Molecular approaches reveal *RUNX1* amplification and a rearrangement of *NTRK3*, a new leukemia putative gene, in a pediatric case of B-ALL: Discussing the intrachromosomal amplification of chromosome 21 (iAMP21)

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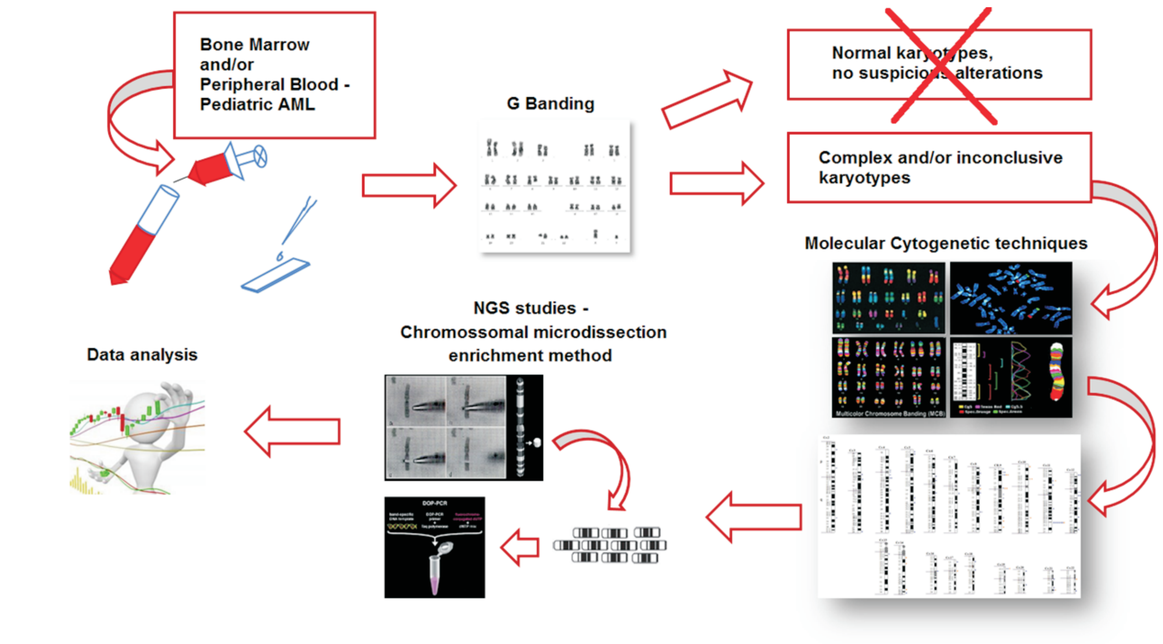
OBJECTIVE

Childhood acute lymphoblastic leukemia (ALL) (~30% pediatric cancers), is a highly heterogeneous disease. Intrachromosomal amplification of chromosome (Chr) 21 (iAMP21) was originally described as multiple copies of the *RUNX1* gene on an abnormal Chr 21. Nowadays it is recognized as a rare (2-5%) distinct cytogenetic subgroup of B-cell ALL which reflects poor outcome and a high rate of relapse. The main cytogenetic features are a normal Chr 21 and a structurally abnormal Chr 21, which varies in G-banded appearance. The current diagnosis is established as those patients who present 3 or more extra *RUNX1* copies in one abnormal Chr 21. Although, the literature has been discussed the limitations regarding a reliable iAMP21 diagnosis. First, in the absence of abnormal metaphase cells, it is difficult to distinguish between multiple copies of the *RUNX1* gene on a single abnormal Chr 21 vs. polysomy of Chr 21. Second, many reports have been showing that *RUNX1* amplification is not only observed in a single abnormal 21, thus, there is a lack of a precise definition.

In this work, we describe the molecular features of a child with B-ALL which presented the rare iAMP21 associated with a rearrangement of the leukemic putative gene *NTRK3*.

MATERIAL AND METHODS



**This project was approved by the research ethics committee of INCA (#088/07)*

Patient

An 11-year-old girl was admitted to the pediatric hematology service at Hospital Federal da Lagoa with a history of bone pain. His laboratory examination showed bicytopenia. Physical examination revealed hepatosplenomegaly. The immunophenotypic examination was consistent with positive intracytoplasmic pre-B Ig positive lymphoblastic leukemia.

The girl was treated according to the BFM 2009 ALL high-risk protocol and is in complete remission for 16 months. The patient is currently awaiting for bone marrow transplantation.

Conventional Cytogenetics

Cytogenetic analysis was performed at diagnosis, before treatment administration, in bone marrow samples cultured for 24 hours according to the standard protocol. The karyotypes were described according to the International System for Human Cytogenetic Nomenclature.

Molecular Assays

Fluorescence *in situ* hybridization-based experiments were conducted using both, homemade and commercial probes, according to the manufacturers' instructions.

Genomic DNA isolated from bone marrow and subjected to array-based comparative genomic hybridization (array CGH) and was performed in accordance with standard procedures.

RESULTS

Conventional cytogenetics by G-Banding technique revealed the karyotype 46,XX,der(21).

FISH assay with the LSI ETV6/RUNX1 dual color, dual fusion probe revealed a normal *RUNX1* signal, plus two extra large *RUNX1* signals (**Figure 1**).

Molecular cytogenetic studies defined the karyotype as: 46,XX,der(21)(21pter->21q22.12::21q22.12amp::21p11.1->21q22.12::21q22.12amp ::15q25.3->14qter),der(15)t(15;21)(q25.3;q22.1) (**Figures 2-4**).

The aCGH analysis identified recurrent acquired CNAs in different regions: gain of 5q22.1q23.1, 7q21.2, amplification of #21 and losses of 2q13, 12q24.11-q24.12, 15p24.2 and 21q22.3.

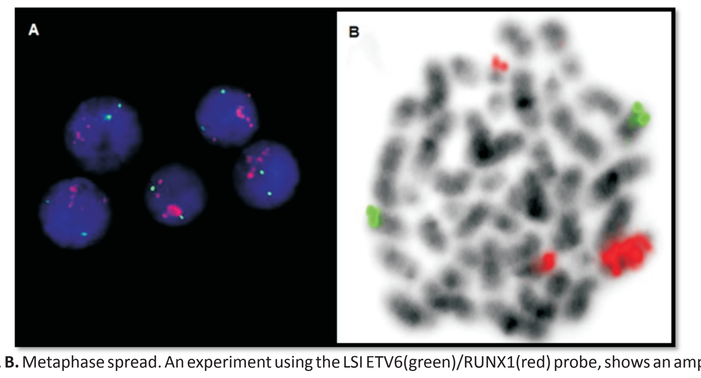


Figure 1: FISH. **A.** Interphase nuclei. **B.** Metaphase spread. An experiment using the LSI ETV6(green)/RUNX1(red) probe, shows an amplification of the *RUNX1* gene.

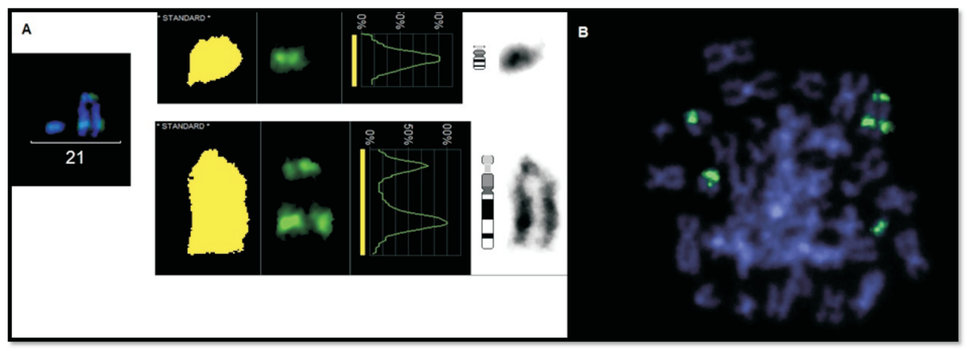


Figure 2: Complementary FISH assay. **A.** Chr. 21 signal pattern. **B.** Metaphase spread. An experiment carried out with the application of a centromeric probe for chromosome 21 (CEP 21) (green signal), which not only confirmed that the marker chromosome was the Chr. 21, but also revealed the participation of the centromere in the translocation, presenting a dicentric Chr. 21.

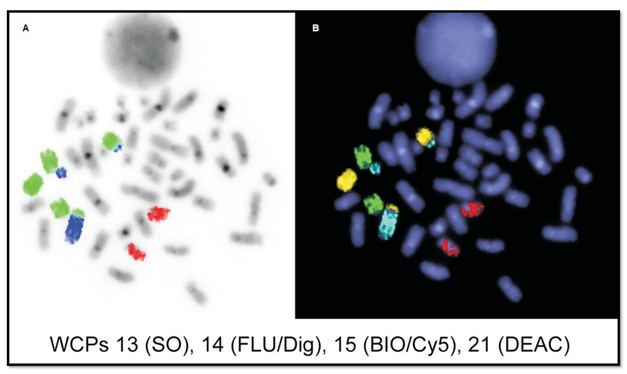


Figure 3: A. Metaphase spread in inverted DAPI. **B.** Metaphase spread in normal DAPI. FISH with WCP probes for chromosomes 13 (red), 14 (green), 15 (yellow) and 21 (blue), to define which acrocentric Chr. from group D was translocated with Chr. 21. The result not only proved to be Chr. 15 which contained part of Chr.21, but also revealed that part of Chr. 15 was also translocated to the marker Chr., thus revealing a reciprocal translocation.

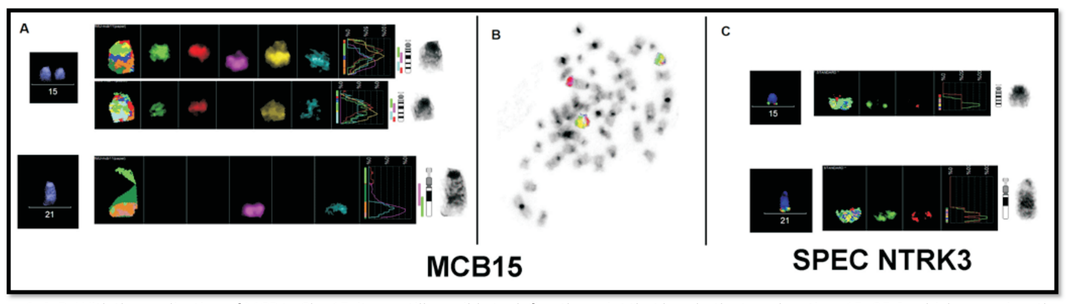


Figure 4: A-B. With the application of MCB to Chr. 15, it was still possible to define the point that breaks the translocation, 15q25.3, which comprises the *NTRK3* gene. **C.** Another FISH experiment, with the LSI NTRK3 probe, has confirmed that this gene is rearranged with the marker Chr.

DISCUSSION AND CONCLUSION

iAMP21 is an independent indicator of poor event-free and overall survival. This abnormality is usually observed by FISH analysis. In our case, we observed *RUNX1* gene amplification by first applying the ETV6/RUNX1 probe. Interestingly, we could observe that it was not only an intrachromosomal amplification, with the involvement of an acrocentric chromosome from group D.

Molecular cytogenetic approaches, using WCP probes, revealed a reciprocal translocation between the aberrant marker Chr 21 and a Chr 15. Johnson and coworkers (2015), in a study with iAMP21 patients, showed cases in which there were extra *RUNX1* signals located in two or three different marker chromosomes. MCB for Chr 15 defined the breakpoint 15q25.3, which comprises the *NTRK3* gene. Current speculation about the oncogenic mechanism of the fused *NTRK3* is related to its putative MAP Kinase pathway activation, with the resulting activation of various downstream proteins, such as transcription factors. Besides, transforming *NTRK3* mutations have already been identified in leukemia patient samples.

Molecular cytogenetics could demonstrate that the formation mechanism of the abnormal Chr 21 was originated by the chromothripsis. Also, we described for the first time the rearrangement of the leukemic putative gene *NTRK3* in a B-ALL pediatric patient with iAMP21.

Acknowledgements

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