CYTOGENOMIC STUDIES HIGHLIGHT A NOVEL PUTATIVE GENE AND SOMATIC HOMOZYGOUS LOSS OF *SH2B3* GENE AND ITS PRESENCE IN CONSTITUTIVE CELLS: DISCUSSING iAMP21 LEUKEMOGENIC MECHANISMS IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

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Objectives

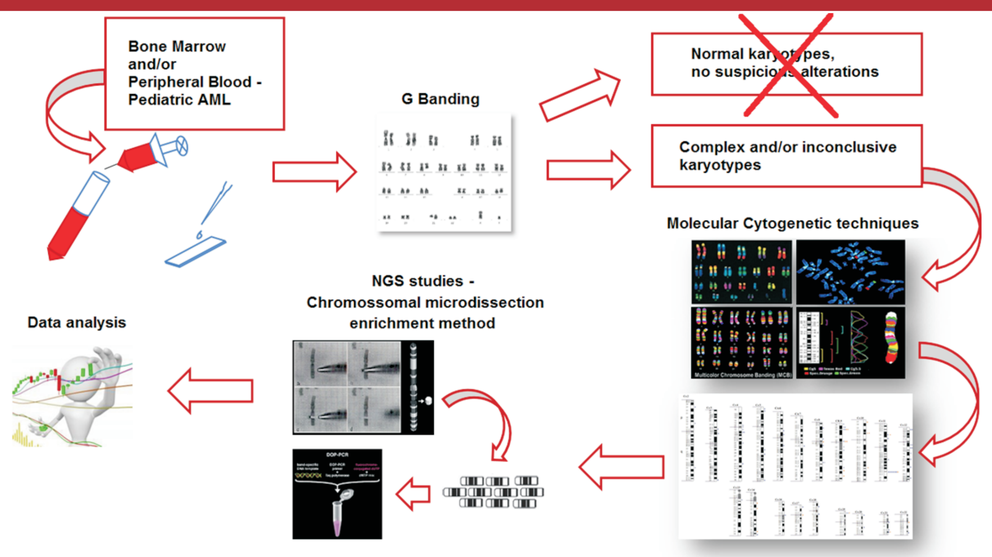
Intrachromosomal amplification of chromosome 21 (iAMP21) is recognized as a new subtype of acute lymphoblastic leukemia (ALL), recently considered as a high-risk entity, is present in 2% of childhood B-ALL.

iAMP21 is so far described as three or more additional copies of the *RUNX1* gene on an abnormal chromosome (chr) 21. Although, there are cases reported in the literature with three or more extra *RUNX1* signals located not only on the abnormal chr 21.

Recently, it has been reported that aberrations in *SH2B3*, a gene which encodes an adaptor involved in the negative regulation of multiple tyrosine kinase and cytokine signaling pathways, are preferentially associated with iAMP21 leukemias. Thus, the identification of novel secondary genetic aberrations may yield important information to potentially indicate novel targets for therapeutic intervention.

Here we describe homozygous deletion of *SH2B3* gene, chromothripsis of chr 21, and a translocation t(15;21)(q25.3;q22.1) with *NTRK3* gene rearrangement, in a child with iAMP21-ALL.

Methods



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

Patient and Methods

A 12-year-old girl was admitted to the Hospital Federal da Lagoa, Rio de Janeiro, Brazil, with a 2-month history of fever, weight loss, arthritis, bone pain, and hepatomegaly (3 cm below the left costal margin). She had bicytopenia with a WBC count of 3.2×10^9/l. Hematocrit was 26.9% and the platelet count was 170×10^9/l. The bone marrow (BM) aspirate was hypercellular, compatible with pre B-ALL phenotype, and the morphologic examination of the BM contents found 95.2% leukemia blast cells.

Flow cytometry analysis revealed a population of leukemic blast cells that expressed CD19+++, CD10+++, cyIgM+ and nuTdT+. The patient was negative for central nervous system involvement. The girl was treated in accordance with a high-risk ALL-BFM-2009 v.13 protocol. She presented a good response to prednisone therapy, and at day 15 of treatment the minimal residual disease was 0.4% of total WBC, and at day 33 it was 0.01% by flow cytometry. The patient is alive, in the maintenance phase, and she has been on remission since week 12 after the initial diagnosis.

Cytogenetic analysis was performed at diagnosis, in BM samples cultured for 24 hours, according to the standard protocol. FISH experiments were conducted using 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.

Transcriptional expression of genes putatively involved in the aberrations *CDK6, RUNX1*, and *SH2B3* was determined by quantitative real-time PCR (RT-qPCR).

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 1a-b).***

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) **(Figure 1c-f).**

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

The RT-qPCR analysis detected elevated expression levels of the *RUNX1* gene (68-fold) and reduced expression of *CDK6* gene (0.057-fold) **(Figure 2c).**

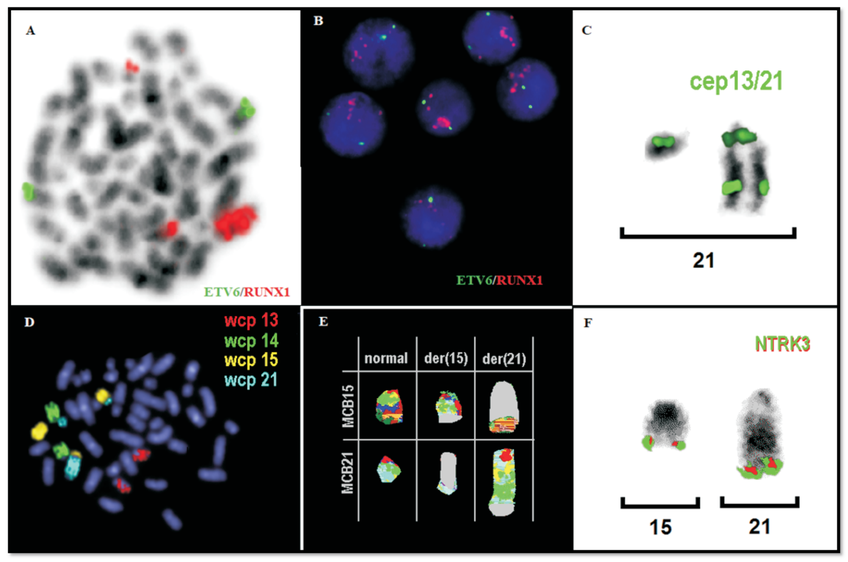


Figure 1: Results of molecular cytogenetic studies. **A,B)** Molecular cytogenetic analysis with FISH using ETV6-RUNX1 probe. (A) FISH showing two normal copies of the *ETV6* gene (green), one normal copy of the *RUNX1* gene (red), one RUNX1 split signal (red), and *RUNX1* amplification (large red signal), in a metaphase spread; **(B)** two normal copies of the *ETV6* gene, and *RUNX1* cluster signals in interphase nuclei. (C) Further FISH analysis with a centromeric probe for chr 21 showing two centromeres (green signals) in the derivative chr 21. **(D)** Molecular cytogenetic analysis using whole-chromosome painting for chrs 13 (red), 14 (green), 15 (yellow), and 21 (light blue), depicting a translocation between derivative chrs 15 and 21. **(E)** MCB panel showing the translocation t(15;21)(q25.3;q22.1). (F) Complementary FISH with a LSI SPEC NTRK3 probe, showing an *NTRK3* gene rearrangement, with a split signal on the derivative chr 21.

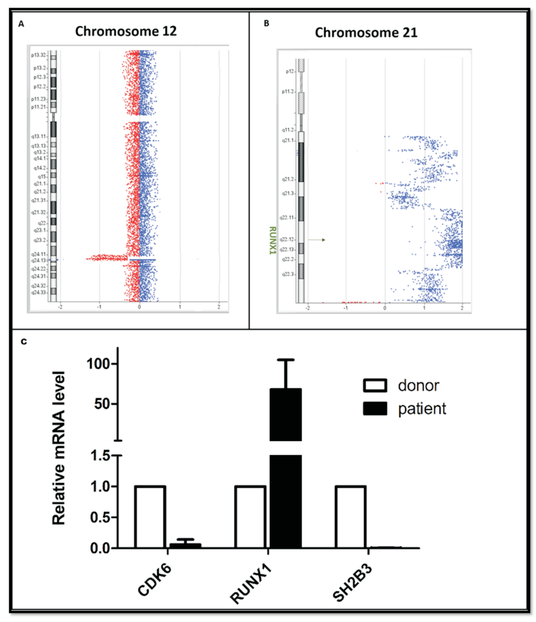


Figure 2: Array-CGH and RT-qPCR results. **A,B)** Array-CGH results. **(A)** aCGH analysis of chr 12 depicted homozygous deletion of *SH2B3* gene at 12q24.12. **(B)** aCGH analysis of chr 21 revealed amplification of #21 and a small deletion at 21q22.3. **(C)** Relative expression of *CDK6, RUNX1* and *SH2B3* in the patient compared with the average values for three healthy donors. Data are presented as means ± SD for three independent experiments.

Discussion and Conclusion

iAMP21 is an independent indicator of poor event-free and overall survival. This abnormality is usually observed by FISH analysis. Interestingly, we observed that *RUNX1* gene amplification was not only intrachromosomal, with the involvement of an acrocentric chromosome from group D.

Molecular cytogenetic approaches 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 with extra *RUNX1* signals located in two or three different marker chromosomes.

Additionally, aCGH showed recurrent acquired CNAs, and rearrangements, in different regions of the aberrant chr 21, compatible with chromotripsis. This suggests that, in iAMP21, the abnormal chr 21 may arise in a catastrophic event, initiated by breakage–fusion–bridge cycles and chromothripsis. Furthermore, the imbalances observed in aCGH prompted us to analyze the putative genes *SH2B3* and *NTRK3*.

According to Baughn and colleagues (2018), the absence of *SH2B3*, a negative regulator of tyrosine kinase signaling, may contribute to poor prognosis in iAMP21-ALL. Therefore, our results corroborate that loss of *SH2B3* may contribute to poor prognosis in iAMP21-ALL.

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.

In this study, we show the involvement of the *SH2B3* gene on the genesis of iAMP21, presenting a homozygous loss in somatic cells and its presence in constitutive cells. Besides, we highlight the presence

Acknowledgements

This work was supported by PROBRAL (DAAD No. 419/14), and FAPERJ (project No. E-26/110: 868/2013 and E-26/200.50/2016), and INCT Para o Controle do Câncer.

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA