A novel variant translocation t(6;8;21)(p22;q22;q22) leading to AML/ETO fusion in acute myeloid leukemia

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Abstract
Acute myeloid leukemia associated with translocation t(8;21) and the underlying AML1-ETO gene fusion is considered as a distinct type of leukemia with characteristic morphologic features. Variant and masked forms of the classic translocation t(8;21) are uncommon and their clinicopathologic features are less well characterized. We report here a patient with a masked translocation involving chromosomes 6, 8, and 21. Chromosomal study at diagnosis initially reported the karyotype as translocation between chromosomes 6 and 8 without visible involvement of chromosome 21. However, fluorescence in situ hybridization studies revealed the involvement of chromosome 21 in the translocation and presence of the AML1-ETO chimeric gene. The complex rearrangement t(6;8;21) observed in our patient was not previously described and could be not detected without combination of techniques. Our case illustrates the challenge of recognizing complex aberrations that occur with variant t(8;21) and further reinforces the utility of fluorescence in situ hybridization applications in more accurate characterization of chromosome abnormalities which can lead to more precise therapeutic stratification.

Key Words
AML1-ETO, fusion gene, variant t(8;21)

Introduction
Acute myeloid leukemia (AML) with the balanced translocation between chromosomes 8 and 21, t(8;21)(q22;q22) is recognized as a distinct type of AML in the WHO classification. The neoplastic cells have characteristic morphologic features, including presence of large blast cells with abundant basophilic cytoplasm, azurophilic granules, numerous and thin Auer rods and dysplastic myeloid elements. The abnormality is associated with young age and most of cases fall into the category of AML-M2 in the FAB classification (1,2).

The result of the translocation is a juxtaposition of the AML1 gene from chromosome 21 to the ETO gene on chromosome 8 creating a new AML1-ETO chimeric gene. The novel chimeric gene produces a transcript casually implicated in leukemogenesis by blocking trans-activation of AML1-responsive hematopoietic target genes critical to normal hematopoiesis (3,4).

By conventional cytogenetics the t(8;21)(q22;q22) is detected in approximately 7% to 8% of patients with acute myeloid leukemia (5,6). Cases with variant form of the translocation involving a (variable) third chromosome are less common and the clinicopathologic features of AML carrying variant t(8;21) are less well characterized.

Fig. 1. Karyotype of the patient with rearranged chromosomes 6 and 8. Chromosomes 21 appear to be normal in contrary to classic t(8;21)(q22;q22) when one chromosome 21 is enlarged due to the juxtaposition of chromosomal segment from 8q22 (inset).
characterized (7). We report here a patient with a variant form of t(8;21)(q22;q22) detected by conventional cytogenetics and fluorescence in situ hybridization (FISH) analysis.

Our case may be of interest due to the (1) rare occurrence of variant form of t(8;21), (2) first described case with complex chromosome rearrangement t(6;8;21)(p21;q22;q22), and (3) illustration of the importance of combination of conventional cytogenetic and FISH studies in identification of cryptic rearrangements.

Case report
A 25-year old female was referred to our hospital with severe anemia, thrombocytopenia, pallor and recent history of fatigue. Initial investigations showed: hemoglobin 3.4 g/l, platelets 13 x 10⁹/l and white blood cell count 18.8 x 10⁹/l. Bone marrow smears were hypercellular with 46% blast cells with open type nucleus with one to two nucleoli with mildly basophilic cytoplasm. There was a mild eosinophilia (9%) and few of the blasts showed Auer rods. Erythropoiesis

Fig. 2. Fluorescence in situ hybridization with whole chromosome painting for chromosomes 8 (red) and 21 (green) illustrating the presence of chromosome 8 material on the short arm of chromosome 6 without visible involvement of chromosome 21.

Fig. 3. Dual-colour FISH with AML1 (green signal) and ETO (orange signal) probes illustrating the single orange/green (AML-ETO) fusion signal on derivative chromosome 8 and the presence of ETO signal on chromosome 6.
was suppressed and megakaryocytes markedly decreased. Cytochemical studies showed Sudan Black, non-specific esterase and myeloperoxidase positive blast cells. Immunophenotyping disclosed positivity for CD45, CD11b, CD11c, CD33 and CD13, CD34 and for the HLA-DR stem cell antigen with negative lymphoid associated markers. On the basis of morphological and laboratory findings, a diagnosis of AML-M2 was made. The patient was treated with conventional chemotherapy regimen and went into hematological remission within two months.

Materials and methods

Chromosome analysis

Cytogenetic analysis was performed on bone marrow specimen obtained at diagnosis and on follow-up using standard techniques. Direct and 24h cultures were established without mitogens and chromosome analysis was performed using G-banding technique. Karyotypes were described according to the International system for Cytogenetic Nomenclature (15).

Fluorescence in situ hybridization studies

The presence of chromosomal abnormalities was confirmed by fluorescence in situ hybridization on slides prepared for cytogenetic analysis. Interphase and metaphase FISH assay was performed using commercially available LSI AML-/ETO Dual Color, Double Fusion, centromeric 8 and WCP (whole chromosome probe) 8 probes (Vysis, Downers Grove, IL) according to manufacturer’s protocol. Hybridization signals were visualized using a Leitz Diaplan fluorescence microscope and images were captured and digitally recorded by Isis software (MetaSystem, Germany). Approximately 250 interphase nuclei and 10 metaphase spreads were examined and scored for each probe.

Results

Conventional cytogenetic analysis from bone marrow aspirate at diagnosis revealed a reciprocal translocation between chromosomes 6 and 8 in all analyzed cells. Both chromosomes 21 appeared to be normal in contrary to a classic t(8;21) when one of chromosomes 21 is enlarged as a result of juxtaposition of chromosomal segment from chromosome 8 (Fig 1.). Fluorescence in situ studies with whole chromosome painting for chromosome 8 on metaphase chromosomes confirmed the presence of chromosome 8 material on the short arm of chromosome 6 without visible involvement of chromosome 21 confirming the cytogenetic result (Fig. 2). However, the location of breakpoint at 8q22 prompted us to search for a cryptic t(8;21)(q22;q22) and involvement of the AML1 and ETO genes. Dual-color FISH with AML1 and ETO probes revealed 2 orange, 2 green, and a single orange/green (AML-ETO) fusion signal in all 200 nuclei, in contrary to the classical translocation t(8;21), when two fusion signals are expected, representing the juxtaposition of ETO and AML1 gene regions on the derivative chromosomes 8 and 21. FISH analysis performed on metaphase chromosomes demonstrated 2 AML1 signals on chromosomes 21, 1 ETO signal on normal chromosome 8, one ETO signal on the derivative chromosome 6, and a single AML1-ETO fusion on the shortened chromosome 8, confirming the involvement of chromosome 21 in a translocation (Fig. 3). Based on karyotypic and FISH findings the karyotype of the patient was revealed as ish t(6;8;21)(p21;q22;q22). This abnormality is considered 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.

Discussion

The chromosomal aberration t(8;21) leading to AML1-ETO gene fusion is recognized as indicator of favorable prognosis and is now classified as a distinct subgroup 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 karyotypic analysis remains the (gold standard) for the detection (5,6). However, in rare cases conventional cytogenetic analyses show no aberration, and the t(8;21)
could be missed or be masked by insertions or variant translocations\(^{(9-14)}\).

Cytogenetic analysis of bone marrow sample in our case revealed a karyotype: 46,XY,t(6;8)(p22;q22) and no involvement of chromosome 21 was discerned at a microscopic level. While 8q22 is a recurrent breakpoint reported in patients with AML, the rearrangement by itself does not lead to the AML1-ETO fusion and hence these cases would not be assigned to favorable prognosis group. To reconcile this major diagnostic discrepancy, FISH analysis was performed to search for a cryptic t(8;21) and AML1-ETO rearrangement. Accordingly, FISH analysis performed on our patient revealed the presence of AML1-ETO fusion on der(8) chromosome. An unusual finding in our case was the juxtaposition of ETO gene to chromosome 6 and the lack of fusion signal on chromosome 21 in contrary to classic t(8;21), confirming previous observations that the crucial event lies on der(8) chromosome which encodes the AML1-ETO fusion product involved in the pathogenesis of AML-M2. Creation of AML1-ETO fusion gene in our patient probably occurred through complex mechanisms, combining translocations of chromosomal segments of three chromosomes.

The occurrence of variant and masked forms of t(8;21) is an infrequent phenomenon and the translocation t(6;8;21)(p22;q22;q22) observed in our patient was not previously described. Our case further highlights the importance of combination of standard karyotyping and FISH techniques for assessing submicroscopic rearrangements, which may eventually lead to more precise treatment stratifications.

References