CHARACTERIZATION OF *RB1* IN PEDIATRIC *TCF3-PBX1*+ ACUTE LYMPHOBLASTIC LEUKEMIA

THAYANA DA CONCEIÇÃO BARBOSA[1], MARCELA BRAGA MANSUR[1], CAROLINE BARBIERI BLUNCK[1], EUGÊNIA TERRA GRANADO PINA[1], CAMILA FERNANDA COSTA GOMES DE ANDRADE[1], MARIANA EMERENCIANO[1], MARIA S POMBO-DE-OLIVEIRA[1] & THE BRAZILIAN STUDY GROUP OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA*

[1] Pediatric Hematology-Oncology Program, Research Center, Instituto Nacional de Câncer, Rio de Janeiro, Brazil. * List of co-authors included as part of BSGCALL: Caroline Pires Poubel, Ana Luiza Tardem Maciel, Bruno de Almeida Lopes, Júlio César Santoro de O. Assis, Alessandra Faro, Bruno Gonçalves Aguiar, Carolina da Paz Zampier, Elda Pereira Noronha, Isis Quezado Magalhães, Patricia Carneiro de Brito, Renato Guedes, Gustavo Ribeiro Neves, Andrea Gadelha Nobrega, Gilson Guedes Filho, Marcelo dos Santos Souza.

INTRODUCTION

- Genomic alterations such as *TCF3-PBX1* are frequently associated with B-cell precursor acute lymphoblastic leukemia (BCP-ALL) risk stratification;
- Additional genomic aberrations, observed in most pre-leukemic clones, are crucial in both BCP-ALL leukemogenesis and treatment management;
- Our aims were to explore the role of deletions in genes involved in key BCP-ALL pathways and to evaluate the gene expression profile of genes involved in cell cycle regulation according to the presence of *RB1* deletions (RB1^{del}).

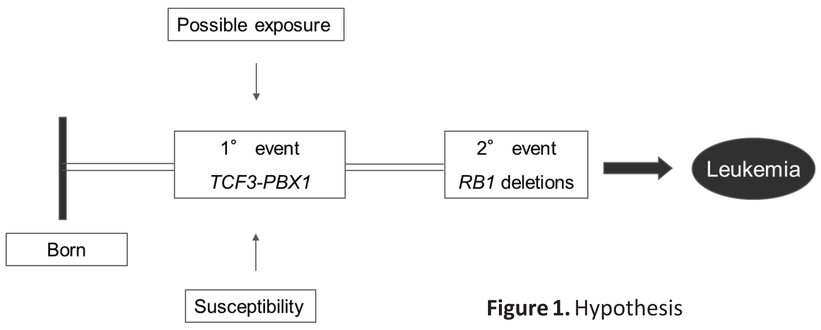


Figure 1. Hypothesis

METHODS AND RESULTS

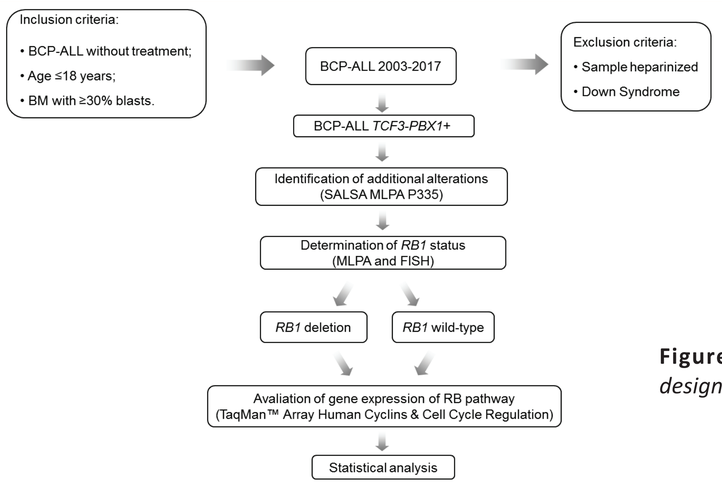
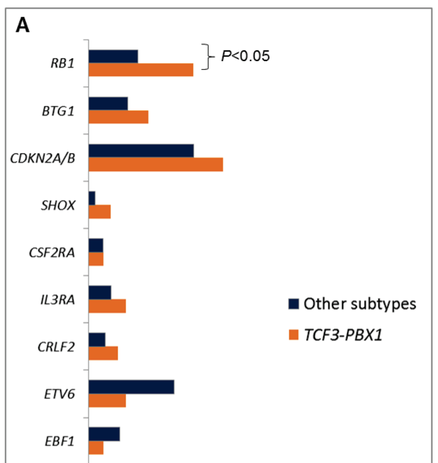


Figure 2. *Schematic design of study.*



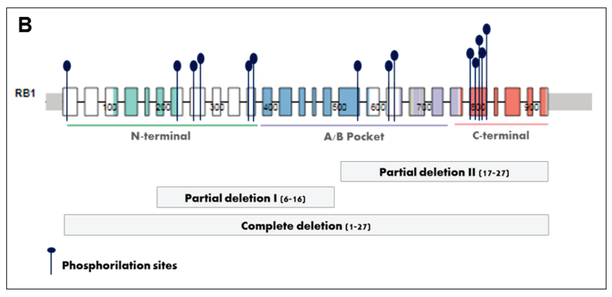


Figure 3. A. Frequency of additional copy number alterations in BCP-ALL according to the presence of TCF3-PBX1. BCP-ALL patients younger than 18 years-old were included in the present study. Fifty cases were identified as *TCF3-PBX1*+ (6.9% of the overall BCP-ALL series). CNAs were identified by SALSA MLPA P335 ALL-IKZF1 probemix, and the most frequent alterations found in those *TCF3-PBX1*+ cases were deletions affecting *CDKN2A/B* (40.0%) and *PAX5* (37.7%), followed by *RB1* (31.1%) and *BTG1* (17.7%). The frequency of *RB1*^{del} in *TCF3-PBX1*+ patients was markedly different from those with other cytogenetic subgroups of BCP-ALL (*P*<0.05). **B.** Schematic design of *RB1* gene showing the type of *RB1* deletions found in our series of cases and the phosphorylation sites of rb1.

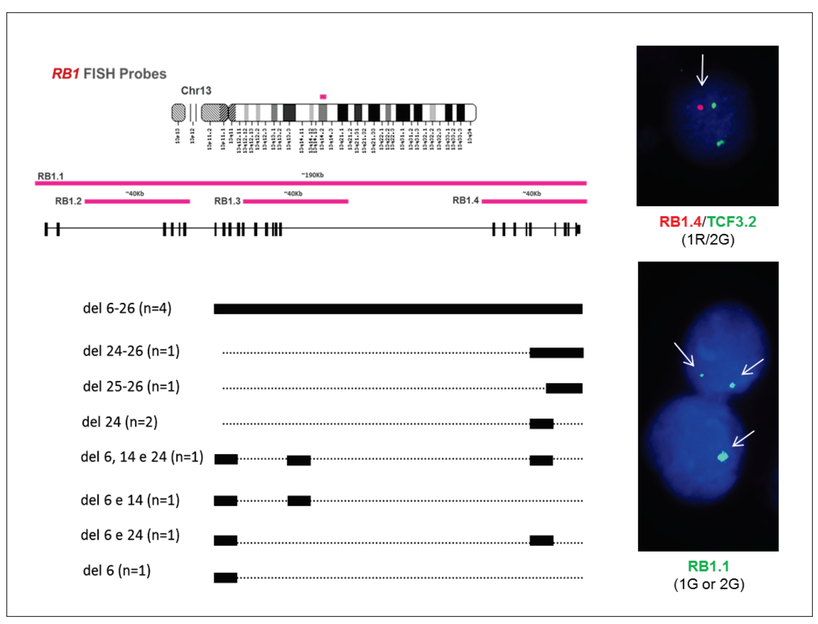


Figure 4. Schematic design of *RB1* BAC probes constructed to FISH screening based in RB1 deletions identified by MLPA. FISH analyses revealed heterogeneity of nuclei harboring *RB1*^{del}, varying from 12% to 97% of the interphase nucleus evaluated.

Table 1. Characteristics of patients with BCP-ALL *TCF3-PBX1* with *RB1* deletions

ID	NCI risk	Gender	WBC count (x10^9/L)	Age (years)	Type of deletion	% of nucleus with deletion
7	Standard	F	62.0	6.2	Complete	-
12	Standard	F	500.0	2.7	Complete	86.1
16	High	F	9.4	18.7	Complete	-
19	Standard	M	39.6	6.4	Partial II	89.0
28	High	F	34.5	10.8	Rare	-
29	High	M	74.6	2.7	Complete	-
33	Standard	F	7.3	6.0	Partial I	-
34	Standard	F	14.0	3.4	Complete	93.1
36	Standard	F	24.2	3.8	Complete	38.6
41	Standard	M	95.6	6.9	Partial II	12.0
44	High	F	9.8	17.1	Partial II	60.0
45	Standard	M	31.7	4.0	Complete	97.0
47	Standard	F	27.0	2.9	Partial II	33.0
49	Standard	F	8.6	0.02	Partial II	75.0
50	Standard	M	25.1	1.2	Partial II	-
51	Standard	F	20.1	2.4	Partial II	64.8

Abbreviations: F, female; L, litre; M, male; Complete deletion: deletion of all exons; Partial I: deletion of exons 6-16; Partial II: deletion of exons 17-26; Rare: deletion of exon 6.

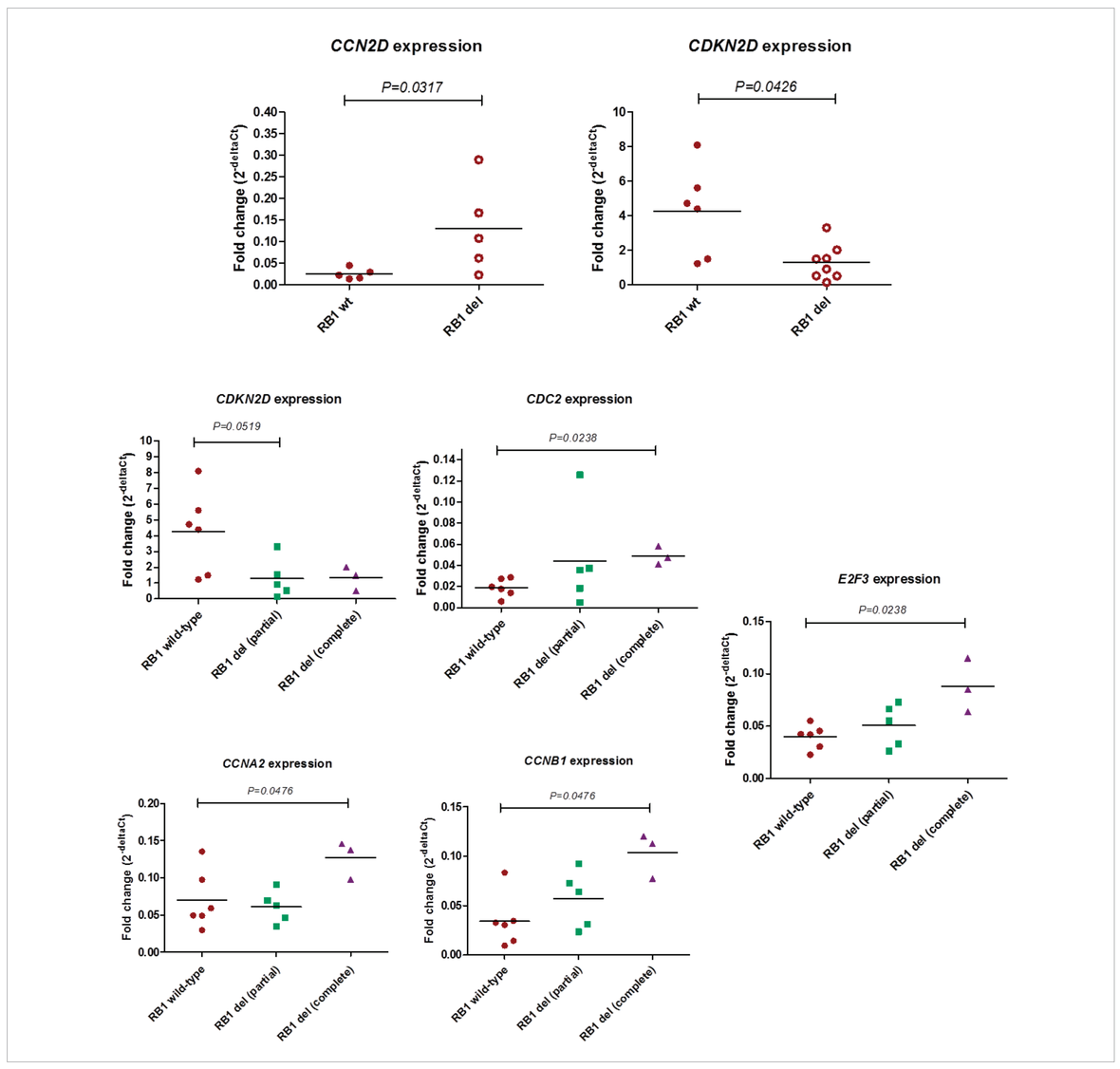


Figure 5. Gene expression profile of target genes using the TaqMan™ Array Human Cyclins & Cell Cycle Regulation, according to the presence of RB1 deletions. The presence of *RB1*^{del} was associated with significantly increased expression of *CCND2* (P=0.032), while the expression of *CDKN2D* was reduced compared to cases without *RB1*^{del} (P=0.043). Additionally, the complete *RB1*^{del} was significantly associated with increased expression of *CCNA2, CCNB1, CDC2* and *E2F3* (*P*<0.05). On the other hand, *RB1* partial deletion (involving exons 17-26) was associated with the reduction of *CDKN2D* expression.

CONCLUSIONS

- *RB1*^{del} were frequently found as an additional aberration, particularly in *TCF3-PBX1*+ patients, and deletions in 9p21 locus were also frequently observed;
- The loss of critical *RB1* phosphorylation sites, as a consequence of complete *RB1*^{del}, deregulate the expression of *E2F3*, an important transcript factor that interacts directly with pRB and regulate the expression of other genes involved in the cell cycle, such as cyclins *A2* and *B1*, and *CDC2*, essentials for G1/S and G2/M phase transitions.

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