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BCL11B, *FLT3*, *NOTCH1* and *FBXW7* mutation status in T-cell acute lymphoblastic leukemia patients

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ABSTRACT

T-cell acute lymphoblastic leukemia is a heterogeneous malignancy originating from developing lymphocyte precursors likely due to mutations in genes regulating thymocyte differentiation. Here, we characterized mutation status of *BCL11B* and *FLT3* genes, presumably involved in T-ALL, together with *FBXW7* and *NOTCH1* as known players in T-ALL in 65 pediatric T-cell acute lymphoblastic leukemia patients. We also aimed at the assessment of prognostic value of *NOTCH1* and *FBXW7* mutations in ALL-IC BFM 2002 protocol. *FLT3* and *BCL11B* mutations were detected in 3% and 2% of patients, respectively. *FBXW7* mutations were observed in 8% of patients, while *NOTCH1* was mutated in 40%. No correlation was found between *NOTCH1* and *FBXW7* mutations and traditionally used clinical factors or molecular features. In total we have detected nine mutations, which have not been previously described by others. Eight of them were found in *NOTCH1* and one in *BCL11B* gene.

Observed frequencies of *NOTCH1* and *FBXW7* are in line with previous reports, thus confirming postulated participation of these two genes in T-ALL pathomechanism. Moreover, we report on mutation frequency of *FLT3* and *BCL11B*, not extensively studied in T-ALL so far. Finally, we suggest a putative role of *BLC11B* as an oncogene in T-ALL pathogenesis.

Introduction

T-cell acute lymphoblastic leukemia (T-ALL) is a malignant disease of thymic cells accounting for 25% and 15% [1] of all childhood and adult acute lymphoblastic leukemia cases, respectively. T-ALL originates from a developing thymocyte, therefore alterations of genes responsible for normal thymocyte development and lineage determination are suspected to contribute to T-ALL pathomechanisms. Since only about half of T-ALL patients carry a faulty karyotype, the attention has been drawn to smaller, not cytogenetically detectable aberrations such as gene mutations.

Among the most groundbreaking findings in T-ALL biology in the recent years was the identification of NOTCH1/FBXW7 pathway disruption in over a half of all T-ALL cases and its possible impact on the patient outcome [2–6]. In the current report we aimed at analyzing mutation status of these two major genes crucial for T-ALL pathobiology in a substantial group of uniformly treated pediatric T-ALL cases with additional focus on mutation status of two more putative contributors: *BCL11B* and *FLT3*.

The products of *NOTCH1* and *FBXW7* are involved in the same signaling pathway and are known to play a major role in the molecular

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pathomechanisms of T-ALL. *NOTCH1* is a cell membrane receptor responsible for signal transduction, thus taking part in the control of hematopoietic cell proliferation, maturation, adhesion, and apoptosis. *NOTCH1* mutations have been reported in about 50% of all T-ALL cases in several reports [2,7–10]. *FBXW7* encodes for a component of ubiquitin ligase complex, which plays a role in the degradation of such proteins as c-MYC, cyclin E and NOTCH1 — molecules crucial for T-ALL pathogenesis [1]. The frequency of *FBXW7* mutations in T-ALL, according to two groups studying *FBXW7* mutations in children so far, ranges between 8.6% and 12% [5,11]. *NOTCH1* mutations together with those of *FBXW7* result in the elevated activity of intracellular NOTCH1 subunit (ICN1) and overexpression of its downstream targets, leading to tumor development. *FBXW7* and *NOTCH1* mutations are also relatively frequently observed in the same patient — 60% of patients carrying *FBXW7* mutation also carry a *NOTCH1* mutation [5,11], suggesting cooperation between these two mutational events leading to the development of T-ALL.

FLT3 encodes a class III membrane receptor tyrosine kinase involved in the regulation of hematopoiesis. It has been postulated that T-ALL patients with *FLT3* mutations could benefit from treatments with small molecule tyrosine kinase inhibitors [12]. However, in two cases studied so far [13] *FLT3* mutations were eradicated in the course of therapy indicating that malignant cells carrying *FLT3* mutations are not resistant to currently administered treatment. In pediatric T-ALL, *FLT3* mutational studies were conducted so far by only three groups, each showing *FLT3* mutation frequency on the level of 2% [13–15]. In contrast to acute myeloid leukemia (AML), where *FLT3* mutations are very frequent (found in 30% of AML patients) and are related to unfavorable prognosis [16], in T-ALL their impact on the outcome is still to be elucidated.

BCL11B is a suspected tumor suppressor/oncogene involved in T-cell lineage differentiation. We have decided to assess its mutation status as it encodes a DNA binding protein expressed in T-cells but not in B-cells and seems to be the key for T lineage development; *BCL11B* is necessary for progression of T-cell precursors from more immature double negative (DN) through more mature double positive (DP) to even more mature single positive (SP) T-cells [17]. The *BCL11B* has been described to be a partner in chromosome translocations observed in T-ALL [18,19]. At the time this study was planned, *BCL11B* mutation status in T-ALL was not known and to date it has only been assessed by two groups [20,21] which have found this gene mutated in 9% of unselected and 16% of *TLX1*-overexpressing T-ALL patients.

Here, we assessed the frequency of mutations of these four genes presumably involved in T-ALL pathomechanism. It is noteworthy, that *FBXW7* and *NOTCH1* mutations have so far been reported to indicate generally good response to treatment but long-term prognosis was reported to differ depending on the protocol administered. Therefore, in the present study we also analyzed the correlation between the mutation status of these two genes and clinical factors traditionally used in ALL-IC BFM 2002 protocol to evaluate prognostic value of these mutations in T-ALL patients treated according to this protocol.

Materials and methods

Sixty five patients aged below 18 years, diagnosed with de novo T-ALL were enrolled in the study, based on the patient/guardian informed consent. The diagnosis was based on cytomorphology and immunophenotyping with standard set of monoclonal antibodies [22]. All but two children were stratified and treated according to the ALL-IC BFM 2002 protocol. The two remaining children were stratified and treated according to the INTERFANT'99 protocol [23]. The study was approved by the Bioethics Committee of Medical University of Silesia, Katowice (Resolution no KNW/0022/KB1/186/08/09 dated 6/01/2009). This resolution covered all centers of Polish Pediatric Leukemia Lymphoma Study Group participating in the study. Patients, according to their age and legal status, and their parents/guardians were informed about the purpose of this research and its potential benefits. Only samples from patients whose parents consented in writing to collection of samples and molecular analysis of their children's samples were included in the study. The average observation time was 1.65 years and for majority of patients was above 2 years.

DNA was extracted from mononuclear cells obtained from the bone marrow collected at diagnosis (QIAamp® DNA Blood Mini Kit, Qiagen, Hilden, Germany). For the mutation analysis, exons 9 and 10 of *FBXW7*, exons 26, 27, 28 and 34 of *NOTCH1* and all four exons of *BCL11B* were first PCR amplified and then directly sequenced. Exons 11 and 12 of *FLT3* gene were amplified and amplicon size was assessed for internal tandem duplication by agarose gel electrophoresis. Primer sequences for *FBXW7*, *FLT3* and exon 4 of *BCL11B* were reported previously [11,20,24,25], and the remaining primers were designed by the Department of Immunology, Erasmus MC, Rotterdam, The Netherlands (Table 1). Sequencing results were checked against Ensembl (http://www.ensembl.org) and Gene Window (NCBI) (http://genewindow.nci.nih.gov) databases. In case of sequence change detection, amplification and sequencing were repeated for confirmation of the event. If synonymous mutations were detected, they were not counted as events, however are listed in Table 2. The newly found mutations have been deposited to GenBank and their accession numbers are provided in Table 2.

Results

The frequency of *NOTCH1* mutations observed in the present study was 40% (26/65) (Table 2). Approximately half of all *NOTCH1* mutations (53%) detected here were found in the heterodimerization (HD) domain, responsible for non-covalent binding of two NOTCH1 subunits. These changes were always in-frame, mostly single nucleotide substitutions or small insertions/deletions (ins/dels) leading to amino acid replacements. Disruption of this region results in ligand independent activation of the ICN1. The other 47% of all *NOTCH1* mutations detected in the present study were found in the region encoding PEST domain, taking part in ICN1 degradation. These changes mostly led to frameshifts and premature introduction of stop codon resulting in transcription termination (10/15 detected changes) and to disruption of the region responsible for tagging ICN1 for degradation. Nine percent of patients studied here had in cis mutations in HD and PEST domain leading to both: uncontrolled ICN1 activation and lack of its degradation.

We have found *FBXW7* mutations in 8% (5/65) of patients (Table 2), all changes being single nucleotide substitutions leading to single amino acid residue exchange. Sixty percent (3/5) of patients with *FBXW7* mutations had also mutations in *NOTCH1* HD domain.

In addition we have observed *FLT3* internal tandem duplication in 2/65 (3%) and *BCL11B* mutations in 2/49 studied patients (smaller number of patients for *BCL11B* analysis was caused by limited material availability). Of the two changes detected in exon 4 of *BCL11B* (Table 2) only one change led to amino acid sequence disruption of this gene. Hence, we report the frequency of *BCL11B* mutations for 2% (1/49) of the studied cases.

Discussion

NOTCH1 and *FBXW7* mutations are predominant in T-ALL. The frequency of 40% found in the present study is in line with other studies, reporting *NOTCH1* mutations in 34–71% of T-ALL cases [2,7–10]. Eight of all changes detected here were new mutations, not reported previously (Table 2). The identified *NOTCH1* changes most often lead to disruption of protein structure by introduction of proline (it reduces stability of α and β structures of NOTCH1) or by elimination of