The chromosome 21: A recurrent target of genetic and epigenetic abnormalities in paediatric acute lymphoblastic leukemia

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RATIONAL

B-cell precursor acute lymphoblastic leukaemia (BCP-ALL) is a heterogeneous disease with recurrent genetic abnormalities. Aberrations involving chromosome 21 (chr 21) are present in approximately 60% of BCP-ALL including hyperdiploidy (HeH), *ETV6-RUNX1* fusion and intrachromosomal amplification of the chromosome 21 (iAMP21). In the other hand, epigenetic mechanisms could regulate the transcriptional programme and induce the leukemogenic process.

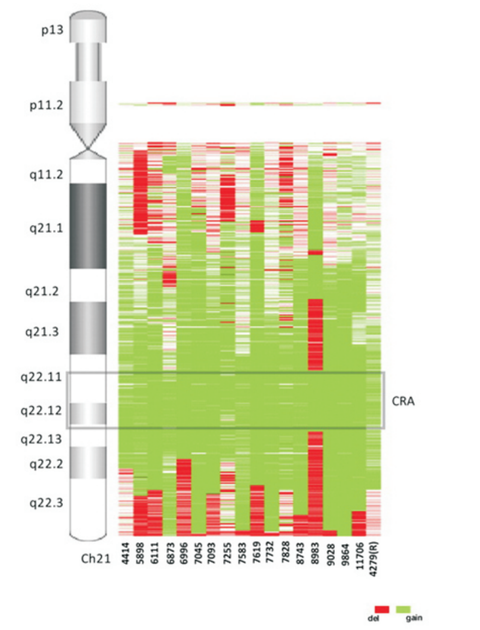

Figure 1: Heat map of chromosome 21 abnormalities in iAMP21 patients. The regions of deletion are in red, gain in green, and normal copy number in white. Rand et al., 2011

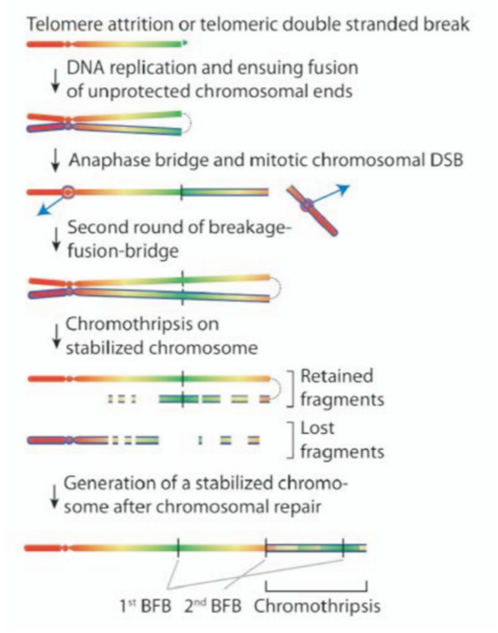

Figure 2: The model proposed to the evolution of the iAMP21 chromosome. Li at al., 2015.

AIM

This study aims to accurate the discrimination of the iAMP21 in BCP-ALL as well as seeks to characterize the DNA methylation profile between ALL with abnormalities involving the chr 21.

MATERIAL AND METHODS

A series of 373 BCP-ALL samples were selected for copy number alterations (CNA) analysis concerning the chromosome 21. Multiplex ligation probe amplification (MLPA, SALSA P327_A1 and P327_B1) was performed according to manufacturer instructions. FISH was performed using the "LPH012 TEL/AML1 translocation, dual fusion probe and centromere probes to the Chr 4, 8, 10, 14, 17, 18, X and Y (Cytocell, Cambridge, UK). Additionally, to the DNA extracted from cases of BCP-ALL, healthy controls and remission samples was modified with EZ DNA Methylation™ Kit (Zymo Research, Irvine, CA) and analysed by the HumanMethylation450 Infinium Assay (Illumina, San Diego, CA). The statistical analysis has been performed using RStudio software with different Bionconductor packages.

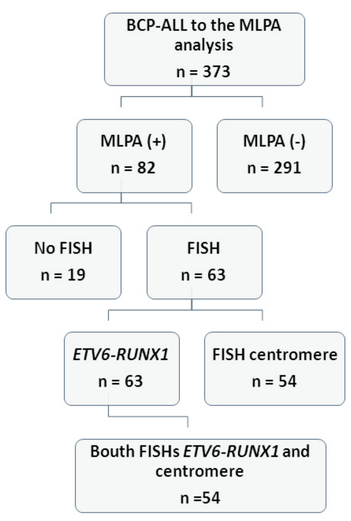

Figure 3: Flowchart of BCP-ALL samples included in the study and analysed by different techniques, Brazil, 2002-2015.

RESULTS

We found evidence of gains in chromosome 21 in 82 samples analysed by MLPA. Most gains were verified by FISH and 11 samples had ≥5 *RUNX1* signals. The centromere FISH probes characterized 53 samples as HeH. The extra copy of the chromosome 21 has been confirmed in all the cases in addition to other gains: chromosomes 4 (58%), 10 (57%), 14 (84%), 17 (53%) , 18 (60%), X (86%) and Y (46%). Losses of the chromosomes 17 (2%) and Y (4%) were also identified. Two BCP-ALL with *ETV6/RUNX1* had extra copy of the chromosome 21 Two patients had iAMP21 identified by MLPA and confirmed by FISH with telomere probe targeted to chromosomes 13 and 21. The case versus. control analysis regarding the DNA methylation array showed 5031 differentially methylated CpG sites (adjusted p <0.001). It has been observed that the methylation profiles of cases and controls were very distinct, while the controls and remission samples had similar profiles within these 5031 sites.

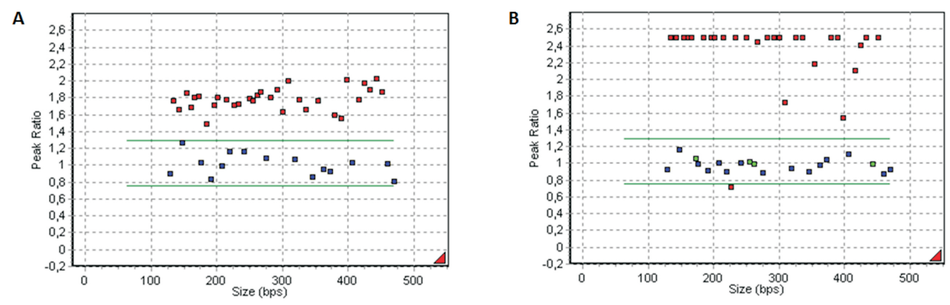

Figure 4: Screen short of the MLPA graphic. and iAMP21. A) Isochromosome 21 in one HeH sample showing the the profile of gain involving all the MLPA probes tested in the chr 21. B) A iAMP21 sample characterized by the high level of amplification (peak ratio higher than 2,13) in a non-homogenous distribution.

Table 1: Demography and Clinical features of B-cell precursor ALL, Brazil, 2002-2015

Characteristics	Positive MLPA n (%)	Negative MLPA n (%)
Total	82 (23)	291 (79)
Gender		
Male	45 (55)	164 (56)
Female	37 (45)	127 (44)
Age range, (years)		
≤1	4 (5)	34 (12)
2-10	66 (80)	177 (61)
>10	12 (15)	80 (27)
Skin color		
White	42 (51)	117 (43)
Non white	39 (49)	157 (57)
WBC, x10⁹/l		
<20	43 (54)	142 (50)
20-50	16 (20)	56 (20)
>50	21 (26)	88 (31)
Immunophenotype		
Pró-B ALL	0 (0)	29 (10)
Common ALL	74 (90)	226 (78)
Pre-B ALL	8 (10)	36 (12)
Risk group (NCI)		
Standard risk	47 (57)	139 (48)
High risk	35 (43)	152 (52)
Status		
Alive	26 (79)	28 (65)
Dead	7 (21)	15 (35)

Abreviation: n, number; WBC, white blood cell count x10⁹/l.

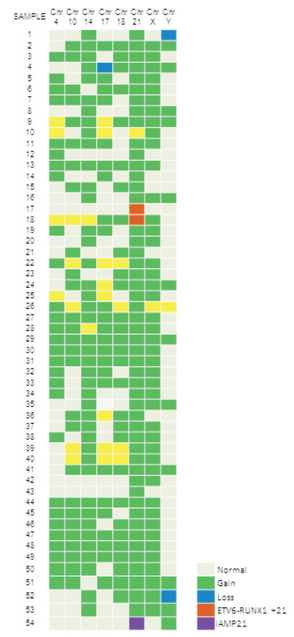

Figure 6: Heat map plotting the FISH results from the telomeric probes depicted by color-coded squares.

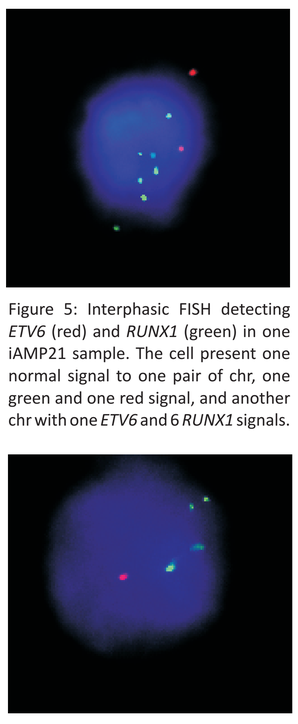

Figure 5: Interphasic FISH detecting *ETV6* (red) and *RUNX1* (green) in one iAMP21 sample. The cell present one normal signal to one pair of chr, one green and one red signal, and another chr with one *ETV6* and 6 *RUNX1* signals.

Figure 7: Interphasic FISH detecting the chr 13/21 (green) and 21q (red) in one iAMP21 sample.

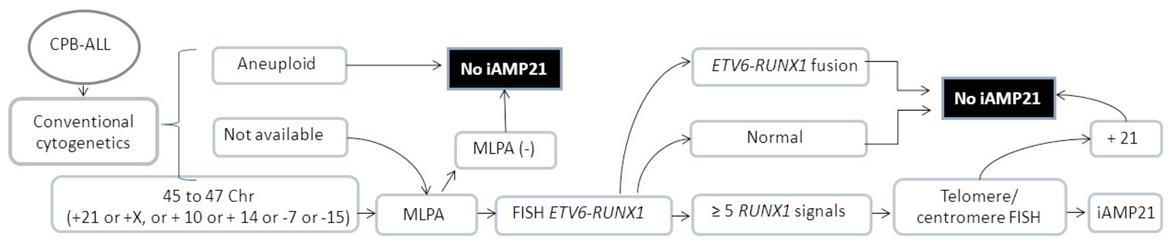

Figure 8: Workflow of tests to identify the iAMP21. BCP-ALL with 45-47 chr or in absence of conventional cytogenetic must be tested by MLPA. Positive MLPA samples have to be confirmed by FISH (*ETV6-RUNX1* probes). Samples who present ≥5 *RUNX1* signals have to be additionally evaluated by centromere/telomere FISH (chr 13/21). In case of ≥5 *RUNX1* signals by FISH not related with gain of chr 21 (centromere/telomere FISH), it is possible to affirm it is really an iAMP21.

Figure 9: Hierarchical clustering of iAMP21 cases, controls and remission samples. A. Case versus control analysis with 5.031 DMR. B. Case versus remission with 6796 DMR. Hypermethylated regions are highlighted in red and hypomethylated regions in green.

CONCLUSIONS AND PERSPECTIVES

To identify iAMP21 cases is recommended to using centromere/telomere FISH probes along with the MLPA tests when it is not possible to access the HeH by conventional karyotyping. The BCP-ALL patients present a distinct DNA methylation, in the next step of this study we intend to perform comparisons between different BCP-ALL subtypes in order to characterize the DNA methylation signature between these groups.

Projeto Gráfico: Serviço de Edição e Informação Técnico-Científica / INCA

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