Screening for leukemia- and clone-specific markers at birth in children with T cell precursor ALL suggests a predominantly postnatal origin

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Short title: Indication for postnatal origin of TCP ALL

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Abstract

Childhood T cell precursor (TCP) ALL is an aggressive disease with a presumably short latency that differs in many biological respects from B cell precursor (BCP) ALL. We therefore addressed the issue of in utero origin of this particular type of leukemia by tracing oncogenic mutations and clone-specific molecular markers back to birth. These markers included various first and second hit genetic alterations (TCRD-LMO2 breakpoint regions, n=2; TAL1 deletions, n=3; Notch1 mutations, n=1), and non-oncogenic T cell receptor rearrangements (n=13) that were derived from leukemias of 16 children, who were 1.5 - 11.2 years old at diagnosis of leukemia. Despite highly sensitive PCR approaches (one cell with a specific marker among 100,000 normal cells), we identified the leukemic clone in the neonatal blood spots in only one young child. These data suggests that in contrast to BCP ALL the vast majority of TCP ALL cases are initiated after birth.

Introduction

TCP-ALL accounts for approximately 15% of childhood ALL and comprises genetically heterogeneous subforms of different maturation stages. Chromosomal translocations leading to the aberrant transcription and expression of proto-oncogenes are important, but rare early events that require additional mutations for progression into clinically overt disease. Moreover, a combination of various other activating and loss of function mutations that concur with the aberrant expression of oncogenic transcription factors are more common steps in the process of thymocyte transformation. Several types of these acquired genomic alterations can serve as specific markers for tracing the respective leukemic clone...
with highly sensitive molecular genetic techniques. In addition, unique T cell receptor (TCR) gene rearrangements that are not causative in the oncogenic process can serve as distinct fingerprint-like markers for individual T cells and their clonal progeny.4

Several studies of children with different genetic BCP ALL subgroups indicated that in a considerable portion of them the initiating event takes place already during fetal life.5-9 An exception to this is the t(1;19) positive ALL.10 Two reports also suggested an in utero origin of at least some cases of TCP-ALL.6,11 To explore the issue of the timing of TCP ALL development more systematically, we used patient specific leukemia- and clone-specific markers to analyze neonatal blood spots as the earliest postnatal sample of children who developed TCP ALL later in life.

Material

Patient samples

Neonatal blood spots from 16 children with a median age of 3.2 years (range 1.5-11.2) at diagnosis of TCP ALL were collected. Diagnosis of TCP ALL was based on standard morphology, immunophenotyping and cytogenetics. Informed consent was obtained from the parents of the cases and controls for inclusion into the study and for obtaining the dried neonatal blood spot (Guthrie card). The study was approved by the ethical committee of the CCRI and St. Anna Kinderspital.

Extraction of DNA from Guthrie cards was performed using the QIAmp Blood Mini Kit (QIAGEN Inc., Valencia, CA), otherwise as reported earlier.6,7 Controls included a peripheral blood (PB) from healthy donors, thymocytes from two young children
undergoing cardiac surgery and Guthrie cards from healthy age-matched anonymous newborns.

DNA was extracted from leukemic cells by QIAamp DNA Mini Kit (QIAGEN) and used for the identification of TCRD-LMO2 breakpoints, TAL1 deletions, Notch1 mutations and TCRD and TCRG rearrangements, as described earlier.\textsuperscript{12,13,14} Detection of the specific TCR rearrangements in DNA from neonatal blood spots was performed by real time quantitative PCR as used for minimal residual disease analysis.\textsuperscript{15} All the other markers were amplified by a two-round nested PCR (supplemental Table 1). Identification of TCRD-LMO2 breakpoint regions was performed by long range ligation-mediated PCR as previously described.\textsuperscript{16} Notch1 mutations were identified by screening the heterodimerization and PEST domains.\textsuperscript{17} For this study only mutations with deletions and insertions were selected based on the likelihood to provide a highly specific molecular target for a sensitive PCR approach.

**Results and Discussion**

In this study leukemia- or clone-specific markers were used for retrospective screening of neonatal blood spots from 16 children with TCP ALL (Table 1, Figure 1). They comprised rare well-defined first hit oncogenic mutations, like the distinct t(11;14)(p13;q11) genomic breakpoints leading to aberrant expression of LMO2, proposed second hit mutations of the TAL1 and Notch1 genes, and TCR rearrangements as “universal” markers for the leukemic clone. In all instances a sensitivity of $10^{-5}$ was achieved, indicating that one cell carrying the specific marker could be detected in a background of 100,000 cells. Further, a third to half of a
Guthrie spot was used from each patient in this study, an amount that would have been sufficient to identify 10 – 100 pre/leukemic cells per Guthrie spot with the applied technique. This number of cells was detected in earlier studies on BCP ALL.5-7,9,10 Despite this optimal sensitivity the molecular target was present at birth just in one of the 16 cases (sample #185, Figure 1). These data indicate that in general, even in young children, the pre/leukemic clone cannot be detected at birth in the majority of cases with TCP ALL implying that it is initiated postnatally. However, we cannot formally exclude the possibility that TCP ALL is initiated in utero but does not reach a critical size detectable in the peripheral blood at birth.

To understand the meaning of the predominant lack of detection of a TCP ALL-associated marker at birth, and thereby also the difference in leukemia development between those of the T and B lineage, several biological factors should be considered: the specific time and type of the initiating event, the kinetic of the preleukemic clone, which includes the timing and sum of secondary hits, the tissue in which the pre/leukemic clone proliferates, and the propensity to spread to other organs and the peripheral blood, the only available source for detection. None of these factors are yet known for humans. Consequently, we evaluated whether the primary site of clonal proliferation could have potentially influenced the low detection rate as opposed to leukemias with a BCP phenotype that expand always in the bone marrow.5,6,8,9 For this purpose we included the two cases reported previously6 and analyzed the results according to the presence or absence of a thymus tumor as well as the extent of bone marrow infiltration (Table 1). There was neither a correlation with any of the above-mentioned parameters nor with the absolute blast cell count in peripheral blood at the time of diagnosis. The latter of which reflected the extent of
the bone marrow infiltration, which was, interestingly enough, independent of a thymus enlargement.

There are several mouse and zebra fish models for T ALL leukemogenesis. They suggest that the initiating event occur in T cells in the thymus or in the bone marrow. The transformed cells then readily spread to other organs and the peripheral blood and a highly malignant T cell leukemia evolves rapidly.

The only hint concerning the duration between initiation and clinical emergence of TCP ALL in humans comes from the emergence of a T ALL-like disease in two children who underwent retroviral gene transfer for SCID-X1.24 Provided this scenario also proves relevant for children without an underlying immunodeficiency, these data suggest that i) the latency for TCP ALL might be as short as 2.5 - 3 years, ii) the leukemia-specific marker in peripheral blood can be detected already 13 months after the initiating event, independent of whether it proliferated in the bone marrow or in the thymus and iii) the TCP ALL-like disease progresses and leads to clinical manifestation within three months. In support of a relatively restricted latency period would be the rather consistent incidence of this particular type of ALL during the entire childhood and adolescence. This incidence, however, only occurs after the age of two, which may point to a peri/postnatal initiation.25

Taken together, the data presented here suggests that TCP ALL develops after birth in the majority of cases. This assumption is consistent with the rapid course of the human disease as well as with various animal models.
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References

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*, I and II, pro- and pre-T (CD7+cyCD3+CD1-); III, cortical T (CD7+cyCD3+CD1+); IV, mature T (CD7+sCD3+CD1-) T cell phenotype; na, not available; §, absolute blast count in peripheral blood x 10⁶/L; #, molecular marker used for tracing the leukemic clone; Pt FU and SC were included in an earlier study† and refer to patients 5 and 4, respectively. Cases with a presumed leukemia initiation before birth and the particular molecular target are marked in bold letters. †, GenBank EF455600; ‡, GenBank EF455601; ††, GenBank EF450768.
**Figure Legend**

**Figure 1. Highly specific and sensitive detection of leukemia- and clone-specific genetic targets.** Numbers and initials refer to the respective patient ID in Table 1. 

A. Typical examples of an albumin real-time quantitative (RQ)-PCR from Guthrie card DNA indicating the range of variability (translating to approximately 100-1,000 cells per sample). External DNA standard at dilutions 100ng, 10ng and 1 ng in grey; Guthrie card DNA in black. 

B. Representative example for quantification of the preleukemic/leukemic clone by allele-specific real-time RQ-PCR of TCR rearrangements. Curves represent of 10-log dilutions of leukemic DNA into PB starting from $10^{-2}$ to $10^{-5}$ in duplicates depicted in different shades of grey. Background amplification in light-grey dotted lines; no specific signal from Guthrie card DNA detectable. 

C-E. Detection of **TAL1** deletions (C), **TCRD-LMO2** breakpoint regions (D) and **Notch1** mutation (E) by a nested PCR approach. 

C. Polyacrylamide gels showing second round PCR products of **TAL1** deletions; S, size marker; lanes 1-6, 10-log dilutions of leukemic DNA in PB from $10^{-1} - 10^{-6}$; lane 7, PB DNA; lane 8, no DNA; A, aliquots of Guthrie card DNA from the particular patient; C, control Guthrie cards; A vertical line has been inserted to indicate where a gel lane was cut. These gels came from different experiments. 

D+E. Polyacrylamide gel electrophoresis of second round PCR products of **TCRD-LMO2** breakpoints (D) and **Notch1** mutation (E); lane 1, $10^{-4}$ dilutions of leukemic DNA in PB; lanes 2-5, $10^{-5}$ dilutions; lane 6, $10^{-6}$ dilution; lane 7, PB DNA; lane 8, no DNA; A and C, as before; T, thymus DNA.
Figure 1.

A

245, 270, 338

B

338

C

115

121

239

S 1 2 3 4 5 6 7 8 S A A A C C C

D

185

121

E

115

1 2 3 4 5 6 7 T 8 S A A A A C S
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