Intrachromosomal amplification of chromosome 21 (iAMP21) detected by ETV6/RUNX1 FISH screening in childhood acute lymphoblastic leukemia: a case report

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Chromosome abnormalities that usually define high-risk acute lymphoblastic leukemia are the t(9;22)/breakpoint cluster region protein-Abelson murine leukemia viral oncogene homolog 1, hypodiploid with < 44 chromosomes and 11q23/myeloid/lymphoid leukemia gene rearrangements. The spectrum of acute lymphoblastic leukemia genetic abnormalities is nevertheless rapidly expanding. Therefore, newly described chromosomal aberrations are likely to have an impact on clinical care in the near future. Recently, the rare intrachromosomal amplification of chromosome 21 started to be considered a high-risk chromosomal abnormality. It occurs in approximately 2-5% of pediatric patients with B-cell precursor acute lymphoblastic leukemia. This abnormality is associated with a poor outcome. Hence, an accurate detection of this abnormality is expected to become very important in the choice of appropriate therapy. In this work the clinical and molecular cytogenetic evaluation by fluorescence in situ hybridization of a child with B-cell precursor acute lymphoblastic leukemia presenting the rare intrachromosomal amplification of chromosome 21 is described.

Keywords: *Leukemia, lymphoid; In situ hybridization, fluorescence; Gene amplification; Leukemia, B-cell; Chromosomes, human, pair 21/genetics; Transcription factors; Case reports*

Introduction

Cytogenetic techniques have galvanized efforts to understand the biology of chromosomal changes in childhood acute lymphoblastic leukemia (ALL) and to identify specific alterations that can predict treatment outcome[1]. Several clinically relevant abnormalities are used in risk stratification for treatment within the context of clinical trials[2]. Some chromosome abnormalities that have defined ALL as very high risk are those with t(9;22)/breakpoint cluster region protein-Abelson murine leukemia viral oncogene homolog 1, those that are hypodiploid with < 44 chromosomes, and with 11q23/myeloid/lymphoid leukemia (MLL) gene rearrangements[3]. The spectrum of genetic abnormalities described in ALL is rapidly expanding; therefore, newly described aberrations are likely to have an impact on clinical care in the near future[4].

Intrachromosomal amplification of chromosome 21 (iAMP21) is a rare high-risk chromosomal abnormality that can occur in approximately 2-5% of pediatric patients with B-cell precursor ALL (BCP-ALL)[4,5]. This abnormality has been associated with a poor outcome in patients treated by a standard protocol[2,6]. Therefore, an accurate detection of this abnormality is very important to the choice of appropriate therapy[7].

Here, a clinical and molecular cytogenetic analysis of a child with BCP-ALL and presenting the rare iAMP21 abnormality is described.

Case report

An 11-year-old girl with a two-month history of joint pain in both hands and shoulders that evolved to arthritis was admitted to the Onco-Hematology Pediatric Service of Hospital Estadual Bauru, São Paulo, Brazil. A physical examination revealed that the patient did not present enlarged palpable lymph nodes in the cervical, submandibular, axillary or inguinal regions. No signs of musculoskeletal abnormalities, including inflammatory joint arthritic changes, were noted. Examinations of the cardiovascular and respiratory systems were also normal. The hemoglobin level was low (11 g/dL), white blood cell count was also low (3 x 10^9/L) with no blast cells, platelet count was 165 x 10^9/L and lactate dehydrogenase (LDH) was 23 U/L. The morphologic evaluation of the bone marrow showed 95% blast cells with lymphoid characteristics compatible with French-American-British (FAB) classification L1. Flow cytometry revealed a population with 71% of blast cells that expressed CD79a, CD19, CD20, CD38, CD58, CD66c heterogeneously, low expressions for CD10, CD13, CD22, and negative expressions for cCD3, sCD3, cCD7, CD45, CD34, CD15, CD33, cIgM, and myeloperoxidase (MPO) thus compatible with BCP-ALL. The patient was initially treated according to the ALL-IC-BFM2009 protocol for medium risk[8]. Peripheral blood showed good treatment response to prednisone on Day 8, and bone marrow examination at the end of the induction therapy showed complete remission.

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The authors declare no competing financial interest

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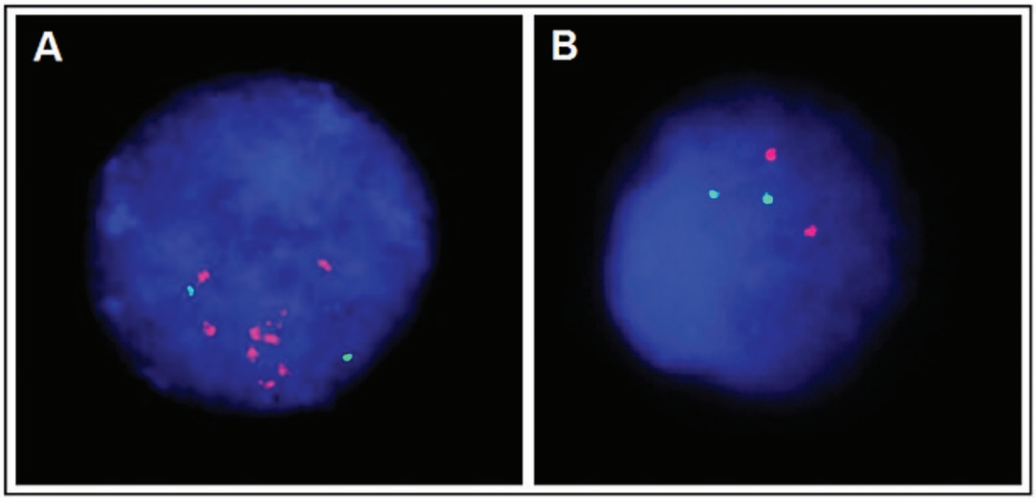

Figure 1 - iFISH with LSI TEL/AML1 dual fusion probe; A) iFISH detected 8 in tandem copies of RUNX1 gene in the present case; B) Negative control for the TEL/AML1 probe showing a normal cell

The patient is clinically well without any evidence of disease five months after diagnosis.

The patient's bone marrow sample was sent for cytogenetic analysis, at the time of diagnosis, to the Cytogenetics Laboratory of the Bone Marrow Unit, National Cancer Institute (INCA). The 24-hour culture technique was unsuccessful in obtaining metaphase cells for chromosome analysis[9]. Fluorescence in situ hybridization (FISH) was performed on interphase cells using the commercially available LSI ETV6/RUNX1 ES dual color translocation probe, LSI MLL dual color break apart rearrangement probe, and the LSI BCR/ABL ES dual color translocation probe (All from Abbott Diagnostics, Maidenhead, United Kingdom), according to the manufacturer's instructions. Two-hundred interphase cells were scored for each probe.

Interphase FISH (iFISH) analysis revealed no ETV6/RUNX1 fusion signals and 6 to 10 signals for the RUNX1 probe in 75% of the analyzed nuclei in this child (Figure 1A & 1B). FISH analyses with MLL and BCR/ABL probes showed normal results.

Discussion

Patients with iAMP21 are characterized by a low white blood cell count, older age (> 5 years old, with a median age of 9-10 years), FAB L1 classification, and common/pre-B cell immunophenotype[4,6,7]. In a multivariate analysis, Moorman et al.[6] observed that the presence of the iAMP21 was an independent indicator of poor event-free and overall survival. Studies have identified iAMP21 as one of the most significant, recently discovered abnormalities in ALL in which genotype has a direct impact on treatment[4,6,7].

iAMP21 was first detected by FISH using the ETV6/RUNX1 probe[7]. iAMP21 can be observed by conventional cytogenetics as a gross abnormality of chromosome 21. This finding is usually observed by FISH analysis, in metaphase, as one signal located on a normal chromosome 21, while the others are seen in tandem duplication along an abnormal chromosome 21[2]. When ETV6/RUNX1 probes are used, patients carrying the iAMP21 usually show two normal copies of the ETV6 signal and multiple RUNX1 signals (three or more additional signals), and are negative for the ETV6-RUNX1 fusion gene. This abnormal chromosome 21 may vary according to its morphological form. In interphase, the signals are clustered together, except for one signal representing the normal chromosome 21 that is usually located apart from the others[2,7].

Recent studies have shown that iAMP21 is mostly found as a single abnormality, suggesting that it is likely to be a primary genetic event given the lack of any other consistent abnormality outside of chromosome 21[2]. Although *RUNX1* is a well-known leukemia-related gene, it does not appear to be the target of iAMP21. iAMP21 is thought to arise through a breakage-fusion-bridge cycle mechanism[10]. These breaks were recently determined by aCGH and long distance inverted (LDI) polymerase chain reaction (PCR) that pointed to an initiating event involving chromothripsis of chromosome 21. This would explain the unexpected nature of the PDE9A rearrangements, the cytogenetic heterogeneity of the iAMP21 marker chromosomes, and the complexity of some array profiles. The common region of amplification on chromosome 21 was confined to a 5.1-mb region that included the *RUNX1*, miR-802, genes mapping to the Down syndrome critical region, and also telomeric breakpoints[11].

Cytogenetic analysis sometimes is hampered by poor mitotic index and poor quality of chromosome preparations, problems that are frequent in ALL studies, as well as the presence of cryptic abnormalities that mask the detection of specific chromosome aberrations by conventional techniques[4,5]. The FISH technique is routinely used to complement cytogenetic studies in pediatric ALL. Our observation underscores the value of FISH analysis as an efficient and fast tool to detect a variety of biomarkers in a single experiment, providing important insights for the management of children with leukemia.

In this work, FISH analysis using the ETV6/RUNX1 probe allowed the identification of a rare chromosome abnormality with important prognostic impact, the iAMP21. Although iAMP21 has

been associated with poor prognosis, new therapies with more intensive treatment may leave behind the prognostic importance of the genetic abnormality.

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