Brief Report

LYMPHOID NEOPLASIA

Genetic profile of T-cell acute lymphoblastic leukemias with MYC-translocations

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Running title: MYC rearrangements in T-ALL

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Abstract word count: 131; Text word count: 1196
References: 25; Table: 1; Figure: 1

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**Key Points**

MYC-translocations represent a genetic subgroup of NOTCH1-independent T-ALL clustered within the TAL/LMO category. MYC-translocations are secondary abnormalities, which appear to be associated with induction failure and relapse.

**Abstract**

MYC-translocations represent a genetic subtype of T-lineage acute lymphoblastic leukemia (T-ALL), which occurs at an incidence of approximately 6%, assessed within a cohort of 196 T-ALL patients (64 adults and 132 children). The translocations were of two types; those rearranged with the T-cell receptor loci (TCR) and those with other partners. MYC-translocations were significantly associated with the TAL/LMO subtype of T-ALL (p=0.018) and trisomies 6 (p<0.001) and 7 (p<0.001). Within the TAL/LMO subtype, gene expression profiling identified 148 differentially expressed genes between patients with and without MYC-translocations, specifically 77 were up- and 71 down-regulated in those with MYC-translocations. The poor prognostic marker, CD44, was among the up-regulated genes. MYC-translocations occurred as secondary abnormalities, present in sub-clones in half of cases. Longitudinal studies indicated an association with induction failure and relapse.

**Keywords**

T-ALL, MYC-translocations, TAL/LMO
Introduction

MYC is one of the main PI3K/AKT targets, thus rearrangements underlying PI3K/AKT activation result in MYC overexpression. Deregulation of the PI3K/AKT pathway plays a pivotal role in T-ALL, being constitutively activated in cases with NOTCH1/FBXW7 (50-60%) mutations, PTEN (10-30%) inactivation and PTPN2 (6%) deletions. These observations have identified MYC as a key T-ALL oncogene and an effective therapeutic target. The potential role of MYC activation in initiating T-ALL tumorigenesis has been demonstrated in transgenic zebrafish and mouse models, where the induced overexpression of MYC lead to T-ALL development with high penetrance and short latency. Moreover, in T-ALL murine models, MYC appeared to be critical for leukemia initiation, maintenance and self-renewal, as its suppression, prevents leukemia development.

We have characterized an emerging group of T-ALL with MYC-translocations, identified as a specific subgroup of NOTCH1-independent TAL/LMO positive leukemia, occurring in about 6% of adult and childhood T-ALL.

Study design and Methods

To assess the incidence of MYC-translocations in T-ALL, we investigated 64 adults and 132 children (supplemental Data). Combined interphase FISH (CI-FISH) and/or Predictive Analysis of Microarrays classified 80% cases into groups according to distinct genetic features: TAL/LMO (57), HOXA (49), TLX3 (31), TLX1 (16), and NKX2-1 (5), whose distribution into age groups reflected previous studies (supplemental Table 1). Karyotyping, CI-FISH, SNP-array, and mutational analysis investigated concurrent genomic abnormalities (supplemental Data). Microarray data have been deposited in GEO, accession number GSE60733.
Results and discussion

**Incidence and type of MYC-translocations.** MYC-translocations were detected in 12/196 cases of T-ALL (6.1%) and were equally distributed between children and adults (Table 1). They involved TCR loci in 6 cases and new partners in the other 6. The 8q24 breakpoints clustered within the telomeric region of MYC in all TCR translocations, while in the non-TCR translocations the 8q24 breakpoints mapped both telomeric and centromeric to MYC (supplemental Figure 1) mirroring non-IGH MYC translocations in B-cell ALL.\(^{13}\)

Here non-TCR translocation partners were assessed in 4 cases. CDK6/7q21.2, rearranged in T-ALL with t(5;7)(q35;q21) and TLX3 overexpression,\(^{14}\) was involved in cases 3 and 4. Hitherto undescribed breakpoints involved 1q32.1, in case 1, within a long intergenic non-coding RNA, about 300Kb downstream of PTPRC; and Xq25, in case 7, in a no gene region 5Kb of upstream SH2D1 (Figure 1A, supplemental Data). Whatever the partner, MYC-translocations resulted in MYC over-expression (Figure 1B). Remarkably, common to all cases was MYC relocation close to genes which are transcriptionally active in T-lymphocytes (supplemental Figure 2).

In T-ALL, high MYC expression is mainly caused by molecular mechanisms acting at the transcriptional or post-transcriptional level.\(^ {15}\) In this study, we have shown that other genes/regions besides TCR may be involved in MYC-translocations and that the incidence of MYC-translocations in T-ALL is higher than previously reported.

**Genetic profile of T-ALL with MYC-translocations.** Similar to other type B abnormalities, MYC-translocations were not seen as isolated changes. In-depth molecular-cytogenetic characterization revealed from 2 to 9 abnormalities per case (median: 3.7) (Table 1 and supplemental Table 2). T-ALL with MYC-translocations clustered within the TAL/LMO category (Pearson Chi-square, p= 0.017) (Figure 1C). Complete or partial trisomies of chromosomes 6 (3/12, 25%) (Chi-square, P<0.001) and 7 (3/12, 25%) (Chi-square,
P<0.001) were significantly associated with MYC-translocations and occurred together in all cases (2, 7, and 11 of Table 1). Other co-occurring abnormalities were CDKN2A/B deletions (CDKN2ABdel) (75%) and PTEN inactivation, resulting from deletion or mutation (PTENdel/mut) (58%). Similar results were found in the MOLT-16 and SKW-3/KE-37 cell lines with t(8;14)(q24;q11)/TCRAD-MYC. In fact, they both carry SIL-TAL1 and/or LMO2-translocations, as primary abnormalities, and CDKN2ABdel and PTENdel/mut as additional hits (supplemental Table 3). PTEN inactivation in primary samples as well as cell lines reflect results from experimental mouse models, which have shown that Myc rearrangements and Ptendel exert a synergistic effect in the development of T-ALL, and appearing to replace the function of Notch1.8,16 Interestingly, PTENdel/mut and NOTCH1 mutation were mutually exclusive in our cases, confirming that they arise in different T-ALL subgroups.17 In a unique TLX1 positive case (no.12), the MYC-translocation was associated with PTPN2 loss. The two PTEN and PTPN2 negative regulators of PI3K/AKT signalling,18 were inactive in approximately 65% of our cases, suggesting that constitutive PI3K/AKT pathway activation is a critical synergistic hit in this T-ALL subgroup.

**CMYC translocations identify a subgroup within the TAL/LMO category.** Within the set of 51 pediatric patients with TAL/LMO positive T-ALL, the 6 with MYC translocations belonged to the group with the highest MYC expression, defined as the fourth quartile (Q4) based on MYC expression. Supervised Gene Expression Profiling analysis of the Q4 group showed that patients with and those without MYC-translocations clustered separately (Figure 1D). A Shrinkage T-test revealed 148 genes differently expressed between the two groups (supplemental Table 4). Namely, 77 were significantly up-regulated and 71 genes down-regulated (lfdr <0.05) in the group with MYC-translocations compared to the group without. Specifically, a >1.3-fold change in CD44 expression was observed in patients with MYC-translocations, while NOTCH1 and genes associated with NOTCH1 activation (PTCRA, NOTCH3, HES4 and CR2) were significantly down-regulated
(Figure 1E,F). In support of these results, Gene Set Enrichment Analysis (GSEA) confirmed enrichment of genes in the NOTCH1 pathway in the group without MYC-translocations (q-value =0.06; NES 1.71) (supplemental Figures 2 and 3A). GSEA further indicated significant enrichment of cell death and apoptosis pathway genes in patients harboring MYC-translocations (supplemental Figure 4B,C).

**CMYC-positive subclones are associated with relapse/induction failure.** In case 12, paired diagnostic and relapse bone marrow samples showed that the size of the subclone with MYC-translocations increased at relapse, rising from 8% to 100% (Table 1), whereas other abnormalities, which were present either in the main clone, i.e. \(^{ETV6}_{\text{del}}\), or in diverse subclones, such as \(^{WT1}_{\text{del}}\) and \(^{BCL11B}_{\text{del}}\), disappeared at relapse (Figure 1G). These findings are in line with results from xenograft models\(^{19}\) which showed that MYC confers a proliferative advantage and resistance to drug toxicity. It is noteworthy that in mice c-Myc plays a crucial role in maintenance and self-renewal of leukemia-initiating cells, which are thought to be resistant to chemotherapy and mediate relapse.\(^{11}\) In case 11, the MYC-translocation, present at relapse, was not detected at diagnosis, implicating that it was acquired during disease progression (Figure 1G). Taken together, these data suggest that identification and possible eradication of small MYC-positive subclones at diagnosis and/or during the early stages of treatment may assist in prevention of disease progression. Notably, MYC-translocations were found in subclones of variable size (range: 8-62%) in 4 additional cases (Table 1).

**Clinical and hematological characteristic of T-ALL with MYC-translocations.** MYC-translocation positive T-ALL is characterized by leukocytosis and cortical/mature differentiation arrest in the majority of cases. It was not possible to evaluate the prognostic implications of MYC-translocations in this retrospective study including children and adults belonging to different treatment protocols. However, poor prognostic markers, such as high CD44 expression and \(^{PTEN}_{\text{inactivation}}\) appeared to be strongly associated with this
Moreover, although determination of minimal residual disease, the most powerful criteria used for risk stratification of pediatric ALL, classified case 2 into the standard risk group, this patient failed induction therapy and died in disease. Similar to B-lineage ALL and AML, in which disease relapse has been related to minor leukemic subclones rather than to the predominant clone at diagnosis, subclones with \textit{MYC}-translocations in T-ALL may be more resistant to therapy and thus sustain relapse.

\textbf{Acknowledgements}

Authors wish to thank Dr Francesca Grillo and Dr Maddalena Paganin for mutational analysis in selected patients belonging to the AIEOP protocol; Dr Giovanni Roti for providing cell lines; Dr Renato Bassan and Dr Cristina Morerio for providing biological samples. C.M. is supported by Fondo per gli Investimenti della Ricerca di Base (FIRB 2011 RBAP11TF7Z_005), AIRC IG 11512, and Fondazione Cassa di Risparmio di Perugia (Cod. 2012.0108.021 Ricerca scientifica e tecnologica). G.teK. is supported by Fondazione Cariparo progetto d’eccellenza

\textbf{Authorship}

Contribution: Conception and design: Roberta La Starza, Cristina Mecucci; Provision of study materials or patient samples: Claire Schwab, Christine Harrison, Anna Leszl, Gianni Cazzaniga, Sabina Chiaretti, Giuseppe Basso; Data analysis and interpretation: Roberta La Starza, Chiara Borga, Gianluca Barba, Valentina Pierini, Geertruy Te Kronnie, Cristina Mecucci; Manuscript writing: Roberta La Starza, Cristina Mecucci; Final approval of manuscript: All authors
Conflicts of Interest disclosure: The authors declare no competing financial interest

Supplemental Information accompanies the paper on the Blood website (http://www.bloodjournal.org)

References


Table 1. Clinical, hematologic and molecular-cytogenetic features of T-ALL with MYC-translocations

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<thead>
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<th>No</th>
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<th>Phenotype</th>
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<th>status</th>
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<th>CATEGORY§</th>
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<td>wt</td>
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<td>died</td>
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<td>TCRAD-TLX1</td>
<td>MYC-translocation (8%)</td>
<td>del(18)(q11)/PTPN2, del(9)(p21)/CDKN2A/B, del(12)(p13)/SET8, del(14)(q32)/BCL11B, del(11)(p13)/WT1</td>
<td>TLX1</td>
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</table>
No, number; *, cases with subclonal MYC-translocations. Between brackets the percentage of cells with MYC-translocation is indicated; WBC, white blood cell; m, male; mmc, cubic millimeters; f, female; n.a., not available; m, male; f, female; n.a., not available; wt, wild type; mut, mutated; §the genetic category was defined by Combined Interphase-Fluorescence In Situ Hybridization and/or Gene Expression Profile; AIEOP, associazione italiana emato-oncologia pediatrica; IR, intermediate risk; SR, standard risk; HR, high risk; MRC, Medical Research Council protocols; GIMEMA, gruppo italiano malattie ematologiche maligne dell'adulto protocols; NILG, Northern Italy Leukemia Group protocol; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; hyperCVAD, cyclophosphamide, doxorubicin, vincristine, prednisone, methotrexate, cytarabine.
**Figure legend**

**Figure 1.** A) Non-TCR partners of 3 cases of T-ALL (nos. 1, 4, and 7 of Table 1) with MYC-translocations. Mapping of super-enhancers at 1q32, 7q21, and Xq25 were indicated with 3 vertical thin bars; B) MYC expression in 83 cases of pediatric T-ALL and in 8 MYC-translocation positive T-ALL (nos. 1-4, 9-12 of Table 1). Cases with translocations had a significantly higher MYC expression; C) Circos plot shows distribution of MYC-translocations according to genetic categories. MYC-translocation positive T-ALL clustered into the TAL/LMO category; D) Supervised GEP analysis of 13 TAL/LMO positive T-ALL with high MYC expression at diagnosis (fourth quartile, Q4): 6 cases with MYC-translocations (nos. 1-4,9,10; Table 1) clustered together and separated from the 7 cases without; E) Q4 TAL/LMO positive T-ALL: CD44 expression was higher in T-ALL cases with MYC-translocation compared to cases without; F) NOTCH1 expression was significantly lower in cases with MYC-translocations compared to cases without; G) Longitudinal FISH studies in 2 cases: in case no. 11 the clone with MYC-translocation was not detected at diagnosis but only at relapse (left); in case no.12, the small subclone (~8%) with the MYC-translocation present at diagnosis was found in 100% of leukemic blasts at relapse.
Genetic profile of T-cell acute lymphoblastic leukemias with MYC-translocations

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