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Brief Reports

Frequency and clinical relevance of DNA microsatellite alterations of the CDKN2A/B, ATM and p53 gene loci: a comparison between pediatric precursor T-cell lymphoblastic lymphoma and T-cell lymphoblastic leukemia

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Although deletions of cell cycle regulatory gene loci have long been reported in various malignancies, little is known regarding their relevance in pediatric T-cell lymphoblastic lymphoma (T-LBL) and T-cell lymphoblastic leukemia (T-ALL). The current study focused on loss of heterozygosity (LOH) analyses of the CDKN2A/B (chromosome 9p), ATM (chromosome 11q) and p53 (chromosome 17p) gene loci. Frequencies of LOH were compared in 113 pediatric T-LBL and 125 T-ALL who were treated uniformly according to ALL-BFM-strategies. Furthermore, LOH findings were correlated with clinical characteristics and tested for their prognostic relevance. LOH at 9p was detected in 47% of T-LBL and 51% of T-ALL, and was associated with male gender in both. In T-ALL, LOH at 9p was associated with favorable initial treatment response. A tendency for favorable event-free-survival was observed in LOH 9p positive T-LBL. The frequency of LOH at chromosomes 11q and 17p was 5% or less for both diseases.

Key words: lymphoblastic lymphoma, lymphoblastic leukemia, LOH, CDKN2A/B, ATM, p53

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ABSTRACT

Although deletions of cell cycle regulatory gene loci have long been reported in various malignancies, little is known regarding their relevance in pediatric T-cell lymphoblastic lymphoma (T-LBL) and T-cell lymphoblastic leukemia (T-ALL). The current study focused on loss of heterozygosity (LOH) analyses of the CDKN2A/B (chromosome 9p), ATM (chromosome 11q) and p53 (chromosome 17p) gene loci. Frequencies of LOH were compared in 113 pediatric T-LBL and 125 T-ALL who were treated uniformly according to ALL-BFM-strategies. Furthermore, LOH findings were correlated with clinical characteristics and tested for their prognostic relevance. LOH at 9p was detected in 47% of T-LBL and 51% of T-ALL, and was associated with male gender in both. In T-ALL, LOH at 9p was associated with favorable initial treatment response. A tendency for favorable event-free-survival was observed in LOH 9p positive T-LBL. The frequency of LOH at chromosomes 11q and 17p was 5% or less for both diseases.

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Introduction

Pediatric lymphoblastic precursor T-cell neoplasms are currently classified as either acute lymphoblastic T-cell leukemia (T-ALL) or lymphoblastic T-cell lymphoma (T-LBL) depending on bone marrow (BM) involvement: in case of ≥25% BM involvement the diagnose T-ALL is made, in case of <25% BM involvement T-LBL is diagnosed. Both are often considered as different manifestations of one and the same disease because of biological and clinical similarities. Nevertheless, T-LBL and T-ALL might harbor different biological potentials due to certain differences of immunophenotypic and molecular genetic parameters which were reported recently.1-3

Beside other molecular mechanisms, chromosomal deletions with consecutive loss of tumor suppressor genes have been reported as contributors for the pathogenesis of ALL.4 The most common deletions in T-ALL are located at chromosome 9p21, which contains the CDKN2A/B gene encoding for cyclin-dependent kinase inhibitors p16INK4A, p15INK4B and the alternative reading frame protein of the CDKN2A locus p14ARF:

These proteins negatively regulate G1 to S transition of cell cycle and thus prevent cell division. The tumor suppressor gene p53 on chromosome 17p represents another key regulator of apoptosis and cell cycle arrest upon DNA damage, which is frequently altered in solid tumors. However, in pediatric T-LBL and T-ALL, alterations of p53 are reported to be rare events.6,7 The ataxia telangiectasia mutated gene (ATM), located on chromosome 11q22, is a tumor suppressor gene acting upstream of p53. ATM is activated by DNA damage and phosphorylates proteins, such as p53. The ATM gene is reported to be frequently deleted in lymphoid malignancies, including childhood ALL.8-10 Data on ATM alterations in T-LBL are lacking until now.

On the whole, deletions and loss of heterozygosity of several key regulator genes of the G1 to S transition of cell cycle have been reported for lymphoid malignancies. However, the frequency of these alterations and their prognostic relevance remains yet to be elucidated for pediatric T-cell lymphoblastic lymphoma and leukemia. The current study focused on LOH analyses of the CDKN2A/B locus, the ATM locus and the p53 locus, comparing the results obtained in 113 pediatric T-LBL cases with the findings obtained in 125 pediatric T-ALL samples.
Design and Methods

Patients

Between 04/1995 and 06/2007 264 T-LBL-patients were registered in the NHL-BFM study-center (Non-Hodgkin Lymphoma Berlin-Frankfurt-Munster-Group) after informed consent. Of these, 113 T-LBL patients with available tumor and germ-line DNA were evaluable for LOH-analysis. Between 08/1999 and 07/2002, 186 pediatric T-ALL-patients were registered in the ALL-BFM study-center after informed consent, from which 125 were evaluable for LOH-analysis. In both, T-LBL and T-ALL there was no difference in clinical characteristics between evaluable and non-evaluable patients (Online Supplementary Tables 1 and 2). The studies were approved by the responsible ethics committees.

Diagnosis and therapy

T-LBL were classified according to WHO-Classification of Haematological Malignancies, and were treated according to an ALL-BFM-type treatment strategy.11 T-ALL, diagnosed by cytomorphology and flow cytometry analyses of bone marrow (BM) and peripheral blood (PB) were also treated according to ALL-BFM treatment strategy.12

DNA microsatellite analysis

The following set of microsatellite markers were analyzed: D9S1869, D9S285, D9S157, D9S162, D9S171 flanking the gene locus CDKN2A/B (chromosome 9p), D11S1339, D11S2179, D11S1294, D11S4206, D11S4090 flanking the ATM gene locus (chromosome 11q), and the common marker TP53 for the p53 gene (chromosome 17p). Primer sequences, were retrieved from Genome Database (GDB http://www.ncbi.nlm.nih.gov) and ensembl database (www.ensembl.org) (Online Supplementary Table 3). The PCR primers were synthesized by MWG Biotech (Ebersberg, Germany). Preparation of DNA and microsatellite analysis was performed as described previously.3,13

Statistical analysis

The probability of event-free survival (pEFS) was calculated according to Kaplan and Meier with differences compared by the log-rank test. pEFS was calculated from the date of diagnosis to the first event (death from any cause, relapse, resistant disease, or second malignancy) or to the date of the last follow-up. Patients lost to follow-up were censored at the time of their last follow-up examination. Differences in the distribution of individual parameters among patient subsets were analyzed using the χ² test or Fisher’s exact test. Statistical analyses were conducted using the SAS statistical program (SAS-PC, Version 9.1, Cary, NC: SAS Institute Inc.). Follow-up data were updated in June 2008.

Results and Discussion

Precursor T-cell lymphoblastic lymphoma

In T-LBL DNA microsatellite marker analyses of germ-line DNA and tumor DNA were successful for a total of 1132 markers. LOH was found in 181 (16%), retention of the heterozygous status in 637 (56%), homozygous pattern in 299 (26%) and microsatellite instability in 15 (1%) markers.

Precursor T-cell lymphoblastic leukemia

For the 125 T-ALL patients, LOH analyses showed a total of 1364 successfully analyzed markers. LOH was detected in 222 (16%), retention of heterozygous status in 846 (62%), homozygous pattern in 293 (21%) and microsatellite instability in 3 (<1%) markers, respectively.

Frequency of DNA microsatellite alterations according to targeted loci

The most frequent alteration in both T-LBL and T-ALL was LOH at chromosome 9p. The rate of LOH 9p positive patients was 47% and 51%, respectively. For T-LBL and T-ALL, LOH rates were 5% and 2% at chromosome 11q, and 4% and 1% at chromosome 17p (Table 1). The comparison of the LOH rates between T-LBL and T-ALL revealed no significant differences. The complete data of all marker results analyzed separately are shown in detail in supplemental Table IV.

In the 113 T-LBL patients, three patients showed LOH 9p and LOH 11q. One patient had LOH 9p and LOH 17p. In 48 T-LBL patients LOH 9p was found, two patients had LOH 11q and another two patients LOH 17p, while 57 patients were LOH negative for all three loci.

For the 125 T-ALL patients, LOH 9p combined with

<table>
<thead>
<tr>
<th>Table 1. Results of the loss of heterozygosity (LOH) analyses per locus in patients with lymphoblastic T-cell lymphoma (T-LBL) and patients with acute T-cell leukemia (T-ALL).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T-LBL patients (n=113)</strong></td>
</tr>
<tr>
<td>--------------------------</td>
</tr>
<tr>
<td><strong>No. of informative pts</strong></td>
</tr>
<tr>
<td>LOH at 9p (CDKN2A/B)</td>
</tr>
<tr>
<td>LOH at 11q (ATM)</td>
</tr>
<tr>
<td>LOH at 17p (TP53)</td>
</tr>
</tbody>
</table>

LOH+: positive findings of loss of heterozygosity; rate of LOH+ pts: rate of patients with loss of heterozygosity. *CI: 95% confidence interval.
LOH 11q was detected in two patients. One T-ALL patient showed LOH at 9p and 17p. 61 T-ALL patients had LOH 9p, one patient had LOH 11q and 60 patients showed no findings of LOH.

**Association of patients' characteristics with DNA microsatellite alterations**

To examine the association of DNA alterations with clinical characteristics, the following parameters were considered: gender, age, CNS involvement, mediastinal tumor, prednisone response day 8 (T-ALL), BM response day 15 (T-ALL), initial BM involvement (T-LBL) and stage of disease (T-LBL).

Table 2 displays the analyses of clinical characteristics for T-LBL and T-ALL patients according to their LOH 9p status. In T-LBL the only statistically significant finding was the association of LOH at 9p with male gender (Table 2A). A similar male predominance was found in LOH 9p positive T-ALL patients (Table 2B). Noteworthy was a trend for an increased rate of prednisone good responders
at day 8 and a statistically significant favorable bone marrow response. LOH analyses of the ATM (11q) and p53 gene loci (17p) were hampered by the low rate of LOH in both T-LBL and T-ALL. Therefore, no statistically significant association with clinical characteristics was found for these loci.

**DNA microsatellite alterations and outcome**

5-year pEFS of LOH 9p positive T-LBL was 84±5% compared with 75±6% in LOH 9p negative T-LBL (p value log rank 0.28) (Online Supplementary Figure 1A). In T-ALL pEFS at 5 years was 80±5% in LOH 9p positive T-ALL versus 74±6% in LOH 9p negative T-ALL (p value log rank 0.53) (Supplemental Figure 1B). There was no statistically significant association between microsatellite alteration and outcome of any of the targeted loci (Table 3).

**Microsatellite instability**

Beside LOH, microsatellite instability (MSI) was detected. MSI is a change in the number of microsatellite repeats, due to abnormal DNA repair mechanisms, resulting in altered fragment lengths. MSI was detected only sporadically and did not allow for statistical analysis (data not shown).

**Discussion and Results**

The current report represents to our knowledge by far the largest series of T-cell lymphoblastic lymphoma (T-LBL) and leukemia (T-ALL) patients evaluable for DNA microsatellite analyses of three DNA loci encoding for critical cell cycle regulatory molecules: CDKN2A/B, ATM and p53. T-LBL and T-ALL patients were treated uniformly according to ALL-BFM strategies, allowing the analysis and comparison of the impact of DNA alterations on prognostic parameters and outcome.

Loss of heterozygosity (LOH) analysis of these loci was performed, as an indirect technique for the search of chromosomal deletions. Advantages of this method are the minimal requirements on amount and quality of samples, which is of particular importance for the systematic analyses of diseases with scarcity of tumor material, e.g. pediatric lymphoblastic lymphoma.

In pediatric T-ALL, deletions of CDKN2A/B examined with molecular genetic methods have been reported in about 30-70% of the cases, with higher frequency in T-ALL compared to PB-ALL. Yet, the prognostic significance of CDKN2A deletions remains inconclusive. Depending on the study design and the composition of the analyzed population, CDKN2A/B deletions were either of no prognostic value or associated with poor prognostic parameters and inferior outcome. However, most studies on childhood ALL included precursor B- and T-cell leukemia, with T-ALL representing less than 20% of the entire population. Focusing on T-cell immunophenotype, the current study revealed LOH in 51% of T-ALL patients. Interestingly, LOH at 9p was associated with male gender, which had not been described in earlier reports. Furthermore an association of LOH at 9p with favorable initial treatment response in T-ALL at day 8 and day 15 could be shown. This association with good response to initial treatment did not translate into a statistically significant superior pEFS, but pEFS was slightly favorable for LOH 9p positive T-ALL patients. The current findings of an association with male gender, good initial response and at least equal pEFS in LOH 9p positive T-ALL might be attributed to the projects research focus on a biologically well defined and uniformly treated group of patients. The necessity of differentiation according to immunophenotype had already been reported earlier by Heerema et al., who showed significant inferior pEFS for pediatric ALL with cytogenetic abnormalities at chromosome 9p; however, this difference in pEFS lost its statistical significance when analyzed for T-ALL patients separately.

Until today, data on the frequency of CDKN2A/B deletions in T-LBL are rare, while such of prognostic value are still lacking. The current report shows data on T-LBL, detecting a LOH 9p rate of 47%, which is similar to 51% in T-ALL. Just as in T-ALL, LOH at 9p was associated with male gender in T-LBL. Interestingly, pEFS for LOH 9p positive T-LBL patients was 84±5% versus 75±6% for LOH 9p negative T-LBL patients (p value 0.28). This might be an indicator that LOH 9p could be associated with good response to treatment and favorable outcome in T-LBL. Unfortunately, valid criteria for response evaluation are lacking in T-LBL, so that it was not possible to compare the finding of good initial response in LOH 9p positive T-ALL with similar benchmarks in T-LBL. For the ATM gene locus and p53 the LOH rates were 5% or less in both diseases which hampered further analysis on association with clinical characteristics or prognosis.

**Table 3. Univariate analysis of 5 year event free survival for pediatric precursor T lymphoblastic lymphoma (T-LBL) cases and precursor T lymphoblastic leukemia (T-ALL) cases according to LOH-status. Data refer to patients with successful investigation of the respective criteria.**

<table>
<thead>
<tr>
<th>LOH at 9p (CDKN2A/B)</th>
<th>LOH at 11q (ATM)</th>
<th>LOH at 17p (TP53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOH-</td>
<td>LOH+</td>
<td>LOH-</td>
</tr>
<tr>
<td>58 pts</td>
<td>52 pts</td>
<td>105 pts</td>
</tr>
<tr>
<td>75±6%</td>
<td>84±5%</td>
<td>79±4%</td>
</tr>
<tr>
<td>0.28</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>p value (LR)</td>
<td>p value (LR)</td>
<td>p value (LR)</td>
</tr>
<tr>
<td>0.28</td>
<td>0.30</td>
<td>0.30</td>
</tr>
</tbody>
</table>

LOH: no findings of loss of heterozygosity, LOH+: positive findings of loss of heterozygosity, LR: log rank test, pEFS: probability of event-free survival at 5 years, 1 due to the small number of LOH+ patients the data must be discussed with caution. The numbers are too small to draw any conclusion on the prognostic significance.
Little is known on the comparison of molecular profiles of pediatric T-LBL and T-ALL. Recent reports noted both, similarities and differences.\(^1\) The current study therefore focused on DNA microsatellite alterations in genomic loci encoding for key molecules of cell cycle regulation comparing frequency, association with clinical characteristics and prognostic relevance within uniformly treated populations. Owing to the robust method of LOH analyses, it was possible to investigate such large populations. The current study revealed comparable figures in T-LBL and T-ALL, suggesting the two diseases might share common mechanisms of cell cycle deregulation resulting in uncontrolled proliferation of the precursor T-cell lymphoblasts.

**Authorship and Disclosures**

BB designed research and takes primary responsibility for the paper. DK and BB performed laboratory work. BB, DK and AR coordinated the research. MZ provided statistical analysis. IO performed central histopathological review and provided samples. AR and BB provided samples and clinical data of T-LBL cases. MS and AM provided samples and clinical data of T-ALL cases. All authors contributed to the interpretation of results. BB, DK and AR wrote the paper. The authors reported no potential conflict of interest.

**References**


