Genetic and functional characterization of *IKZF1* deletion in acute lymphoblastic leukemia

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INTRODUCTION

IKZF1 deletion (Δ*IKZF1*) is an important predictor of relapse in childhood B-cell precursor acute lymphoblastic leukemia (BCP-ALL). Therefore, PCR systems to generate a rapid identification of Δ*IKZF1* are of clinical importance. We previously mapped the breakpoints of intragenic deletions and developed a multiplex PCR (MP-PCR) assay to detect Δ*IKZF1* (Δ2-3, Δ2-7, Δ2-8, Δ4-7, Δ4-8). Because this assay was not able to detect other types of Δ*IKZF1*, we first investigated the genetic scenario of *IKZF1* Δ1-8, and revealed that monosomy 7 and large interstitial deletions on chromosome 7 are the main causes of *IKZF1* Δ1-8. Detailed genomic breakpoint analysis showed that 13% of patients with *IKZF1* Δ1-8 had large interstitial deletions starting within *Cordon-Bleu* gene (*COBL*), which is ~611 Kb downstream of *IKZF1*. *COBL* rearrangements (*COBL*-r) lead to both *IKZF1* complete deletions and tail-to-tail fusions between *IKZF1-COBL*. In order to improve the coverage of the MP-PCR assay for detection of Δ*IKZF1*, we aimed at investigating the characteristics of other intragenic deletions. Also, we settled an international collaboration in order to better characterize *COBL*-r in leukemia, and to explore the oncogenic role of alterations between *IKZF1* and *COBL*.

METHODS

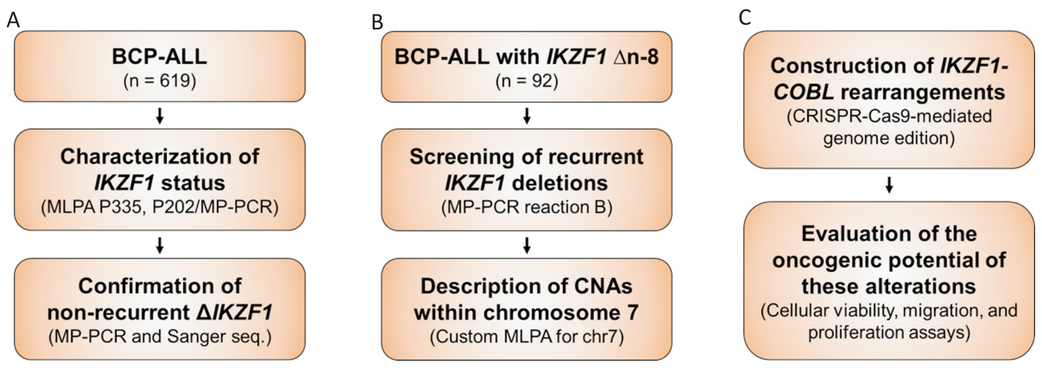

Figure 1. This study is divided into three parts. (A) We investigated patients with BCP-ALL and Δ*IKZF1*, which was evaluated by multiplex ligation-dependent probe amplification (MLPA) P335 and confirmed using MLPA P202. PCR amplification of sequences flanking Δ*IKZF1* will be used for mapping breakpoint hotspots. (B) We studied BCP-ALL with *IKZF1* Δn-8. Patients without recurrent deletions (Δ2-8, Δ4-8) were evaluated by a customized MLPA for the detection of copy number alterations within chromosome 7. (C) We performed the CRISPR-Cas9-mediated genome edition model for the construction of *IKZF1-COBL* rearrangements, and will evaluate oncogenic potential of these alterations.

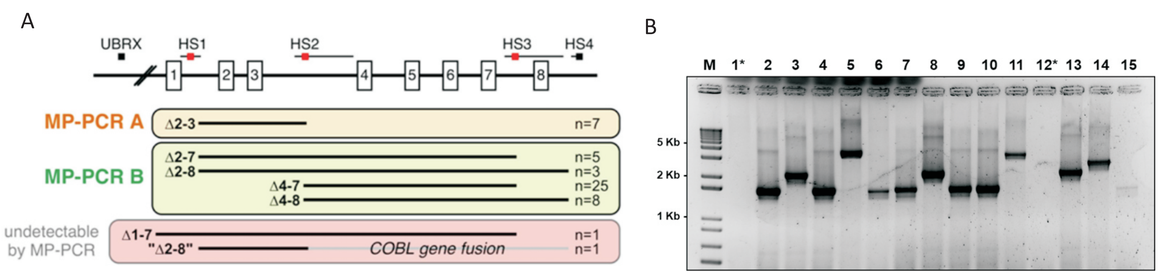

Figure 2. We screened recurrent *IKZF1* deletions (Δ2-8, Δ4-8) using MP-PCR, reaction B. (A) Four different recombination hotspots (HS1-4) and a single upstream breakpoint (UBRX) are described within *IKZF1*. MP-PCR reactions A and B can identify the most frequent *IKZF1* deletions, respectively. (B) We used the MP-PCR reaction B in order to identify recurrent Δn-8 *IKZF1*. Certain samples (*) did not present recurrent deletions, and were further analyzed by custom MLPA. M, 1 Kb ladder; 1-15, samples with either Δ2-8 or Δ4-8 as characterized by SALSA MLPA P335/P202.

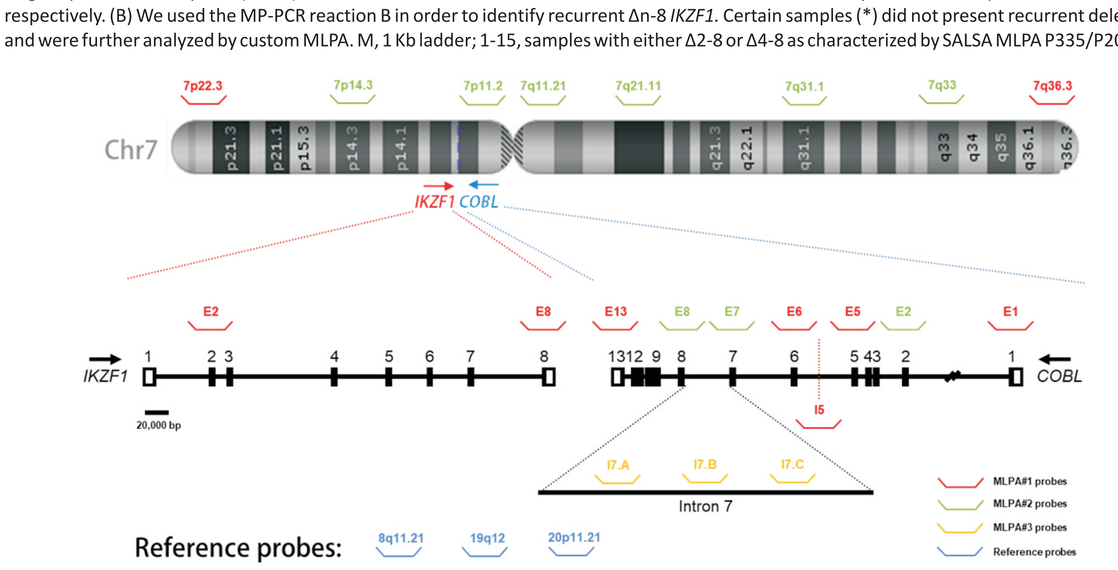

Figure 3. Custom MLPA strategy for analysis of copy-number alterations (CNAs) within chromosome 7. Three MLPA assays were used for analyses, and they were composed by MLPA#1 (red), MLPA#2 (greeen), MLPA#3 (orange) probes mixed with reference probes (blue) located on chromosomes with rare aberrations in BCP-ALL. E, exon; I, intron.

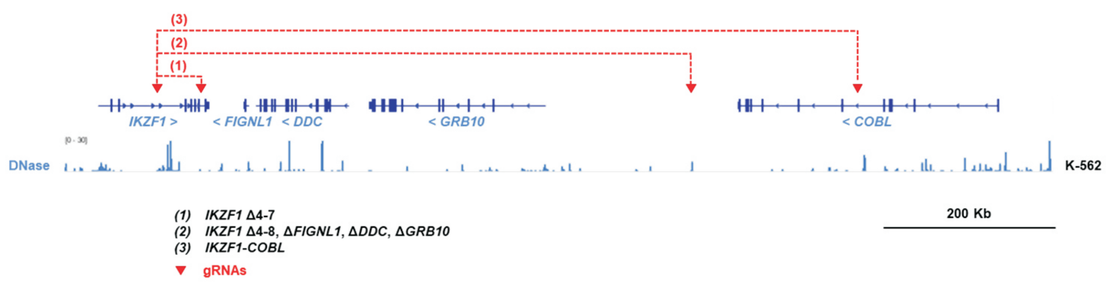

Figure 4. For the experimental assays, we performed the CRISPR-Cas9-mediated genome edition model for the construction of rearrangements that mimic (1) the most frequent intragenic deletion of *IKZF1* (*IKZF1* Δ4-7), (2) the loss of *FIGNL1* and *GRB10* (located between *IKZF1* and *COBL*), and (3) the *IKZF1-COBL* tail-to-tail fusion. The guide RNAs (gRNAs) were designed preferentially within DNase highly sensitive sites of K-562 cell line (ENCODE database).

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RESULTS

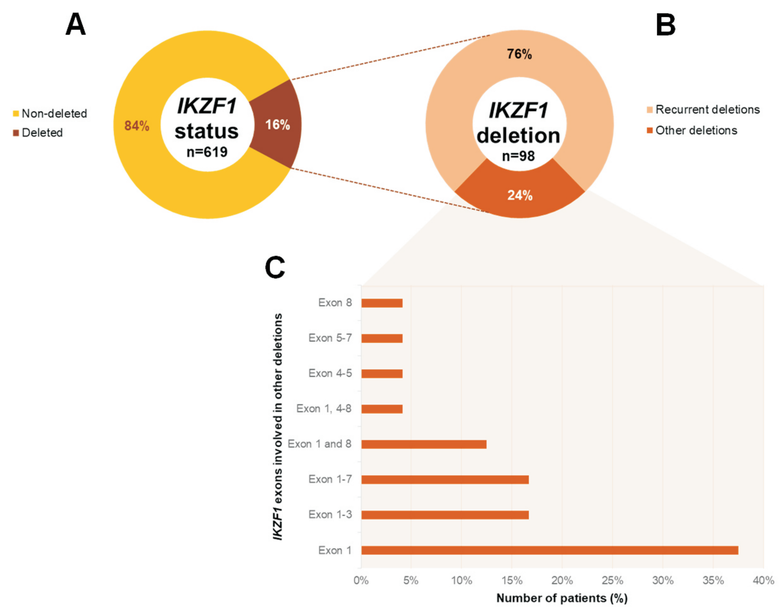

Figure 5. Overview of *IKZF1* deletions in childhood BCP-ALL. (A) *IKZF1* deletions were found in 16% of patients with BCP-ALL. (B) Although most of the patients with *IKZF1* deletions displayed recurrent deletions (Δ1-8, Δ2-3, Δ2-7, Δ2-8, Δ4-7, Δ4-8), 24% of them presented other types of deletions, which were characterized by (C) miscellaneous deletions: Δ1-3 (17%), Δ1-7 (17%), 4-5 (4%), Δ5-7 (4%), and one-exon deletions (58%). Most of the one-exon deletions ranged from *IKZF1* promoter until exon 1.

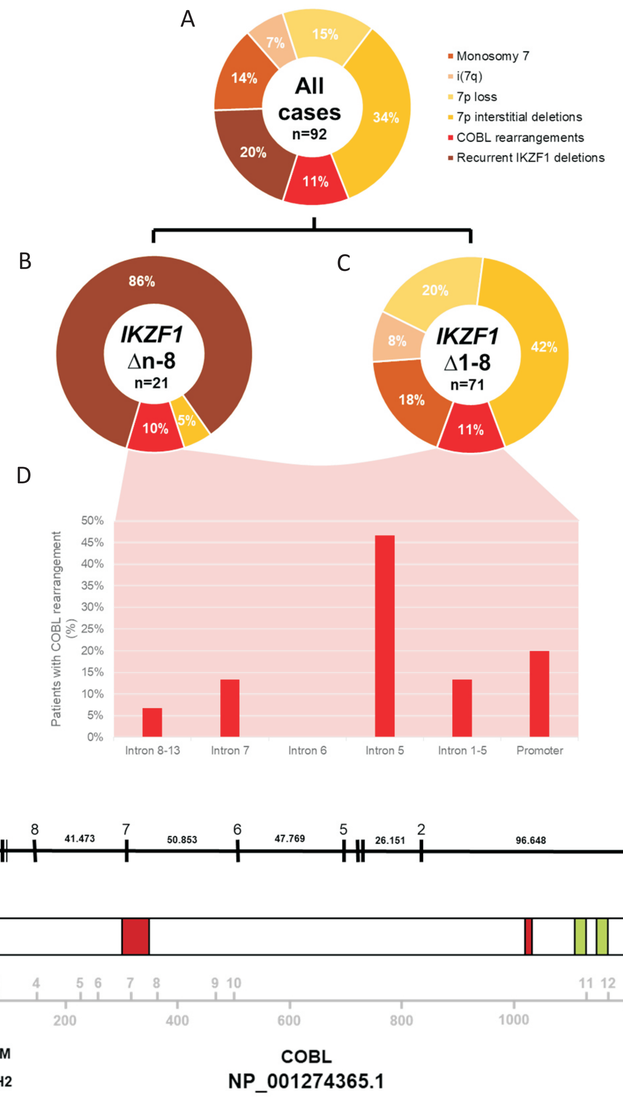

Figure 6. Chromosome 7 alterations in patients with *IKZF1* Δn-8 are represented for (A) all cases with BCP-ALL, (B) *IKZF1* Δn-8 (Δ2-8, Δ3-8, Δ4-8, Δ6-8), and (C) complete *IKZF1* deletions (Δ1-8). (D) In total, 15 patients had *IKZF1* deletions with *COBL*-r, and their breakpoints located close to *COBL* promoter (20%) or within its gene-body (80%), mainly on *COBL* intron 5 (47%) and intron 7 (13%). (E) Structure of *COBL*, which is composed of 13 exons and is located on the minus DNA strand. (F) COBL protein and its proline-rich (PRM, red), and WASP homology domains (green), which are important for its role on acting nucleation. Protein domain information retried from UniProt: COBL (O75128).

CONCLUSION

IKZF1 deletions are characterized by a diverse spectrum of alterations. Although 26% of intragenic deletions are not detected by MP-PCR methods, we show that this group deserves special attention in order to define its importance for diagnosis and risk stratification. Besides, we have shown that deletions involving exon 8 differ according to the type of *IKZF1* deletion; while intragenic deletions (Δn-8) present a breakpoint hotspot ~11.6 Kb downstream *IKZF1*, complete deletions (Δ1-8) are defined by monosomy 7, 7p loss, and interstitial deletions involving *COBL*.

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