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Is intrachromosomal amplification of chromosome 21 (iAMP21) always intrachromosomal?

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Recurrent chromosomal abnormalities in childhood B-cell acute lymphoblastic leukemia (B-ALL) provide prognostic information that is useful in determining treatment stratification. iAMP21 is a more recently recognized cytogenetic entity of B-ALL that was originally described as multiple copies of the *RUNX1* gene on a structurally abnormal chromosome 21. Subsequent studies elucidated a common region of highest-level amplification that includes *RUNX1*. Fluorescence in situ hybridization (FISH) is the most common method used to identify iAMP21, which is defined as the presence of five or more total copies of *RUNX1*, with three or more extra *RUNX1* signals on a single abnormal chromosome 21. More recently, chromosomal microarray (CMA) and next generation sequencing have uncovered a characteristic chromosome 21 copy number profile in cases of iAMP21.

We present a case of iAMP21 that does not fit the formal FISH definition. However, CMA uncovered the characteristic chromosome 21 copy number profile that is seen in iAMP21, demonstrating that CMA is helpful for the detection of this entity when FISH results are ambiguous. Furthermore, CMA showed that the highest level of amplification in this case did not include the *RUNX1* gene, consistent with current evidence that *RUNX1* is not the primary target of amplification.

Keywords iAMP21, *RUNX1*, acute lymphoblastic leukemia, FISH, chromosomal microarray

Introduction

Intrachromosomal amplification of chromosome 21 (iAMP21) was originally described as multiple copies of the *RUNX1* gene on a structurally abnormal chromosome 21 (1,2). Subsequently, iAMP21 was recognized as a distinct cytogenetic subgroup of B-cell acute lymphoblastic leukemia (B-ALL) that is associated with a high risk of relapse (3–5). This entity comprises 2% of childhood B-ALL, and is frequently associated with an older age at presentation and a low white cell count. In cases of iAMP21, there is usually one normal chromosome 21 and one structurally abnormal chromosome 21. The abnormal chromosome 21 varies in G-banded appearance between different patients, and even sometimes between clones within a patient.

Multiple studies uncovered a common region of highest level amplification in cases of iAMP21 that includes *RUNX1* (6,7). Thus, fluorescence in situ hybridization (FISH) using a probe that includes *RUNX1* has become the most common method for identification of this entity (6). The formal definition of iAMP21 currently consists of the finding of three or more extra copies of *RUNX1* on a single abnormal chromosome 21, with a total of five or more *RUNX1* signals per cell (8,9). FISH can be problematic for the detection of iAMP21 in cases in which abnormal metaphase cells are absent, as it can be difficult to distinguish between multiple copies of *RUNX1* on a single abnormal chromosome 21 versus polysomy for chromosome 21 by interphase FISH. A characteristic chromosome 21 copy number profile in cases of iAMP21 has also been observed by chromosomal microarray (CMA) or next generation sequencing, and is described as copy number changes from centromere to telomere along chromosome 21, with the highest level of amplification just proximal to a telomeric deletion (10–12). In sporadic cases, the mechanism behind the formation of the abnormal chromosome 21 and the characteristic copy number profile occurs through breakage-fusion-bridge

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cycles followed by chromothripsis and other complex structural rearrangements (13). The case presented here illustrates that CMA may be useful for identifying iAMP21 in cases that are ambiguous by FISH.

Materials and methods

G-banded chromosome analysis

Four cultures of a bone marrow specimen from the patient were initiated at a concentration of 1 million white blood cells per ml of Marrow Max complete bone marrow medium (Gibco®, Thermo Fisher Scientific, Waltham, MA) and harvested after overnight incubation. Slides G-banded with Wrights stain were scanned and metaphase cells were captured using a GSL 10 (Leica Biosystems).

FISH

Interphase FISH was performed using the following probes: Vysis BCR/ABL1/ASS1 dual fusion probe; Vysis MLL break apart probe; Vysis ETV6/RUNX1 (TEL/AML1) ES probe; and Vysis CEP4 and CEP10 probes (Abbott Laboratories, Abbott Park, IL). Previously G-banded metaphase cells were destained and subsequently hybridized with the Vysis ETV6/RUNX1 ES probe, as well as chromosome 7 and 21 whole chromosome paints and a chromosome 7 alpha satellite probe (Cytocell, Tarrytown NY) as described previously (14).

CMA

DNA was extracted from bone marrow using a MagNA Pure Compact Instrument (Roche Diagnostics Corp, Indianapolis, IN). CMA was performed using the CytoScan™ HD Suite following the manufacturer protocol and data was analyzed using ChAS Software (Affymetrix, Inc, Santa Clara, CA).

Results

Clinical presentation

The patient was a 12 year-old female who presented with left shoulder and chest pain, and mild splenomegaly. She had pancytopenia with a white cell count of 4900 cells/μl with 13% circulating blasts. A bone marrow aspirate was diagnostic of precursor B-ALL with 80% blasts by morphology. The blasts were CD19, CD10, HLA-DR and TdT positive by flow cytometry. She was negative for central nervous system disease involvement. Induction therapy consisted of prednisone, weekly vincristine and daunorubicin, and PEGasparaginase. The end of induction bone marrow demonstrated minimal residual disease (MRD) by flow cytometry (0.013% of total white blood cells). Following consolidation therapy, her marrow was MRD negative by flow cytometry. After two courses of high dose methotrexate, she underwent a matched unrelated donor myeloablative transplant and is currently in remission.

G-banded chromosome analysis and FISH

A single abnormal clone was found by G-banded chromosome analysis, with aberrations consisting of monosomy 7, additional material of unknown origin attached to 12p13, and a structurally abnormal chromosome 21 (Figure 1A). Interphase FISH showed no evidence of *BCR/ABL1* or *MLL* rearrangements and centromere probes for chromosomes 4 and 10 demonstrated a normal, disomic hybridization pattern. Interphase FISH with an ETV6/RUNX1 probe was negative for an *ETV6/RUNX1* fusion; however, there were two abnormal cell populations. One population (37% of nuclei) demonstrated three *RUNX1* signals and two *ETV6* signals per nucleus and the second population (32% of nuclei) demonstrated five *RUNX1* signals and one *ETV6* signal per nucleus. The extra *RUNX1* signals were not clustered, but they raised concern for iAMP21 (Figure 1B). The presence of five copies of *RUNX1* prompted metaphase FISH on destained abnormal G-banded cells. All five abnormal G-banded cells that were hybridized with the ETV6/RUNX1 probe demonstrated only three copies of *RUNX1* (one copy on the normal chromosome 21 and two copies on the abnormal 21, Figure 1C); none had five copies of *RUNX1*. The ISCN for this case is as follows:

45,XX,-7,add(12)(p13),der(21)del(21)(q11.2q21),dup(21)(q22q22)[5]/46,XX[15]. ish add(12)(ETV6+,wcp7+),der(21)(RUNX1++)[5].nuc ish(ETV6x2,RUNX1x3)[37/100]/(ETV6x1,RUNX1x5)[32/100]

Metaphase FISH on previously G-banded cells was also performed with additional probes to further characterize the chromosomal abnormalities. A whole chromosome 21 paint probe hybridized along the entire length of both the normal and the abnormal chromosome 21; no signal was observed on other chromosomes. A whole chromosome 7 paint probe hybridized to the normal chromosome 7 and to the additional material on the add(12p), although the CMA results for chromosomes 7 and 12 indicated that the rearrangement cannot be explained as a straightforward der(12)t(7;12). There was only a single chromosome 7 centromere signal, which was present on the normal chromosome 7.

CMA

CMA showed a characteristic chromosome 21 copy number profile for iAMP21, with stepwise gain of copy number involving chromosome 21, and a telomeric deletion adjacent to the highest level of gain (Figure 2). CMA demonstrated between 3 and 4 copies of *RUNX1*, but this is an underestimate of the highest *RUNX1* copy number because of dilution with normal cells, and the presence of two different clones; one with three total *RUNX1* copies per cell and one with five total *RUNX1* copies per cell (see FISH results). Nonetheless, CMA demonstrated that the highest level of amplification of chromosome 21, a 5.55 Mb region containing 50 genes, was distal to *RUNX1* (Figure 2, Table 1). CMA also revealed aberrations involving other chromosomes. Chromosome 7 showed a copy number profile consistent with chromothripsis. There was mosaic loss of all of 9p, with further loss of an 11.5 Mb interstitial segment that includes *CDKN2A*. Chromosome 12 also showed evidence of complex rearrangements, with a terminal mosaic deletion from 12p12.3 to 12pter, an interstitial deletion from 12q12 to 12q13.11, and terminal, copy neutral loss of