Original Article

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Characterization of a Partially Cryptic 3-way Translocation t (8;6;21) (q22.1;q27;q22.3) in AML by Reflex FISH.

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Abstract

Context: Variants of standard translocations in leukemia are occasionally suspected initially by fluorescence in-situ hybridization (FISH), due to a different signal pattern. In many cases, this is due to the involvement of an additional chromosome, leading to a complex three-way translocation. If the breakpoints are in the telomeric region, the cryptic translocation may not be visible on karyotyping. We describe here the characterization of a variant AML1/ETO fusion involving 6q in a patient with AML-M2

Aims: To identify and confirm the extra chromosome involved in a variant AML1/ETO translocation in a case of AML-M2.

Settings and Design: First routine FISH for AML1/ETO and conventional karyotyping was carried out. Reflex FISH was set up on previously Giemsa-banded metaphases in two rounds to confirm that chromosome 6 was additionally involved in the AML1/ETO translocation.

Material and Methods: Heparinized bone marrow aspirate was obtained from a suspected case of AML-M2. Cytogenetic analysis by routine FISH for AML1/ETO and conventional karyotyping was carried out. Reflex FISH was set up on Giemsa-banded metaphases in two rounds using probes for AML1/ETO followed by CEP 6.

Results: FISH results showed the presence of a variant signal pattern for translocation AML1/ETO. Karyotyping of Giemsa-banded metaphases showed suspicion of a three way translocation t (8;6;21) (q22.1;q27;q22.2) which was confirmed by reflex FISH analysis.

Conclusions: In cases with suspicion of a cryptic three way translocation, reflex FISH on previously Giemsa-banded metaphases gives a clue about the additional partner chromosome which can be different in each case.

Keywords: Reflex FISH, Three-waytranslocation, AML1/ETO, cryptic translocation

Introduction

In leukemias, while performing diagnostic fluorescence in-situ hybridization (FISH), a variant signal pattern is occasionally observed instead of the typical positive pattern. In many cases, this is generally due to the involvement of an additional chromosome, leading to a complex three-way translocation. Routine FISH on interphase nuclei cannot identify the third chromosome. Sometimes, involvement of the third chromosome is visible on conventional karyotyping. However, if the breakpoints of translocated chromosomes are in the telomeric region, the cryptic translocation may

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not be visible on karyotyping. In such cases, a reflex FISH set up in multiple rounds on previously G-banded, karyotyped and subsequently destained metaphases is useful to identify the partner chromosomes and the regions involved in the complex translocation. Variants of the translocation t (8;21)(q22;q22) involving chromosomes 8, 21, and a third chromosome account for approximately 4% of all t (8;21)(q22;q22) cases in acute myeloid leukemia (AML) patients [1-4]. We describe here the characterization of a variant AML1/ETO fusion involving a third partner 6q in a patient with AML-M2. A 44 year old female with severe anemia, thrombocytopenia, pallor and fatigue was evaluated in the clinic with hematologic, immunophenotypic, tests. Initial investigations showed hemoglobin 5.9 g/dl, platelets 15.0 thou/cu.mm and leucocyte count 1.02 thou/cu.mm. Bone marrow smears were cellular with 43% blasts, some showing folded nuclei with granular cytoplasm. Long and slender Auer rods were frequently seen. There was mild dyserythropoiesis with decreased megakaryocytes and no eosinophilia. There was no enlargement of liver, spleen or lymph nodes, and no involvement of central nervous system. A six colour flow cytometric immunophenotypic analysis was performed on bone marrow aspirate specimens using a FACSCanto II (BD Biosciences, San Jose, CA). On analysis the blast cells (42%) were positive for stem cell markers, CD34+ 85%, HLA-DR+ 85%, negative for T lymphoid markers CD2, CD3, CD7, CD8, CD5 except for CD4+ 61%. The B lymphoid markers CD19, CD10, CD22, and CD20 were negative. Myeloid markers were positive for CD33+ 93%, CD117+ 91%, MPO+ 86% except for CD13 which was negative. CD64 was negative and CD56 was positive in 86%. Based on these findings and using the European Group for Immunological Characterization (EGIC) criteria for acute leukemias, the patient was diagnosed with Acute Myeloid Leukaemia (AML). The patient was further referred for cytogenetic evaluation for AML by FISH and conventional karyotyping.

Subjects and Methods

Heparinized bone marrow aspirate was obtained from the newly diagnosed AML case. FISH for the AML panel [PML-RARA, AML1-ETO, inv [16], MLL and BCR-ABL] was performed on interphase nuclei and metaphases of a direct harvest using Vysis (Abbott molecular) probes with overnight hybridization. Approximately 100 interphase nuclei and a few metaphases were examined and scored for each probe using Zeiss Axioscop 2 photomicroscope with Metasystems 'isis' software. FISH for t (8;21) was performed using the LSI AML1-ETO dual-color, dual-fusion translocation probe. With this probe, a normal cell will show two orange signals, one on each chromosome 8 and two green signals, one on each chromosome 21., i.e. (ETO,AML1)x2. Cells that contain the classical t (8;21) translocation will show one orange signal, one green signal, and two orange-green (yellow) fusion signals. The fusion signals represent the juxtaposition of the translocated portions of the ETO and AML1 gene regions on the derivative chromosomes 8 and 21, i.e (ETO×3),(AML1×3)(ETO con AML1×2). Conventional karyotyping was carried out on GTG (G banding with Trypsin using Geimsa stain) banded metaphases obtained from direct harvests, as well as 48 and 72 hour unstimulated cultures using standard protocol. After karyotyping, the images of well spread G-banded metaphases were captured using a Zeiss Axioscope 2 microscope and Metasystems Ikaros software. The immersion oil was removed with Xylene, slides were destained with methanol:acetic acid (3:1) fixative and dehydrated. Reflex FISH was set up with the AML1-ETO translocation probe in the first round. Slides were hybridized overnight, washed in hot buffered detergent and counterstained with DAPI. After capturing the FISH signals, the slides were washed to strip the probes, dehydrated and a second round of FISH was repeated on the same slide using the Centromere 6 (CEP 6) probe in Aqua colour to confirm the three-way translocation. No ethical issues were involved.

Results

FISH with the AML1-ETO dual colour dual fusion probe showed two green, two orange and a single fusion signal (2G2O1F)(Figure 1a) in 81% cells instead of the regular translocation pattern (1G1O2F) (Figure 1b), suggesting a variant AML1-ETO translocation involving a third chromosome. The regular AML1-ETO translocation signal pattern was seen in 5% cells, while 14% cells were normal (2G2O). FISH with the inversion 16 breakapart probe showed monosomy 16 in 5% cells, while the MLL breakapart probe showed monosomy 11 in 4% cells, suggesting hypodiploidy. FISH with the PML-RARA and BCR-ABL probes was negative for the translocations. Twenty Giemsa banded metaphases analysed by karyotyping showed a translocation between the long arms of chromosomes 6 and 8 in 15 (75%)

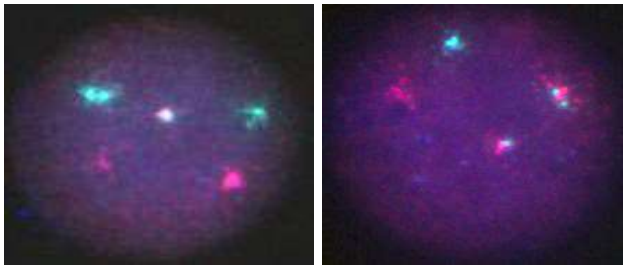

Fig. 1: Interphase FISH with the Vysis AML1/ETO dual colour, dual fusion probe showing a variant fusion positive signal pattern of 2 Green, 2 orange, 1 Fusion (2G2O1F) in 81% cells (Fig. 1a) and the regular 1G1O2F pattern in 5% cells (Fig. 1b)

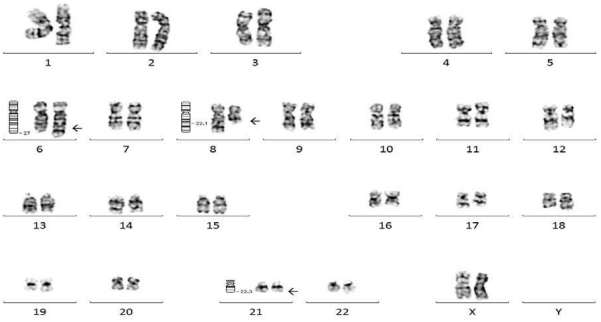

Fig. 2: Bone marrow karyotyping showed a suspicion of a three-way translocation t (8;6;?21)(q22.1;q27;q22) as FISH showed a variant AML1/ETO fusion

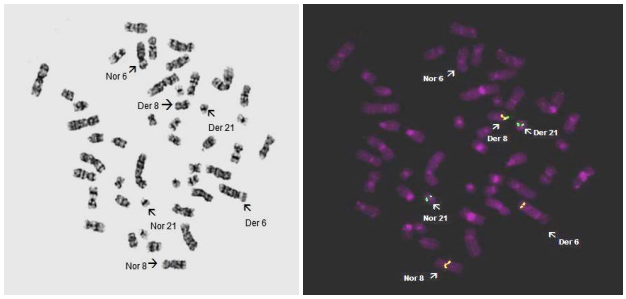

Fig. 3: Reflex FISH on the G-banded metaphase of Figure 2, with the Vysis AML1/ETO dual colour, dual fusion probe set shows that the fusion is on the derivative 8 (Fig. 3a) and one orange signal for ETO is on derivative 6 (Fig. 3b).

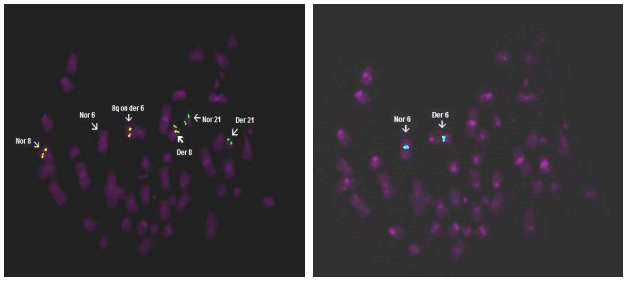

Fig. 4: Sequential FISH on another metaphase using the Vysis AML1/ETO dual colour, dual fusion probe set (Fig. 4a) and the CEP 6 probe (aqua) on the same metaphase (Fig. 4b), shows that one orange signal for ETO is on the normal 8, the AML1/ETO fusion signal is on derivative 8 and the other orange signal is on derivative 6.

metaphases, mainly from the 48 and 72 hour cultures. Both copies of chromosome 21 appeared normal. (Figure 2). A normal karyotype of 46,XX was seen in 2 (10%) metaphases. Hypodiploidy (44-45 chromosomes) with random loss was seen in 3 (15%) metaphases. In order to detect the location of the single fusion signal and confirm the three-way translocation, FISH set up with the AML1-ETO probe on previously captured G-banded metaphases showed that the single fusion signal was on derivative 8 and the third orange signal was on chromosome 6 (partner chromosome in translocation) (Figure 3a, b). This was reconfirmed on another metaphase using the centromere probe for chromosome 6(aqua) in a second round of hybridization on the same slide (Figure 4a, b).

Discussion

The chromosomal aberration t (8;21) leading to AML1-ETO gene fusion is recognized as an indicator of a favourable prognosis and is classified as a distinct sub group of AML. The detection of this rearrangement is thus not only important diagnostically, but also allows patients to be assigned to the appropriate risk group for treatment stratification and clinical management. As the translocation t(8;21) is cytogenetically visible, conventional karyotyping remains the gold standard for its detection. However in rare cases conventional cytogenetic analysis shows no typical aberrations and the t (8;21) in this case could be missed or masked by an insertion or variant translocation as in the present case. [2] In our case, using dual-color, dual fusion FISH with the AML1-ETO probe, we demonstrated two green, two orange and only one fusion signal in 81% cells suggestive of a variant t (8;21) fusion. The cytogenetic analysis showed a suspicion of a three-way translocation between chromosomes 8, 6 and 21, the karyotype being 46, XX, t (8;6;21) (q22.1;q27;q22.2) in 75% metaphases which was further confirmed by reflex FISH, thus explaining the variant t (8;21) fusion signal pattern seen initially by FISH. In one such case, the cytogenetic analysis of bone marrow revealed a karyotype of 46, XX, t (6;8)(p22;q22) and no involvement of chromosome 21. [3] Both chromosomes 21 appeared to be normal in contrast to a classic t (8;21), where one of the chromosomes 21 is enlarged as a result of juxtaposition of a segment from chromosome 8. The location of the break point at 8q22 prompted them to search for a cryptic t (8;21) (q22;q22) and involvement of AML1-ETO genes. Dual colour FISH with AML1-ETO probe revealed two orange, two green and a single orange-green fusion signal as in the present case, in all 200 nuclei in contrast to the classical t (8;21) when two fusion signals were expected. Based on karyotype and FISH findings the translocation of the patient was denoted

as t (6;8;21) (p21;q22;q22). This abnormality is considered as a masked t(8;21) as the aberrant location of AML1-ETO gene and the final karyotype of 46, XX, t (6;8;21)(p22;q22;q22) could not be determined without molecular cytogenetic analysis. Absence of eosinophilia and presence of frequent slender Auer rods were differentiating morphological features in our case, compared to that of Al Bahar et al.[3] In our patient, the breakpoint on chromosome 6 was in the long arm at 6q27, while in the case reported by Al Bahar et al, the breakpoint was in the short arm at 6p21. [3]

Maseki et al. had described many cases with t. (8;V;21) in AML which were similar to what we found showing involvement of third chromosome with t (8;21). [5] They had used Southern Hybridization technique for the detection of masked t (8;21) whereas we have used FISH as a molecular cytogenetic test for confirmation of cryptic translocation t (8;21). Various subtypes of AML and specific translocations related to them have now been identified. Based on the unique phenotype under AML, the translocation t (8;21)(q22;q22) is one of the most common cytogenetic changes. The morphological presentation of leukemic cells in AML classifies t (8;21) as M2 subtype based on French-American-British (FAB) classification. [6] AML with the t (8;21)(q22;q22) is recognized as a distinct type of AML in the WHO classification. Its occurrence is mostly observed in young patients who respond well to treatment. Variants of the t (8;21) are uncommon and may display three or four way translocations involving 8q22 and 21q22. Occasionally, conventional cytogenetic analyses show no aberration, and only FISH and RT-PCR detect cryptic t (8;21) insertions. Deletion of 9q is the most frequent structural additional abnormality in patients with AML associated with t(8;21) and is found in nearly 10% of the cases [7].

Though the patient was in remission within 2 months after treatment with Daunorubicin and Cytarabine, she died in a year due to neutropenic sepsis.

Conclusion

The occurrence of variants of t (8;21) is an infrequent phenomenon and t (8;6;21) (q22.1;q27;q22.2) has not been described previously. Our case further highlights the importance of a combination of standard karyotyping and FISH techniques together with reflex FISH for assessing complex rearrangements and more precise treatment stratification.

Key Message

Many times, standard translocations in leukemia can involve an extra chromosome which cannot be identified on routine FISH analysis. Reflex FISH on previously Giemsa-banded metaphases is helpful to identify and confirm the additional partner chromosome involved in the complex 3-way translocation.

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