrow usually relates to leukemic involvement.[33] In addition, our patient with tetraploid karyotype and an FLT3 mutation represents low frequency of this cytogenetic aberration between all cytogenetically studied AML patients. Similar to other studies, the sex of this patient correlates with a predominance of males vs females with AML having near tetraploidy and FL3 mutations.[27-35]

We previously detected both FLT3/ITD and FLT3/D835 mutations in favorable and intermediate-risk cytogenetic categories.[29] FLT3/ITD was also detected in an adverse-risk category.[36] An FLT3 mutation can also be associated with inversion of chromosome 16 in leukemic transformation of myelofibrosis, and the patient in this particular case study had a long clinical remission.[34] The cytogenetic analyses of 21 patients with FLT3 mutations in AML showed that 12 of them (57%) have the normal karyotype. These findings suggest that at least 1 additional mutation besides the 1 in the FLT3 gene is indispensable for leukemic transformation. Therefore, it is essential to further investigate molecular alterations in patients with AML and the normal karyotype.[29,37] The literature indicates that mutations in FLT3/ITD can be associated with poor prognosis.[27-32]

Our patient with the FLT3/ITD mutation and tetraploidy had a high WBC count, high presence of blasts in the bone marrow, and a very short survival. FLT3/ITD was evidenced in 17%-24% of patients with the highest frequency

in acute promyelocytic leukemia (APL).[38] Point mutations of Asp835 within the FLT3 tyrosine kinase domain have been previously reported in only 7% of AML analyzed patients.[38]

Association of *FLT3/ITD*s and *RAS* mutations as well as *FLT3* and *p53* mutations in the same patients is rare because they share the identical cellular transduction pathway.[39-41] Mutations in the *p*53 gene and subsequent deficiency of the *p*53 protein can be associated with the development of tetraploidy *in vitro* and *in vivo*. The role of *p*53 as a component of a spindle checkpoint can be corrupted by its mutation in exons 5-8. This allows a premature round of DNA replication with mitotic arrest, thereby leading to tetraploidy.[3-5]

FLT3 plays an important role in stem cell proliferation, differentiation, and survival. In normal hematopoiesis, FLT3 ligand binding to the FLT3 receptor causes dimerization of the receptor, autophosphorylation, activation of tyrosine kinase, and induction of multiple intracellular signaling pathways, which are involved in cell proliferation and leukemogenesis.[10,15]

FLT3/ITD confers proliferative or survival advantage to cells through activation of RAS/MAPK or STAT pathways. These mutations do not affect cell differentiation. In a study by Stirewalt and colleagues, only 2 of the 47 patients with FLT3/ITDs also had a RAS mutation, indicating a negative association between FLT3 and RAS mutations.[35] Our case would tentatively support the presumption that abnormal karyotypes in our patients belonged to a mixed population of more differentiated polyploid and diploid blast cells lacking a mutation at the oncogene loci studied. Literature data suggest that near-tetraploidy with secondary chromosome aneuploidy is predictive of a more aggressive course and is resistant to chemotherapy.

Based on these considerations, we suggest the detection of FLT3 mutations as an additional diagnostic procedure for all AML patients routinely at the time of cytogenetics. LM

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