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CASE REPORT

Diagnosis of intrachromosomal amplification of chromosome 21 (iAMP21) by molecular cytogenetics in pediatric acute lymphoblastic leukemia

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Key Clinical Message

Intrachromosomal amplification of chromosome 21 (iAMP21) defines a distinct cytogenetic subgroup of B-cell precursor acute lymphoblastic leukemia (BCP-ALL) with poor prognosis that should be investigated in routine practice. Single-nucleotide polymorphism (SNP)-array provides a useful method to detect such cases showing a highly characteristic profile.

Keywords

BCP-ALL, cytogenetics, iAMP21, RUNX1, SNP-array.

Images in Hematology

We report the case of a 7-year-old girl with no previous medical history, presenting in our department with fever, fatigue, weakness, bruising, and tumor syndrome (lymphadenopathy and hepatosplenomegaly). A total blood count showed: leukocytes 29×10^9/L (including neutrophils 3%, lymphocytes 17%, and blasts 80%), hemoglobin concentration 82 g/L, and platelet count 63×10^9/L. The bone marrow aspirate was of high cellularity with a decreased number of megakaryocytes and 97% peroxidase-negative staining blast cells. Immunophenotyping confirmed the diagnosis of B-cell precursor acute lymphoblastic leukemia (BCP-ALL) with expression of CD34, HLA-DR, and B-lymphoid markers CD19, CD10, CD20, and CD22. T-lymphoid markers were absents. Blasts also aberrantly expressed the myeloid markers CD33 and CD117. Molecular studies did not show any rearrangement usually observed in BCP-ALL (*ETV6-RUNX1*, *TCF3-PBX1*, *BCR-ABL1*, or *KMT2A* fusions). Cytogenetics analyses revealed a female karyotype with 46 chromosomes including a grossly abnormal chromosome supposed to derive from chromosome 21 in all metaphases (Fig. 1A). All other chromosomes appeared normal. Interphase fluorescent *in situ* hybridization (FISH) using RUNX1/RUNX1T1 probe (Vysis®) was performed (Fig. 1B) and revealed extra signals of *RUNX1* clustered together. The same analysis performed on metaphases (Fig. 1C) showed *RUNX1* extra copies all along the abnormal chromosome 21, leading to the identification of intrachromosomal amplification of chromosome 21 (iAMP21). Single-nucleotide polymorphism (SNP)-array was performed on the same sample using Affymetrix® Cytoscan High Density Array. Analysis revealed only few abnormalities. Leukemic cells were characterized by small deletions targeting *ETV6* (location: 12p13, size: 242 kb), *RB1* (location: 13q14; size: 98 kb), and *BTLA* genes (location: 3q13; size: 164 kb) without *IKZF1* deletion, and a highly rearranged chromosome 21 with multiple regions of gain, amplification, and deletion (Fig. 1D). Notably,

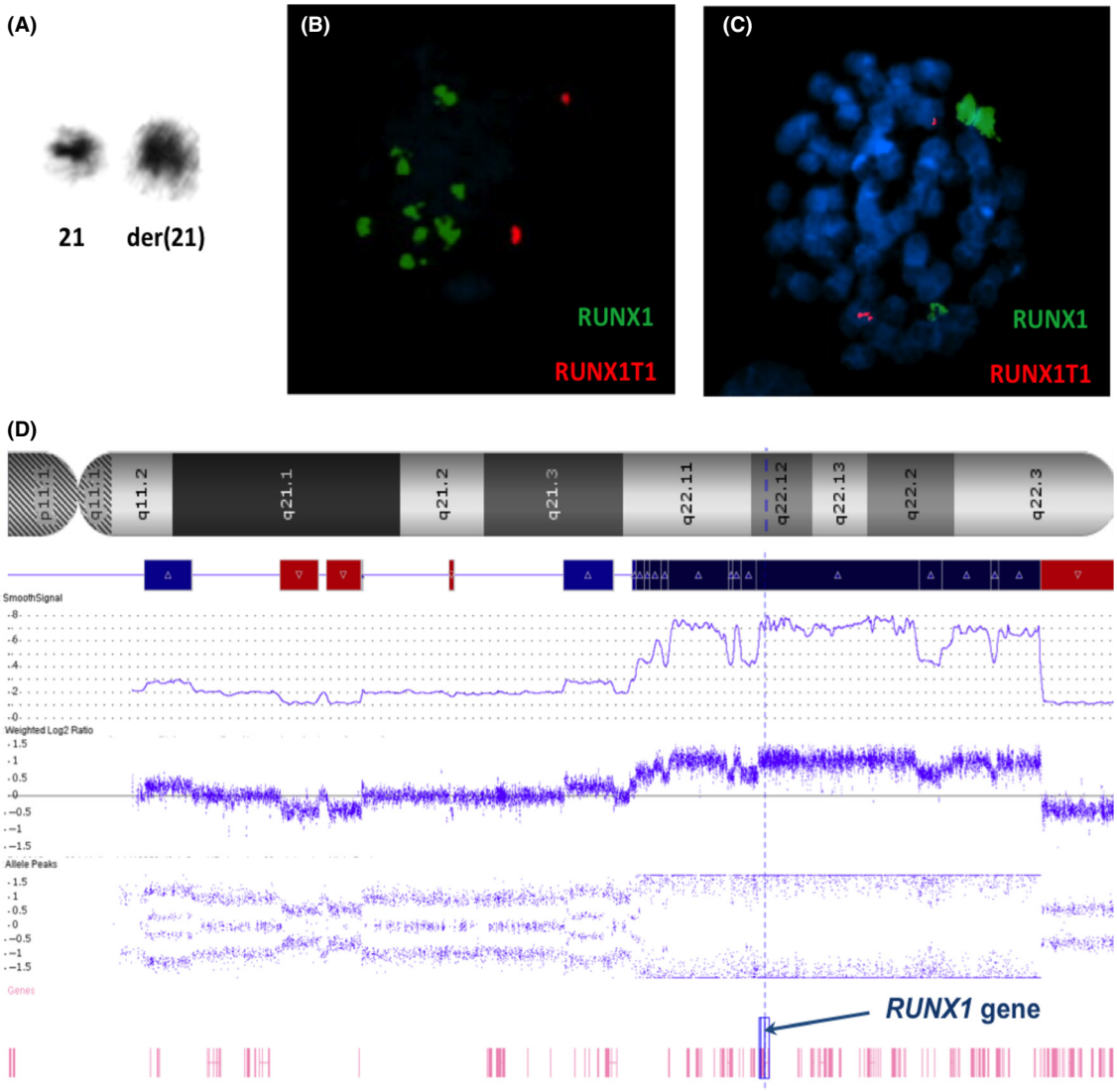

Figure 1. Cytogenetics study of bone marrow sample at BCP-ALL diagnosis. Karyotype was: 46,XX,der(21) [20] and displayed a grossly abnormal derivative chromosome 21 (A). FISH analysis on interphase (B) and metaphase (C) using a probe specific to *RUNX1* (green signals) on chromosome 21q22 confirmed the amplification of *RUNX1* with a median of 6 extra copies and implication of the derivative chromosome 21. SNP-array revealed complex copy number variations with the highest level of amplification located within the *RUNX1* locus (screenshot from Chromosome Analysis® suite). Copy number (referred to *SmoothSignal*) is 2 for normal situation, <2 for loss (red boxes), and >2 for gain/amplification (blue/dark blue boxes) (D).

the highest level of amplification spanned the *RUNX1* locus with a copy number of eight in accordance with the FISH analysis. The patient was enrolled in the very high-risk (VHR) group of the EORTC 58081 protocol and achieved complete remission with complete molecular response.

iAMP21 is now considered as a distinct cytogenetic subgroup of BCP-ALL which has been confirmed to be a primary genetic event [1, 2]. This abnormality has been reported in about 2% of childhood BCP-ALL with a poor event-free survival rate when treated with standard chemotherapy, which justifies to assign such patients in the very high-risk group and treat them intensively [3]. Despite its strong prognostic association, iAMP21 is not systematically investigated in routine practice and its detection may be difficult by conventional cytogenetics. Especially, the morphology of the abnormal chromosome 21 is highly heterogeneous between patients. FISH analysis provides an efficient detection method to evaluate the number of copies of *RUNX1*. Finding three or more extra copies of *RUNX1* is very suggestive of iAMP21. However, interpretation of cases with FISH analysis on interphase

alone should be made with caution, since *RUNX1* extra copies could also be associated with hyperdiploid BCP-ALL. In this context, SNP-array is of great interest as no cell culture is needed, and it provides a distinctive profile of iAMP21, with the highest level amplification of a region spanning *RUNX1* and the lowest level of copy number at the telomeric region of the chromosome 21. Recent findings have highlighted the mechanism giving rise to iAMP21. The abnormality occurs nonrandomly as its formation starts by telomere attrition initiating breakage-fusion-bridge cycles that explains highly amplified regions juxtaposed to a deleted telomeric region [4, 5]. Overall, looking for iAMP21 should be performed in routine practice in BCP-ALL patients, especially in cases without recurrent cytogenetic abnormalities, or when an abnormal chromosome 21 is suspected or when a small marker chromosome of undetermined origin is found at karyotype, particularly in older children (median age of 9 years) [4] or poor responders to standard chemotherapy. In this context, SNP-array is a useful method to confirm the accuracy of iAMP21 diagnosis with its pathognomonic profile. Moreover, its use is of increasing importance to refine risk stratification in pediatric BCP-ALL [6].

Conflict of Interest

None declared.

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