LYMPHOID NEOPLASIA

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. (Blood. 2009;114:647-650)

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] 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 *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.[7] 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.
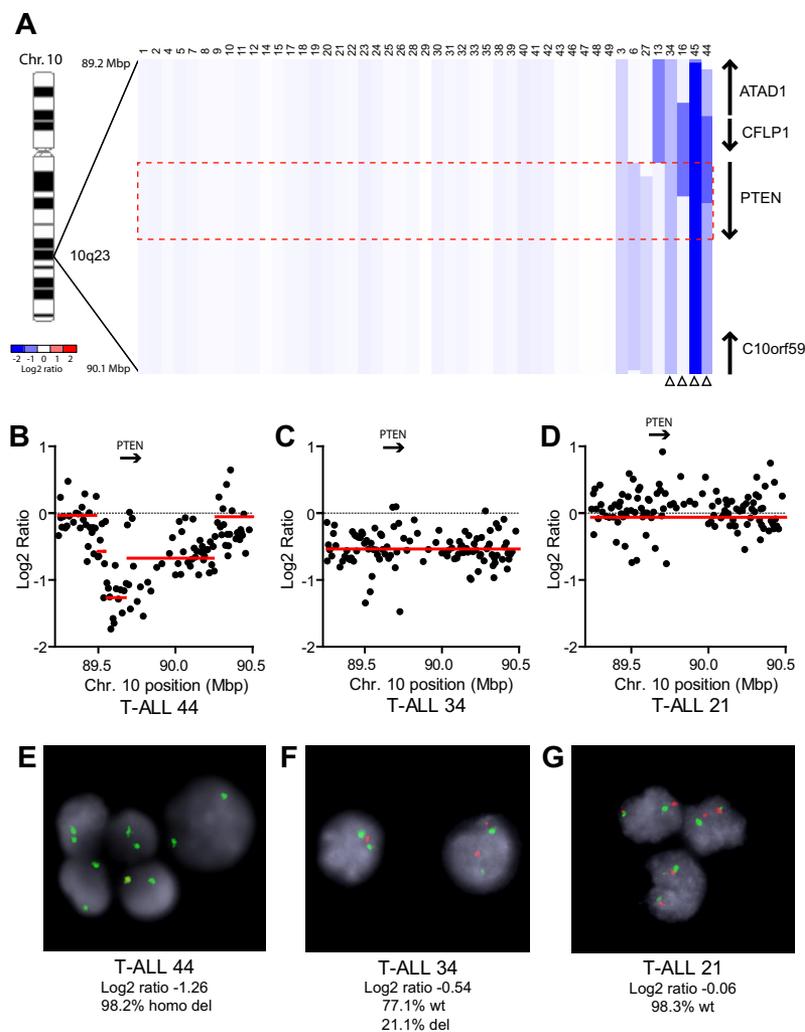
To identify other genetic lesions that could activate PI3K-AKT signaling, we carefully examined the CGH data but did not find focal copy number alterations involving the *PI3K* or *AKT* genes, or the *PDK1* and *p70s6k* genes, which encode other components of

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 $\log_2$ 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 $\log_2$ 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-D) Raw CGH data from representative patient samples. Red lines represent the segmented $\log_2$ copy number ratio shown in panel A. (E-G) FISH analysis of representative cases confirmed the deletions identified by CGH. Orange, *PTEN* probe; green, centromere 10 probe. Images were obtained with an Axio Imager A1 fluorescence microscope (Carl Zeiss) using a 100× Alpha Apochromatic Plan oil-immersion objective (Carl Zeiss), a JAI CV-M4+CL progressive scan camera (JAI Inc), and Genus acquisition software version 3.92 build 7 (Genetix USA). Note that the Genus cytogenetic image acquisition software applies an automated "thresholding" algorithm that sets a signal intensity threshold below which any signal is considered background and thus excluded from the final composite image. During image acquisition, all images generated by the software were compared to the view from the microscope to confirm that they were fully representative. (B,E) Homozygous deletions had $\log_2$ 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 ($\log_2$ ratio, −0.54) correlated with the detection of *PTEN* deletion by FISH on 1 allele in 21% of the cells examined. (D,G) Case 21 retained both PTEN alleles intact by FISH and CGH.

the pathway known to be amplified in other types of human cancers. We then sequenced the entire *PTEN* coding region in 44 of the 47 samples on which CGH arrays were performed, as well as selected *AKT* and *PI3K* exons known to harbor oncogenic mutations in human cancers. These included exons 9 and 20 of *PIK3CA* (encoding the catalytic subunit of class IA PI3K),[11,12] exons 12 and 13 of *PIK3RA* (encoding the regulatory subunit of class IA PI3K),[13] and exon 2 of the *AKT1-3* genes.[14] We additionally sequenced exons 1 and 2 of *NRAS* and *KRAS* and exons 3 and 13 of *PTPN11*, which act upstream of PI3K-AKT signaling and are each known to harbor mutations in some cases of ALL,[15-17] as well as exons 26, 27, and 34 of *NOTCH1*, and exons 9 and 10 of *FBXW7*.[18,19]

Nonsynonymous sequence alterations in *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 resulting from premature termination of translation. Of the 12 T-ALL cases with *PTEN* sequence alterations, 3 harbored biallelic alterations resulting from compound heterozygous mutations, whereas the other 9 harbored simple heterozygous mutations. No mutations were identified in cases with heterozygous *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; supplemental Table 1). Impor-

tantly, the PTEN phosphatase core domain, encoded by exon 5 and targeted by half of the *PTEN* mutations described in other types of primary human tumor samples,[20] was not disrupted by mutations identified in our T-ALL samples. Although *PTEN* mutations can occur in exon 7 in other cancers, including glioblastoma, endometrial, breast, and prostate carcinomas,[20] detection of mutations exclusively in this region has not been described in other types of human cancer.[20-22] Interestingly, the clustering of mutations within exon 7 is specific to primary T-ALL samples, as *PTEN* mutations in T-ALL cell lines frequently disrupt the phosphatase domain.[5,7,23]

Nonsynonymous sequence alterations were also identified in the *PI3K* and *AKT* genes (supplemental Table 1). An E17K *AKT1* activating mutation was identified in one case, whereas 2 others harbored activating mutations of *PIK3CA*, encoding the catalytic subunit of class IA PI3K. Two additional cases harbored novel in-frame insertions/deletions in the PI3K regulatory subunit *PIK3R1*, in a region of the gene frequently mutated in human cancers.[13,24] Alterations of *PTEN*, *AKT*, and *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 PTEN-PI3K-AKT pathway did not predict event-free survival (Figure 2C), in contrast to *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

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 Figure 1 identified nonsynonymous 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 because of the presence of 2 simultaneous frameshift sequences. (B) Targeted sequencing of *PIK3R1, PIK3CA,* and *AKT1-3* exons known to be mutated in human cancer identified nonsynonymous 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.
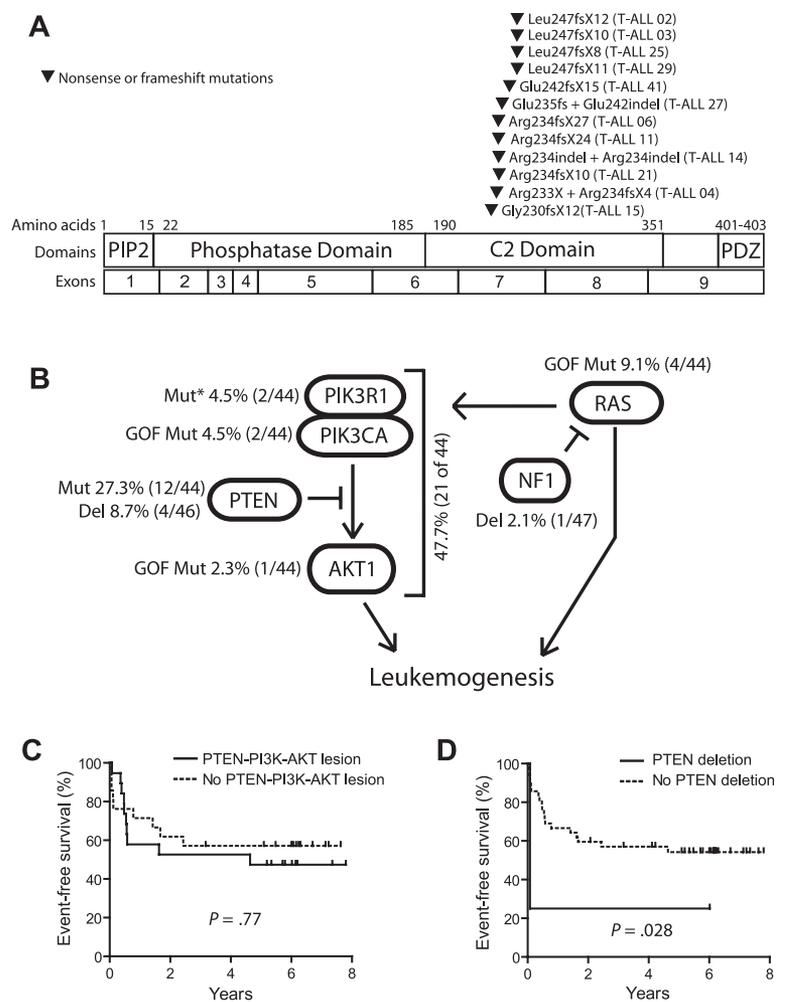
the 4 patients with *PTEN* deletions, including both cases with homozygous deletions, compared with none of the 12 cases with *PTEN* exon 7 mutations ($P = .007$). Nevertheless, the number of patients with *PTEN* deletions we have identified is small, and it will be important to confirm the prognostic 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,[25] as therapeutic agents for T-ALL.

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

Authorship

References

1. Goldberg JM, Silverman LB, Levy DE, et al. Childhood T-cell acute lymphoblastic leukemia: the Dana-Farber Cancer Institute acute lymphoblastic leukemia consortium experience. *J Clin Oncol.* 2003;21:3616-3622.

2. Bassan R, Gatta G, Tondini C, Willemze R. Adult acute lymphoblastic leukaemia. *Crit Rev Oncol Hematol.* 2004;50:223-261.

3. Einsiedel HG, von Stackelberg A, Hartmann R, et al. Long-term outcome in children with relapsed ALL by risk-stratified salvage therapy: results of trial acute lymphoblastic leukemia-relapse study of the Berlin-Frankfurt-Munster Group 87. *J Clin Oncol.* 2005;23:7942-7950.

4. Chow LM, Baker SJ. PTEN function in normal and neoplastic growth. *Cancer Lett.* 2006;241:184-196.

5. Maser RS, Choudhury B, Campbell PJ, et al. Chromosomally unstable mouse tumours have genomic alterations similar to diverse human cancers. *Nature.* 2007;447:966-971.

6. Mullighan CG, Goorha S, Radtke I, et al. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. *Nature.* 2007;446:758-764.

7. Palomero T, Sulis ML, Cortina M, et al. Mutational loss of PTEN induces resistance to NOTCH1 inhibition in T-cell leukemia. *Nat Med.* 2007;13:1203-1210.

8. Silva A, Yunes JA, Cardoso BA, et al. PTEN posttranslational inactivation and hyperactivation of the PI3K/Akt pathway sustain primary T cell leukemia viability. *J Clin Invest.* 2008;118:3762-3764.

9. Winter SS, Jiang Z, Khawaja HM, et al. Identification of genomic classifiers that distinguish induction failure in T-lineage acute lymphoblastic leukemia: a report from the Children's Oncology Group. *Blood.* 2007;110:1429-1438.

10. Clinicaltrials.gov identifier: NCT00165178. http://clinicaltrials.gov/ct2/show/NCT00165178?term=NCT00165178&rank=1.

11. Samuels Y, Wang Z, Bardelli A, et al. High frequency of mutations of the PIK3CA gene in human cancers. *Science.* 2004;304:554.

12. Gymnopoulos M, Elsliger MA, Vogt PK. Rare cancer-specific mutations in PIK3CA show gain of function. *Proc Natl Acad Sci U S A.* 2007;104:5569-5574.

13. Philp AJ, Campbell IG, Leet C, et al. The phosphatidylinositol 3'-kinase p85alpha gene is an oncogene in human ovarian and colon tumors. *Cancer Res.* 2001;61:7426-7429.

14. Carpten JD, Faber AL, Horn C, et al. A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. *Nature.* 2007;448:439-444.

15. Tartaglia M, Martinelli S, Cazzaniga G, et al. Genetic evidence for lineage-related and differentiation stage-related contribution of somatic PTPN11 mutations to leukemogenesis in childhood acute leukemia. *Blood.* 2004;104:307-313.

16. Liang DC, Shih LY, Fu JF, et al. K-Ras mutations and N-Ras mutations in childhood acute leukemias with or without mixed-lineage leukemia gene rearrangements. *Cancer.* 2006;106:950-956.

17. Perentesis JP, Bhatia S, Boyle E, et al. RAS oncogene mutations and outcome of therapy for childhood acute lymphoblastic leukemia. *Leukemia.* 2004;18:685-692.

18. Weng AP, Ferrando AA, Lee W, et al. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science.* 2004;306:269-271.

19. O'Neil J, Grim J, Strack P, et al. FBW7 mutations in leukemic cells mediate NOTCH pathway activation and resistance to gamma-secretase inhibitors. *J Exp Med.* 2007;204:1813-1824.

20. Bonneau D, Longy M. Mutations of the human PTEN gene. *Hum Mutat.* 2000;16:109-122.

21. Kurose K, Gilley K, Matsumoto S, Watson PH, Zhou XP, Eng C. Frequent somatic mutations in PTEN and TP53 are mutually exclusive in the stroma of breast carcinomas. *Nat Genet.* 2002;32:355-357.

22. National Center for Biotechnology Information. OMIM: online Mendelian inheritance in man. http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=601728. Accessed January 22, 2009.

23. Sanger Institute Cancer Cell Line Project. http://www.sanger.ac.uk/genetics/CGP/CellLines. Accessed January 22, 2009.

24. McLendon R, Friedman A, Bigner D, et al. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature.* 2008;455:1061-1068.

25. Garcia-Echeverria C, Sellers WR. Drug discovery approaches targeting the PI3K/Akt pathway in cancer. *Oncogene.* 2008;27:5511-5526.