Characterization of a pediatric T-cell acute lymphoblastic leukemia patient with simultaneous *LYL1* and *LMO2* rearrangements

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ABSTRACT

Translocation of the *LYL1* oncogene are rare in T-cell acute lymphoblastic leukemia, whereas the homologous *TAL1* gene is rearranged in approximately 20% of patients. Previous gene-expression studies have identified an immature T-cell acute lymphoblastic leukemia subgroup with high *LYL1* expression in the absence of chromosomal aberrations. Molecular characterization of a t(7;19)(q34;p13) in a pediatric T-cell acute lymphoblastic leukemia patient led to the identification of a translocation between the *TRB@* and *LYL1* loci. Similar to incidental T-cell acute lymphoblastic leukemia cases with synergistic, double translocations affecting *TAL1/2* and *LMO1/2* oncogenes, this *LYL1*-translocated patient also had an *LMO2* rearrangement pointing to oncogenic cooperation between *LYL1* and *LMO2*. In hierarchical cluster analyses based on gene-expression data, this sample consistently clustered along with cases having *TAL1* or *LMO2* rearrangements. Therefore, *LYL1*-rearranged cases are not necessarily associated with immature T-cell development, despite high *LYL1* levels, but elicit a *TALLMO* expression signature.

Key words: T-ALL, pediatric, LYL1, LMO2, rearrangements.

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Introduction

T-cell acute lymphoblastic leukemia (T-ALL) is characterized by chromosomal rearrangements that activate several oncogenes, such as *TAL1*, *LMO2*, *HOXA*, *TLX1* and *TLX3*, which predominantly occur in a mutually exclusive pattern. In our previous study, we used a supervised gene-expression profiling approach to cluster T-ALL patients with these chromosomal aberrations.[1] Patients with *HOXA*, *TLX1* and *TLX3* abnormalities formed 3 separate T-ALL clusters. Patients with *TAL1* and/or *LMO2* rearrangements formed a single, fourth *TALLMO* cluster, explained by the fact that TAL1 and LMO2 participate in the same transcription complex and affect similar downstream pathways. Co-clustering of 45 additional patients who lack *TAL1*, *LMO2*, *HOXA*, *TLX1* or *TLX3* aberrations, led to the identification of 2 additional T-ALL genetic subgroups that are characterized by *NKX2-1/NKX2-2* or *MEF2C*-activating rearrangements.[1] The *MEF2C*-deregulated subgroup overlapped with the early thymic progenitor ALL (ETP-ALL) subgroup, as previously described by Dario Campana and coworkers.[2] Nineteen of these 45 patient samples strongly co-clustered with *TAL1*- or *LMO2*-rearranged patients in supervised and unsupervised cluster analyses, pointing to a common pathogenic mechanism. These 19 cases were denoted as *TALLMO*-likes, and we hypothesized that these patients might harbor rearrangements involving factors homologous to *TAL1* or *LMO2*, or factors that participate in the TAL/LMO transcription complex. This hypothesis was confirmed when we identified translocations that involved *LMO3*,[3] *LMO1* or *TAL2* in 3 of these *TALLMO*-like patients.[4] A fourth patient had double translocations affecting *TAL2* and *LMO1* oncogenes.[4] To identify aberrations in the remaining 15 *TALLMO*-like patients, we screened for T-cell receptor driven translocations for which the translocation partner was unknown.

Design and Methods

Patient material

Viably frozen diagnostic bone marrow or peripheral blood samples from 117 pediatric T-ALL patients was used.[1,4] Clinical and immunophenotypic data were provided by the German Co-operative study group for childhood Acute Lymphoblastic Leukemia (COALL) and the Dutch Childhood Oncology Group (DCOG). The patients' parents or their legal guardians provided informed consent to use leftover material for research purposes in accordance with the declaration of Helsinki, and the study was approved by the ethical committee of the Erasmus Medical Center. Leukemic cells were isolated and enriched from these samples as previously described.[5] All resulting samples contained 90% or more

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leukemic cells, as determined morphologically by May-Grünwald-Giemsa-stained cytospins (Merck, Darmstadt, Germany). Cytospin slide preparation and DNA and RNA extraction were performed as previously described.[5]

Fluorescent in situ *hybridization (FISH)*

FISH analysis was performed on cytospin slides using the TCRalpha/delta and TCRbeta split signal probes according to the manufacturer's protocol (DAKO, Glostrup, Denmark). Split signal FISH on the *LYL1* locus was performed using the following BAC clones as previously described:[6] RP11-352L7, RP11-356L15.

Ligation mediated PCR (LM-PCR) and real-time quantitative PCR (RQ-PCR)

LM-PCR for *TRB@* breakpoint hotspots (*TRB@D1* and *TRB@D2*), and RQ-PCR for *LYL1* were performed as previously described.[5,7,8] For LM-PCR, briefly, genomic DNA was digested with either one of four different restriction enzymes (PvuII, HincII, StuI, DraI) and ligated to adapters. Adaptor primers were then used in combination with *TRB@* loci specific primers to amplify the breakpoint region in two PCR rounds. For the detection of the reciprocal *LYL1-TRB@* breakpoint, the following specific primers located near *LYL1* were used. First: 5'-CGG GCT GGA GGA GAG AAG-3', nested: 5'-GTG GCT GAC GAC GTG TAA TTT-3'.

Results and Discussion

A FISH strategy was performed to identify novel *TRB@*- or *TRAD@*-driven oncogenic rearrangements in 15 *TAL-LMO*-like patients. These 15 cases strongly clustered in

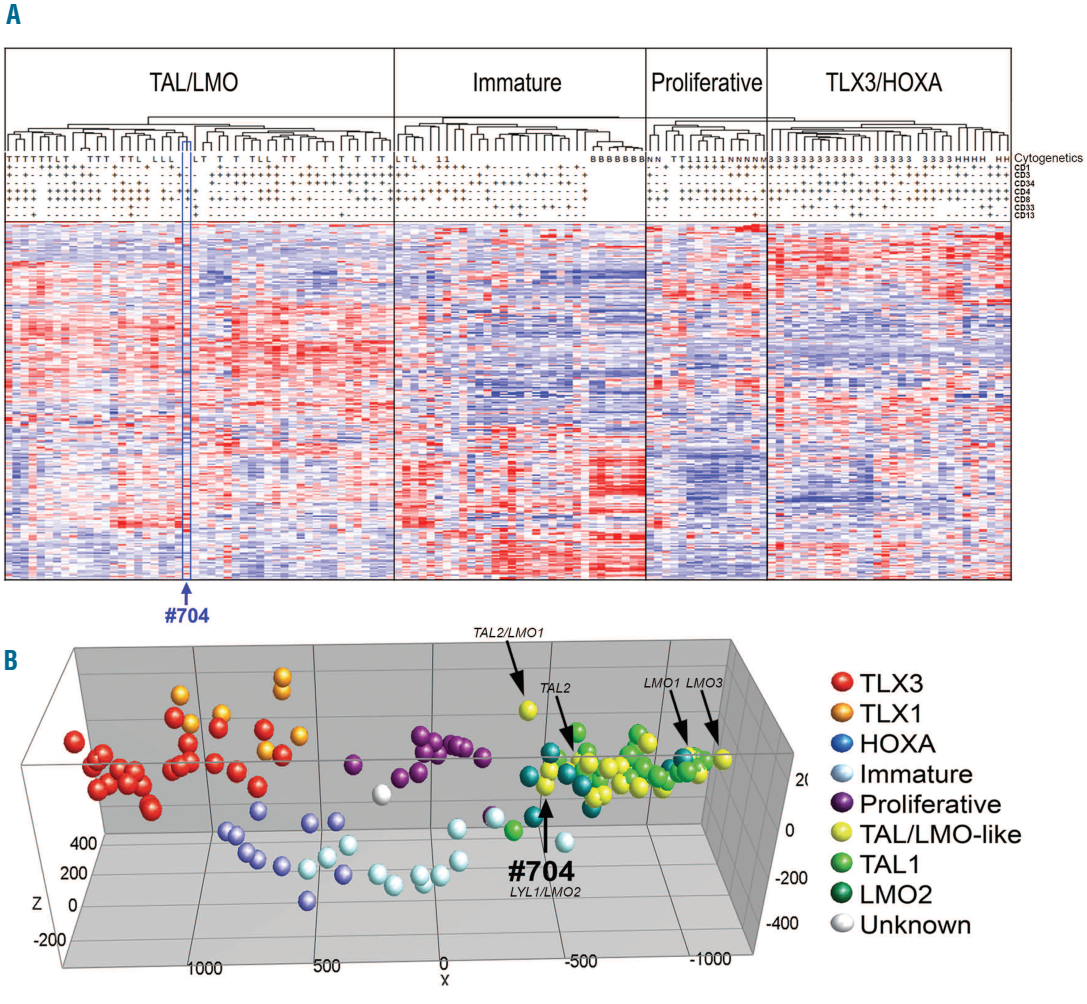

Figure 1. Unsupervised and supervised hierarchical clustering of 117 pediatric T-ALL samples and 7 normal bone marrow samples. (**A**) Unsupervised hierarchical clustering of 117 pediatric T-ALL samples and 7 normal bone marrow samples (horizontal axis), according to microarray gene-expression (genes on vertical axis, gene names not shown).[1] Red corresponds to high expression, blue to low expression. CD surface markers are shown as present (>25%, "+"), absent (<25% "-") or not performed (white). Complete immunophenotype for #704: CD1-, CD2+, CD3-, CD4+, CD5-, CD7+, CD8+, cytoplasmatic CD3+, CD33-, CD14-, CD34-, CD71+, HLA_DR-, TDT+. Cytogentic abnormalities are shown as follows. T: SIL-TAL deletion or TAL1 translocation; L: *LMO2* translocation/deletion; 1: *TLX1* translocation; 3: *TLX3* translocation; B: normal bone marrow; N: *NKX2-1* translocation/inversion/duplication; M: *MYB* translocation; H: HOXA activating abberation (*CALM-AF10*, *SET-NUP*, *HOXA* inversion). Patient #704 is highlighted by a blue box. (**B**) Principal component analysis of supervised analyses of gene-expression data of 117 pediatric T-ALL samples.[1] The position of the yellow dots representing *LMO1*, *TAL2*, *LMO3*, *TAL2/LMO1* rearranged cases and sample #704 (LYL1/LMO2) are indicated by arrows.

hierarchical cluster analyses with T-ALL cases having *TAL1/2* and/or *LMO1/2/3* rearrangements (Figure 1A and B). One sample (#704), from a 7-year old male patient, showed a *TRB@* split signal pointing to a translocation that had not been revealed by karyotypic analysis (47,XY,+8[6]/46,XY[7]).

We then performed ligation-mediated PCR (LM-PCR) from two *TRB@* translocation hotspots (*TRB@D1* and *TRB@D2)* on DNA from this patient and identified a translocation between *TRB@D1* and the last intron of the nuclear factor *I/X* (*NFIX*) gene for the derivative chromosome 7 (der(7)) (Figure 2A and B). The reciprocal breakpoint of the derivative chromosome 19 couples part of the last intron of the *NFIX* gene to an area between *TRB@J1* and *TRB@J2* (der(19); Figure 2A). The *Lymphoid leukemia 1* (*LYL1*) gene is located 240bp centromeric of *NFIX* and is, therefore, placed under the influence of the *TRB@* enhancer as a consequence of this translocation (Figure 2A). *NFIX* was not expressed in any of our patient samples based on microarray data (raw fluorescent intensities<50, 5 probesets; *data not shown*) indicating that changes in *NFIX* are not contributing to leukemogenesis. Positioning of the *LYL1* gene under the influence of the *TRB@* enhancer may explain the relatively high expression level of *LYL1* in this patient (Figure 2C). FISH analysis of the *LYL1* locus on the remaining 14 *TALLMO*-like patients revealed no additional *LYL1* rearrangements.

Various research groups including ours have reported that *LYL1* and *LMO2* are highly expressed in T-ALL patients with an immature immunophenotype,[10-12] despite the fact that *LMO2* rearrangements that are also associated with ectopic *LMO2* expression are exclusively associated with the *TALLMO* subgroup which has a more advanced immunophenotype.[1,13] In another study,[2] immature T-ALL cases were described with an early thymic progenitor expression profile that was associated with poor prognosis, and were denoted as ETP-ALL cases. Based on combined expression profiling and molecular-cytogenetic analyses, we recently identified an immature T-ALL subset that was predominantly characterized by rearrangements that activate the *MEF2C* oncogene.[1] This subset could also be predicted by the ETP-ALL profile. For these immature, ETP-ALL cases, MEF2C has been shown to directly activate expression of *LYL1*, *LMO2* and *HHEX*[1] that may explain the high *LYL1* expression in immature T-ALL cases. So far, we and others have been unable to reveal *LYL1* rearrangements in these immature, ETP-ALL cases.[1,2,10] In line with this, the single reported T-ALL case with an *LYL1* translocation had a mature ($CD3^+$, $CD1^-$, $CD4^+$, $CD8^+$ and $CD34^-$) immunophenotype.[14]

LYL1 is a basic helix-loop-helix (bHLH) transcription factor that shows 82% amino acid homology in the bHLH domain with TAL1.[15] TAL1 and LYL1 also show overlapping expression patterns in hematopoietic development[16] and in some pathways they can exert identical functions.[17] The strong co-clustering of this patient sample (#704) along with *TAL1*-rearranged T-ALL cases indicates that *LYL1* rearrangements elicit a similar expression profile as *TAL1* rearrangements during T-cell oncogenesis.

Using array-CGH, we further identified a small del(11)(p12p13) near the *LMO2* locus in this patient #704[13]

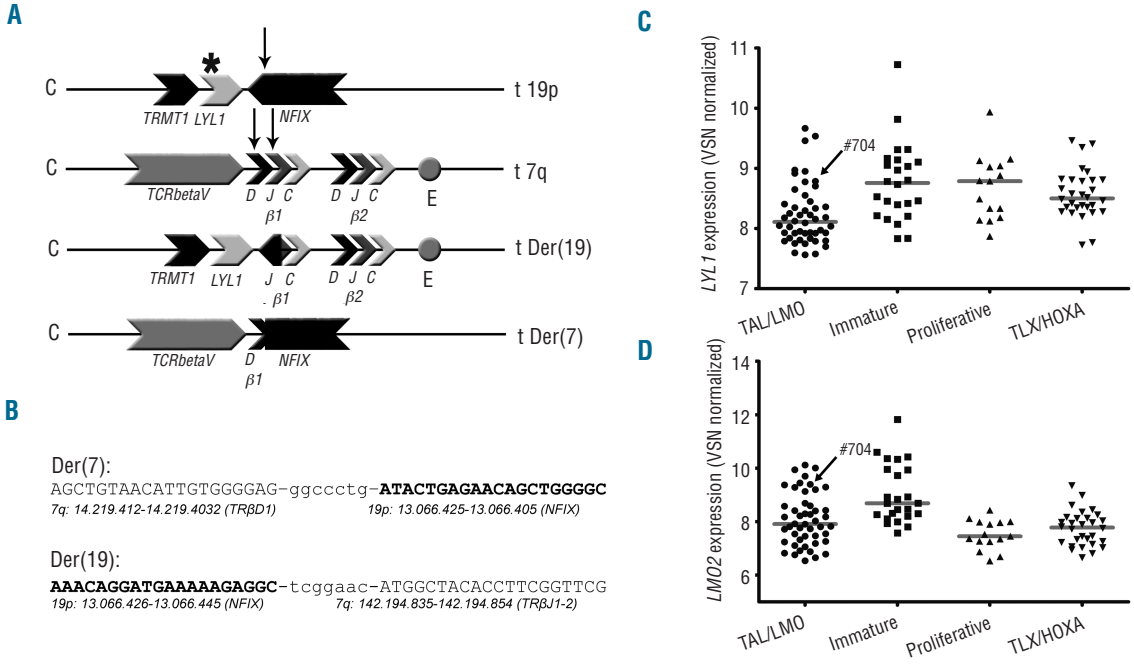

Figure 2. Schematic overview of *LYL1-TRB@* translocation and breakpoint sequences. (A) Schematic overview of breakpoint loci on germline chromosomes 19 and 7, and the der(19) and der(7). Arrows indicate approximate breakpoint locations; *: approximate breakpoint of previously described *LYL1* translocation;[9] c: centromeric side; t: telomeric side of chromosal region. (B) Reciprocal breakpoint sequences of the t(7;19)(q34;p13). In caps sequences corresponding to chromosomal regions as described below, in non-caps; randomly inserted nucleotides. *LYL1* (C) and *LMO2* (D) expression according to VSN normalized array data in the four subgroups as shown in Figure 1A.

(*data not shown*), accompanied by ectopic *LMO2* expression (Figure 2D). No copy number changes were found at the TAL1 locus. *LMO2* rearrangements (translocations or del(11)(p12p13)) occur in approximately 9% of pediatric T-ALL[13] and have been exclusively associated with the *TALLMO* subgroup.[1,13] The identification of an *LYL1* translocation, as well as an *LMO2* rearrangement in this *TALLMO*-like patient implies that LYL1 and LMO2 synergize in T-cell oncogenesis. Other incidental cases harbor *TAL1/2* as well as *LMO1/2* aberrations,[4] and 2 additional cases out of 55 *TALLMO* patients (including the *TALLMO*-like patients) as present in our T-ALL cohort (n=117) had combined rearrangements of *TAL* and *LMO* family members: one case had an *SIL-TAL1* deletion and the *LMO2*-activating del(11)(p12p13), and one had a *TAL2/TRB@* translocation in combination with an *LMO1/TRAD@* translocation.[4] This points to strong synergistic effects between these oncogenic family members in line with their participation in similar transcriptional complexes.[18-20] Lmo1/Lmo2 and Tal1 have also been shown to synergize to T-cell leukemogenesis in mice studies.[18,21-23]

To conclude, we suggest that *LYL1* rearranged cases are not part of the immature, ETP-ALL subgroup, but belong to the *TALLMO* subgroup. *LYL1* translocations fulfill a *TAL1*-like role that can synergize with *LMO2* aberrations in T-cell oncogenesis.

Authorship and Disclosures

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