Characterization of *RB1* in pediatric *TCF3-PBX1* lymphoblastic leukemia

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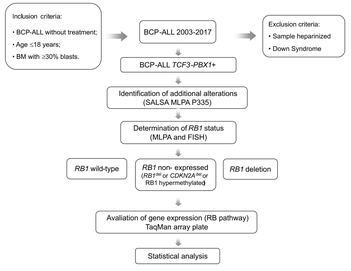
INTRODUCTION

Genomic alterations are frequently associated with acute lymphoblastic leukemia (ALL) prognosis. *TCF3-PBX1* fusion is one of these alterations associated with B-cell precursor ALL (BCP-ALL) subtype. Additional genomic aberrations are observed in most pre-leukemic clones, and they may play a crucial role in BCP-ALL biology and in the treatment management.

AIMS

- To explore the role of deletions affecting genes involved in lymphoid differentiation, cell cycle regulation and cytokine receptors on BCP-ALL *TCF3-PBX1* positive patients;
- To evaluate the difference in the gene expression profile of genes involved in cell cycle regulation when comparing patients with and without *RB1* deletions (RB1^{del});
- To correlate the alterations found with the clinical-laboratorial characteristics and with the risk stratification variables.

MATERIAL AND METHODS



RESULTS

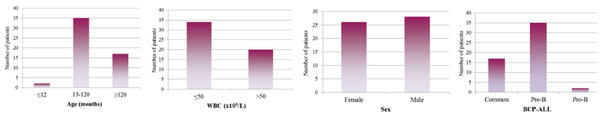


Figure 1. Characteristics of our BCP-ALL *TCF3-PBX1+* patients.

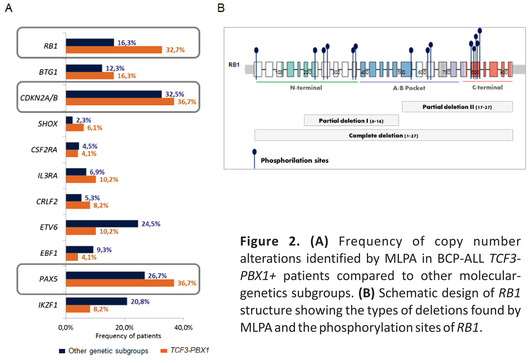


Figure 2. (A) Frequency of copy number alterations identified by MLPA in BCP-ALL *TCF3-PBX1+* patients compared to other molecular-genetics subgroups. **(B)** Schematic design of *RB1* structure showing the types of deletions found by MLPA and the phosphorylation sites of *RB1*.

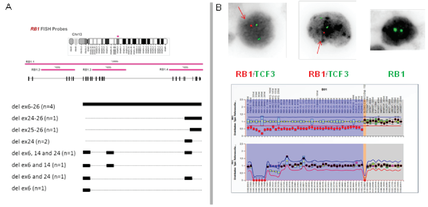


Figure 3. (A) FISH strategy with in-house probes used to confirm *RB1* deletions initially identified by SALSA MLPA P335 ALL kit. **(B)** Characterization of *RB1* deletions by FISH and MS-MLPA (Methylation-Specific Multiplex Ligation Probe Amplification).

Table 1. Demographic, clinical and molecular characteristics of BCP-ALL *TCF3-PBX1+* patients with *RB1* deletions.

ID	Sex	WBC	Age (months)	NCI risk	FISH *RB1*^{del} Probe	FISH *RB1*^{del} % of nucleus with deletion[§]	MLPA P047[+]	Type of *RB1*^{del}
7	F	62	74.4	Standard	-	-	del 13q	complete
12	F	500	32.7	Standard	RB1.1	1S (86.1%)/2S (13.9%)	del 13q	complete
16	F	9.4	224.7	High	-	-	-	partial
17	F	64.1	17.2	Standard	-	-	del 13q	complete
19	M	39.6	77.2	Standard	RB1.4	1S (89.0%)/2S (11.0%)	-	partial
29	M	74.6	32.9	High	-	-	del 13q	complete
33	F	7.3	71.5	Standard	-	-	del 13q	complete
34	F	14	40.9	Standard	RB1.1	1S (93.1%)/2S (6.0%)/3S (0.9%)	del 13q	complete
36	F	24.2	45.1	Standard	RB1.1	1S (38.6%)/2S (66.0%)/3S (1.4%)	del 13q	complete
41	M	95.6	82.6	Standard	RB1.4	1S (12.0%)/2S (87.0%)/ 3S (1.0%)	-	partial
44	F	9.8	205.7	High	RB1.4	1S (60.0%)/2S (39.0%)/3S (1.0%)	-	partial
45	M	31.7	48.3	Standard	RB1.1	1S (97.0%)/2S (3.0%)	del 13q	complete
47	F	27	34.8	Standard	RB1.4	1S (33.0%)/2S (66.0%)/3S (1.0%)	no alteration	partial
49	F	8.6	60.5	Standard	RB1.1	1S (75.0%)/2S (25.0%)	del 13q	complete
50	M	25.1	14.1	Standard	-	-	-	partial
51	F	20.1	28.9	Standard	RB1.4	1S (64.8%)/2S (35.2%)	-	partial

Abreviations: del. deletion; F. female; ID. Identification of patient; WBC, white blood cell; L. litre; M. male.
[§]1S: 1 signal; 2S: 2 signals. 3S: 3 signals.
[+]del 13q: dele tion of long arm of chr13 including *RB1. ENOX1. ITM2B. RCBTB2. DLEU1* and *PCDH8* genes.
[#]partial deletion: deletion of exons 17-27; complete deletion: deletion of exons 1-27.

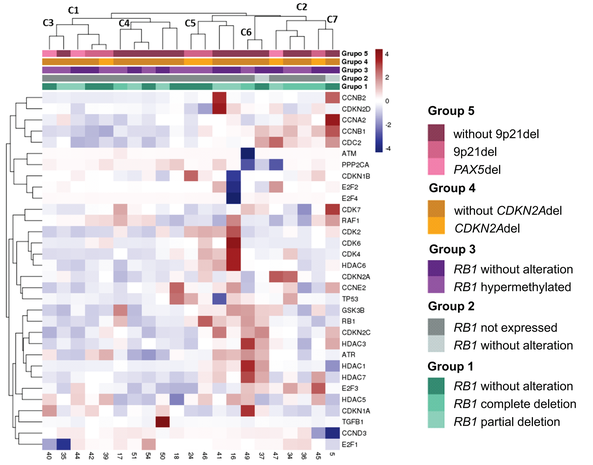


Figure 4. Gene expression profile of cell cycle regulation genes in BCP-ALL *TCF3-PBX1+* patients. Patients were grouped according to *RB1, CDKN2A* and *PAX5* status.

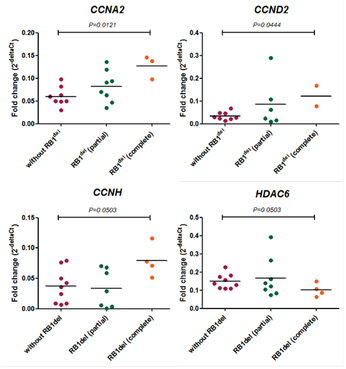


Figure 5. Differences in gene expression profile of cell cycle regulation genes when comparing the type of RB1deletions (partial and complete) vs the group without *RB1* deletions in BCP-ALL *TCF3-PBX1+* patients.

CONCLUSIONS

- *RB1*^{del} were frequently found as an additional aberration, particularly in *TCF3-PBX1+* patients;
- Deletions in 9p21 locus (*CDKN2A/B* and *PAX5*) were also frequently observed;
- In consequence of complete *RB1*^{del} or *RB1* "non-expressed" (*RB1*^{del}, *CDKN2A*^{del} or *RB1* hypermethylated), we identified a disrupted expression profile of genes directly involved with RB1 function, which would potentially affect the G1/S and G2/M transition.

The authors declare no potential conflict of interest.

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