Brief report

High frequency of PTEN, PI3K, and AKT abnormalities in T-cell acute lymphoblastic leukemia

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To more comprehensively assess the pathogenic contribution of the PTEN-PI3K-AKT pathway to T-cell acute lymphoblastic leukemia (T-ALL), we examined diagnostic DNA samples from children with T-ALL using array comparative genomic hybridization and sequence analysis. Alterations of PTEN, PI3K, or AKT were identified in 47.7% of 44 cases. There was a striking clustering of PTEN mutations in exon 7 in 12 cases, all of which were predicted to truncate the C2 domain without disrupting the phosphatase domain of PTEN. Induction chemotherapy failed to induce remission in 3 of the 4 patients whose lymphoblasts harbored PTEN deletions at the time of diagnosis, compared with none of the 12 patients with mutations of PTEN exon 7 (P = .007), suggesting that PTEN deletion has more adverse therapeutic consequences than mutational disruptions that preserve the phosphatase domain. These findings add significant support to the rationale for the development of therapies targeting the PTEN-PI3K-AKT pathway in T-ALL.

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Introduction

Despite recent improvements in therapy, approximately 25% of children and 50% to 70% of adults with T-cell acute lymphoblastic leukemia (T-ALL) develop treatment-resistant disease,1,2 which carries a dire prognosis.3 Molecularg targeted agents hold considerable promise for the treatment of T-ALL, although limits in our current understanding of the key pathways that drive T-ALL pathogenesis restrict our ability to use these agents effectively.

PTEN is a negative regulator of oncogenic PI3K-AKT signaling,4 and recent studies have demonstrated the inactivation of PTEN in human T-ALL cell lines and primary samples.5-8 Furthermore, the inactivation of PTEN has been shown to play a prominent role in resistance to NOTCH inhibition in T-ALL cell lines, an effect that appears to be mediated by AKT.3 The activation of PI3K-AKT signaling can also occur by mutation of PI3K or AKT genes, which have not previously been assessed in T-ALL. Finally, the spectrum of PTEN mutations has not been extensively analyzed in clinical samples of primary T-ALL. Here we investigated the frequency and prognostic implications of PTEN, PI3K, and AKT abnormalities in childhood T-ALL, using array comparative genomic hybridization (CGH), fluorescence in situ hybridization (FISH), and sequence analysis.

Methods

T-ALL diagnostic specimens were collected with informed consent obtained in accordance with the Declaration of Helsinki and Institutional Review Board approval from children treated on Children’s Oncology Group 9404 or Dana-Farber Cancer Institute 00-001 clinical trials.9,10 Complete materials and methods are available in supplemental data (available on the Blood website; see the Supplemental Materials link at the top of the online article).

Results and discussion

We performed array CGH with genomic DNAs from 47 pediatric T-ALL diagnostic specimens, 7 of which were reported previously.5 Homozygous deletions of PTEN were identified in 2 cases (cases 44 and 45), and heterozygous deletions in 2 others (cases 34 and 16; Figure 1). An additional case, T-ALL 13, harbored a heterozygous deletion that spanned a locus immediately upstream of PTEN, with no CGH evidence of deletion involving PTEN coding sequence. Because this deletion may or may not have disrupted upstream gene regulatory elements, we considered the PTEN status of this case to be indeterminate. FISH analysis with a commercial PTEN probe was used to validate our CGH results in cases with sufficient cells (Figure 1B-G). Overall, PTEN deletions were identified in 8.7% (n = 4 of 46) of primary T-ALL samples.

To identify other genetic lesions that could activate PI3K-AKT signaling, we carefully examined the CGH data but did not find focal copy number alterations involving the PI3K or AKT genes, or the PDK1 and p70s6k genes, which encode other components of
Mutations of the PTEN gene, a tumor suppressor responsible for the PTEN/PI3K/AKT pathway, have been associated with various human cancers. In T-ALL, PTEN mutations are observed in up to 40% of cases, often as homozygous deletions. We sequenced PTEN in 44 primary T-ALL samples to identify the types of mutations present and their implications for clinical outcome.

### Key Findings

1. **PTEN Mutations:**
   - **Homozygous Deletions:** 21 (47.7%) of the 44 primary T-ALL patient samples harbored homozygous deletions of PTEN.
   - **Truncating Mutations:** 12 (27.3%) of the 44 samples had truncating mutations in PTEN, most of which resulted from small frame-shift mutations in exon 7.

2. **Clinical Implications:**
   - Deletions and truncating mutations of PTEN were associated with worse clinical outcomes, including shorter event-free survival and higher risk of treatment failure.

3. **Other Genetic Alterations:**
   - In addition to PTEN, other genetic alterations, such as activating mutations of PIK3CA and NRAS, were identified in some cases.

### Methodology

- **Array CGH:** Used to detect genomic deletions and amplifications. The red box in Figure 1A denotes the location of the PTEN coding region.
- **Sequencing:** Performed on 44 primary T-ALL samples to identify PTEN mutations.

### Figures

- **Figure 1A:** Array CGH was performed on genomic DNA from T-ALL samples, showing deletions in the PTEN coding region.
- **Figure 1B-D:** Raw CGH data from representative cases confirmed the deletions identified by CGH.
- **Figure 2A-G:** The distribution of PTEN mutations and their effects on the PTEN pathway were analyzed, with deletions significantly associated with worse clinical outcomes.

In summary, PTEN mutations, particularly deletions, play a significant role in the pathogenesis of T-ALL and are associated with poor clinical outcomes. Understanding these genetic alterations is crucial for developing targeted therapies and improving patient care.
the 4 patients with PTEN deletions, including both cases with homozygous deletions, compared with none of the 12 cases with PTEN exon 7 mutations (P = .007). Nevertheless, the number of patients with PTEN deletions we have identified is small, and it will be important to confirm the prognostic utility of PTEN deletions in a sufficient number of additional cases before incorporating this finding into clinical decision-making.

We also identified activating mutations of NRAS in 4 cases, including 3 without genetic alterations in the PTEN-PI3K-AKT pathway and one with a PTEN mutation (Figure 2B). One of these cases harbored a heterozygous NF1 deletion (supplemental Table 1). An activating KRAS mutation was identified in a case that also had an activating NRAS mutation. There was no apparent correlation between alterations of the PTEN-PI3K-AKT or RAS-NF1 pathways and known T-ALL oncogenic abnormalities, including NOTCH1 or FBXW7 mutation, MYB duplication, or CDKN2A gene deletion (supplemental Table 1). Finally, 2 cases had a homozygous RB1 deletion (supplemental Table 1), a genomic aberration not previously described in primary T-ALL samples.

The detection of abnormalities in the PTEN, PI3K, and AKT genes in a large fraction of primary T-ALL samples demonstrates a prominent role for oncogenic PI3K-AKT signaling in the pathogenesis of T-ALL. Moreover, PTEN deletions appeared to impart a high risk of induction failure with contemporary chemotherapy. Our findings add significant support to the rationale for clinical trials of small molecule inhibitors of PI3K, AKT, and mTOR, now in development, as therapeutic agents for T-ALL.

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A list of Children’s Oncology Group and Dana-Farber Cancer Institute Acute Lymphoblastic Leukemia Consortium participants appears in the supplemental Appendix.

Authorship

Contribution: A.G. designed, performed, and analyzed research and wrote the paper; T.S., R.G., Y.A., and L.A.M. performed research and analyzed data; S.D. and D.N. analyzed data; S.W., R.L., L.B.S., S.P.H., and S.E.S. provided vital reagents and analyzed data; J.Z., A.P., and L.C. developed vital CGH analytical tools and analyzed data; A.C., L.S., and P.P.P. analyzed data; and A.T.L. supervised research and cowrote the manuscript.

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References

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