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

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Abstract

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 CGH 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 = 0.007), 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.
Introduction

Despite recent improvements in therapy, about 25% of children and 50-70% of adults with T-cell acute lymphoblastic leukemia (T-ALL) develop treatment-resistant disease,\(^1,2\) which carries a dire prognosis.\(^3\) Molecularly 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 \textit{PTEN} in human T-ALL cell lines and primary samples.\(^5-8\) Furthermore, the inactivation of \textit{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.\(^7\) The activation of PI3K-AKT signaling can also occur by mutation of \textit{PI3K} or \textit{AKT} genes, which have not previously been assessed in T-ALL. Finally, the spectrum of \textit{PTEN} mutations has not been extensively analyzed in clinical samples of primary T-ALL. Here we investigated the frequency and prognostic implications of \textit{PTEN}, \textit{PI3K} and \textit{AKT} abnormalities in childhood T-ALL, using array CGH, 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 on the Blood website; see the Supplementary 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 \textit{PTEN} were identified in 2 cases (44 and 45), and heterozygous deletions in 2 others (34 and 16; Figure 1). An additional case, T-ALL 13, harbored a heterozygous deletion that spanned a locus immediately upstream of \textit{PTEN}, with
no CGH evidence of deletion involving \textit{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 \textit{PTEN} probe was used to validate our CGH results in cases with sufficient cells (Figure 1B-G). Overall, \textit{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 \textit{PI3K} or \textit{AKT} genes, or the \textit{PDK1} and \textit{p70s6k} genes, which encode other components of the pathway known to be amplified in other types of human cancers. We then sequenced the entire \textit{PTEN} coding region in 44 of the 47 samples on which CGH arrays were performed, as well as selected \textit{AKT} and \textit{PI3K} exons known to harbor oncogenic mutations in human cancers. These included exons 9 and 20 of \textit{PIK3CA} (encoding the catalytic subunit of class IA PI3K),\textsuperscript{11,12} exons 12 and 13 of \textit{PIK3RA} (encoding the regulatory subunit of class IA PI3K),\textsuperscript{13} and exon 2 of the \textit{AKT1-3} genes.\textsuperscript{14} We additionally sequenced exons 1 and 2 of \textit{NRAS} and \textit{KRAS} and exons 3 and 13 of \textit{PTPN11}, which act upstream of PI3K-AKT signaling and are each known to harbor mutations in some cases of acute lymphoblastic leukemia,\textsuperscript{15-17} as well as exons 26, 27 and 34 of \textit{NOTCH1}, and exons 9 and 10 of \textit{FBXW7}.\textsuperscript{18,19}

Non-synonymous sequence alterations in \textit{PTEN} were identified in 12 (27.3\%) of the 44 primary T-ALL patient samples that were sequenced. Each mutation consisted of a unique nonsense or frame-shift mutation in exon 7, most of which resulted from small insertions or insertion/deletions that were predicted to cause truncation of the protein due to premature termination of translation. Of the 12 T-ALL cases with \textit{PTEN} sequence alterations, 3 harbored biallelic alterations resulting from compound heterozygous mutations, while the other 9 harbored simple heterozygous mutations. No mutations were identified in cases with heterozygous \textit{PTEN} deletions. Strikingly, all of the mutations identified were predicted to disrupt the PTEN protein within an 18-amino acid region of the C2 domain, leading to a carboxy-terminal truncation (Figure 2A; Table S1 available on the Blood website). Importantly, the PTEN phosphatase core domain, encoded by exon 5 and targeted by half of the \textit{PTEN} mutations described in other types of
primary human tumor samples,\textsuperscript{20} was not disrupted by mutations identified in our T-ALL samples. Although \textit{PTEN} mutations can occur in exon 7 in other cancers, including glioblastoma, endometrial, breast and prostate carcinomas,\textsuperscript{20} detection of mutations exclusively in this region has not been described in other types of human cancer.\textsuperscript{20-22} Interestingly, the clustering of mutations within exon 7 is specific to primary T-ALL samples, as \textit{PTEN} mutations in T-ALL cell lines frequently disrupt the phosphatase domain.\textsuperscript{5,7,23}

Non-synonymous sequence alterations were also identified in the \textit{PI3K} and \textit{AKT} genes (Table S1). An E17K \textit{AKT1} activating mutation was identified in 1 case, while 2 others harbored activating mutations of \textit{PIK3CA}, encoding the catalytic subunit of class IA PI3K. Two additional cases harbored novel in-frame insertions/deletions in the PI3K regulatory subunit \textit{PIK3R1}, in a region of the gene frequently mutated in human cancers.\textsuperscript{13,24} Alterations of \textit{PTEN}, \textit{AKT} and \textit{PI3K} were mutually exclusive and occurred in 21 (47.7 \%) of the 44 primary T-ALL patient samples analyzed by both array CGH and sequencing (Figure 2B). When analyzed together, genetic alterations in the \textit{PTEN-PI3K-AKT} pathway did not predict event-free survival (Figure 2C), in contrast to \textit{PTEN} deletions, which were significantly associated with early treatment failure (Figure 2D). This suggests that deletions and truncating mutations may have different implications for clinical outcome in T-ALL. Indeed, induction chemotherapy failed in 3 of the 4 patients with \textit{PTEN} deletions, including both cases with homozygous deletions, compared with none of the 12 cases with \textit{PTEN} exon 7 mutations (\textit{P} = 0.007). Nevertheless, the number of patients with \textit{PTEN} deletions we have identified is small, and it will be important to confirm the prognostic utility of \textit{PTEN} deletions in a sufficient number of additional cases before incorporating this finding into clinical decision-making.

We also identified activating mutations of \textit{NRAS} in 4 cases, including 3 without genetic alterations in the \textit{PTEN-PI3K-AKT} pathway and 1 with a \textit{PTEN} mutation (Figure 2B). One of these cases harbored a heterozygous \textit{NF1} deletion (Table S1). An activating \textit{KRAS} mutation was identified in a case that also had an activating \textit{NRAS} mutation. There was no apparent correlation between alterations of the \textit{PTEN-PI3K-AKT} or \textit{RAS-NF1} pathways and known T-ALL oncogenic abnormalities, including \textit{NOTCH1} or \textit{FBXW7} mutation, \textit{MYB} duplication, or \textit{CDKN2A}
gene deletion (Table S1). Finally, 2 cases had a homozygous $RB1$ deletion (Table S1), 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,$^{25}$ as therapeutic agents for T-ALL.

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For a list of Children’s Oncology Group and Dana-Farber Cancer Institute Acute Lymphoblastic Leukemia Consortium participants, see the Supplemental Appendix.
Authorship

A. Gutierrez designed, performed and analyzed research and wrote the paper. T. Sanda, R. Grebliunaite, Y. Ahn and L.A. Moreau performed research and analyzed data. S. Dahlberg and D. Neuberg analyzed data. S.S. Winter, R. Larson, L.B. Silverman, S.P. Hunger and S.E. Sallan provided vital reagents and analyzed data. J. Zhang, A. Protopopov and L. Chin developed vital CGH analytical tools and analyzed data. A. Carracedo, L. Salmena, and P.P. Pandolfi analyzed data. A.T. Look supervised research and co-wrote the manuscript. The authors have no relevant conflicts of interest to disclose.

References


Figure legends:

Figure 1. **PTEN** deletions in T-ALL. (A) Array CGH was performed with genomic DNA from diagnostic specimens collected from 47 children with T-ALL. The data are shown as a dChip plot of CGH segmented log2 copy number ratios at the **PTEN** locus. The red box denotes the location of the **PTEN** coding sequence. White arrowheads point to cases with segmented log2 copy number ratios of less than -0.5 involving the **PTEN** coding sequence. Two samples on which CGH was unsuccessful (T-ALL 36 and 37) were excluded from analysis. (B,C,D) Raw CGH data from representative patient samples. Red lines represent the segmented log2 copy number ratio shown in panel (A). (E,F,G) FISH analysis of representative cases confirmed the deletions identified by CGH. Orange, **PTEN** probe; green, centromere 10 probe. Note that the Genus cytogenetic image acquisition software utilized applies an automated “thresholding” algorithm that sets a signal intensity threshold below which any signal is considered background and is excluded from the final image generated. All images shown were carefully compared to the view from the microscope to confirm that they were fully representative of what was seen. (B,E)
Homozygous deletions had Log2 ratios of -1.26 (case 44) and -4.11 (case 45). Cells were available for FISH on case 44 and clearly showed homozygous loss of PTEN. (C,F) The CGH detection of a heterozygous deletion in case 34 (log2 ratio, -0.54) correlated with the detection of PTEN deletion by FISH on one allele in 21% of the cells examined. (D,G) Case 21 retained both PTEN alleles intact by FISH and CGH.

Figure 2. Mutations of PTEN and the PI3K-AKT pathway in T-ALL. (A) Sequencing of PTEN in 44 of the primary samples shown in Fig. 1 identified non-synonymous sequence alterations in 12 of these samples, all of which were predicted to disrupt the PTEN protein within an 18 amino acid region of the C2 domain. Note that the specific mutations in cases 14 and 27 were impossible to determine due to the presence of two simultaneous frameshift sequences. (B) Targeted sequencing of PIK3R1, PIK3CA, and AKT1-3 exons known to be mutated in human cancer identified non-synonymous sequence alterations in PTEN and the PI3K-AKT pathway in 47.7% of primary T-ALL cases. Lesions within the PTEN-PI3K-AKT pathway were mutually exclusive. Abnormalities in the NF1 and RAS genes were also identified, but were not solely associated with PTEN-PI3K-AKT pathway abnormalities. Novel in-frame insertion/deletions. (C,D) Kaplan-Meier event-free survival curves for the 44 cases analyzed by CGH and sequencing demonstrate that, overall, genetic alterations of the PTEN-PI3K-AKT pathway did not predict event-free survival, whereas deletions of PTEN were significantly associated with early treatment failure.
### Table

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#### Nonsense or frameshift mutations
- Leu247fsX12 (T-ALL 02)
- Leu247fsX10 (T-ALL 03)
- Leu247fsX8 (T-ALL 25)
- Leu247fsX11 (T-ALL 29)
- Glu242fsX15 (T-ALL 41)
- Glu235fs + Glu242indel (T-ALL 27)
- Arg234fsX27 (T-ALL 06)
- Arg234fsX24 (T-ALL 11)
- Arg234indel + Arg234indel (T-ALL 14)
- Arg234fsX10 (T-ALL 21)
- Arg233X + Arg234fsX4 (T-ALL 04)
- Gly230fsX12 (T-ALL 15)

### Figure 2

#### A

- ▼ Nonsense or frameshift mutations

#### B

- **Mut** 4.5% (2/44)
- GOF Mut 4.5% (2/44)
- Mut 27.3% (12/44)
- Del 8.7% (4/46)
- GOF Mut 2.3% (1/44)
- PTEN-PI3K-AKT lesion
- No PTEN-PI3K-AKT lesion
- Leukemogenesis
- RAS
- NF1
- Del 2.1% (1/47)
- 47.7% (21 of 44)
- GOF Mut 9.1% (4/44)

#### C

- PTEN-PI3K-AKT lesion
- No PTEN-PI3K-AKT lesion
- Event-free survival (%)
- Years
- P = 0.77

#### D

- PTEN deletion
- No PTEN deletion
- Event-free survival (%)
- Years
- P = 0.028
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