Available online at www.sciencedirect.com
SCIENCE DIRECT®
Leukemia Research 29 (2005) 633–639
Leukemia Research
www.elsevier.com/locate/leukres

TEL/AML1 and immunoreceptor gene rearrangements—which comes first?

Jan Zuna[a,b,*], Ondrej Krejci[a,b], Jozef Madzo[a,b], Eva Fronkova[a,b], Lucie Sramkova[a,b], Ondrej Hrusak[a,c], Tomas Kalina[a,c], Martina Vaskova[a,c], Jan Stary[b], Jan Trka[a,b]

The Czech Paediatric Haematology Working Group (CPH)

[a] *CLIP - Childhood Leukaemia Investigation Prague, 2nd Medical School, Charles University Prague, V Uvalu 84, Prague, Czech Republic*
[b] *Department of Paediatric Haematology and Oncology, 2nd Medical School, Charles University Prague, V Uvalu 84, 150 06 Prague 5, Czech Republic*
[c] *Department of Immunology, 2nd Medical School, Charles University Prague, V Uvalu 84, Prague, Czech Republic*

Received 10 August 2004; accepted 23 November 2004
Available online 18 January 2005

Abstract

TEL/AML1 fusion gene is present in 20–25% of childhood acute lymphoblastic leukaemias. In order to unravel at which stage of B-cell precursor development the fusion is originated, we analysed frequency and pattern of immunoreceptor (immunoglobulin and T-cell receptor) gene rearrangements in 47 TEL/AML1-positive and 43 TEL/AML1-negative cases of the same CD10+ immunophenotype. Moreover, we compared corresponding immunoreceptor gene rearrangements in 11 cases of TEL/AML1-positive leukaemia at diagnosis and relapse. More mature immunogenotype of TEL/AML1-positive cases and changes in 37% of rearrangements between diagnosis and relapse suggest that in most cases the TEL/AML1 fusion is formed during immunoreceptor gene rearrangement process.

Keywords: Acute lymphoblastic leukaemia; TEL/AML1; Immunoglobulin; T-cell receptor; Rearrangement; Relapse

1. Introduction

Leukaemias with TEL/AML1 (ETV6/RUNX1) fusion gene (resulting from *t*(12; 21) translocation) form the most common genetically defined subgroup of childhood acute lymphoblastic leukaemia (ALL) with 20–25% of all cases [1]. This subgroup is relatively homogeneous and a significant part of its characteristics regarding biological and clinical features, behaviour, prognosis and also aetiology has been revealed. However, a number of questions still remain unanswered. One of these questions concerns the timing of initiating event of the leukaemic process. It is believed that the fusion of TEL and AML1 genes is the first or initiating hit in these leukaemias. It has been shown that this hit occurs already during prenatal development in most cases [2–4] and recent data suggest that it does not necessarily lead to overt leukaemia [5]. However, it is still not clear at which stage of B-cell precursor (BCP) development this fusion takes place.

In our study we compare the number and pattern of immunoglobulin (Ig) and T-cell receptor (TCR) gene rearrangements at initial diagnosis in the groups of TEL/AML1-positive and -negative patients diagnosed with ALL of the same CD10+ immunophenotype and, moreover, we compare Ig/TCR rearrangements at diagnosis and relapse of the TEL/AML1-positive leukaemia. In discussion, we offer scenarios that are compatible with our own data as well as the other data published so far—particularly, we analyse hypotheses concerning the question in the background of Ig/TCR rearrangements and TEL/AML1 fusion relationship: which comes first?

* Corresponding author. Tel.: +420 22443 6580; fax: +420 22443 6521.
E-mail address: jan.zuna@lfmotol.cuni.cz (J. Zuna).

0145-2126/$ – see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.leukres.2004.11.007

2. Materials and methods

2.1. Immunoreceptor gene rearrangements analysis

We examined pattern of Ig/TCR gene rearrangements in bone marrow samples of ALL patients using the set of 18 reactions covering the vast majority (>90%) of Ig-heavy chain (IgH), Ig-kappa (Igκ), TCR-delta (TCRδ) and TCR-gamma (TCRγ) rearrangements in B-cell precursor ALL (IgH: VHl/7–JHcons, VH2–JHcons, VH3–JHcons, VH4–JHcons, VH5–JHcons; Igκ: VκI–Kde, VκII–Kde, VκIII–Kde, VκIV–Kde, intron RSS–Kde; TCRδ: Vδ2–Dδ3, Dδ2–Dδ3; TCRγ: VγI–Jγ1.1/2.1, VγI–Jγ1.3/2.3, VγII–Jγ1.1/2.1, VγII–Jγ1.3/2.3, VγIV–Jγ1.1/2.1, VγIV–Jγ1.3/2.3). Sequences of primers and PCR conditions were specified elsewhere [6,7]. To reliably distinguish clonal PCR products from polyclonal we performed heteroduplex analysis of fragments using polyacrylamide gel. Clonal PCR products were excised and purified using QIAquick gel extraction kits (QIAGEN, Valencia, CA). Purified PCR fragments were sequenced directly on ABI PRISM® 310 capillary sequencer using Big Dye Terminator Chemistry (Applied Biosystems, Foster City, CA). Variable (*V*), diversity (*D*) and joining (*J*) regions of immunoreceptor genes were identified by comparison with sequences in GeneBank using the ImMunoGeneTics (IMGT) Database (http://imgt.cines.fr, IMGT, European Bioinformatics Institute, Montepellier, France) and the IGBlast search (http://www.ncbi.nlm.nih.gov/igblast/, National Center for Biotechnology Information, Bethesda, MD).

2.2. Patients

All patients included in this study were treated in one of the Czech Paediatric Haematology Working Group (CPH) centres according to Berlin–Frankfurt–Munster (BFM) ALL protocols. Informed consent was obtained from patients or their guardians. We compared the number and pattern of Ig/TCR rearrangements at initial diagnosis in the groups of 47 TEL/AML1-positive and 43 TEL/AML1-negative patients diagnosed with ALL of the same CD10+ immunophenotype (TEL/AML1-positive: common ALL $n = 30$, prae-B ALL $n = 17$; TEL/AML1-negative: common ALL $n = 27$, prae-B ALL $n = 16$) and of the same age at presentation. Moreover, we examined Ig/TCR rearrangements in corresponding diagnostic and relapse samples of 11 patients diagnosed with relapsed TEL/AML1-positive ALL. The TEL/AML1 status was determined using reverse transcriptase (RT)-PCR method as described elsewhere [1].

2.3. Cell cycle analysis

CycleTESTt PLUS DNA Reagent Kit (Becton Dickinson Immunocytometry Systems, CA, USA) was used for analysis of nuclear DNA from cell suspension according to the manufacturer's instructions. The distribution of cell cycle phases was analysed with CELLQuest (Becton Dickinson) and ModFit (Verity House, Topsham, ME, USA) software applications.

2.4. Statistical analysis

Fisher's exact test and Mann–Whitney test were used for statistical analysis.

3. Results

3.1. Ig/TCR rearrangements in TEL/AML1+ and TEL/AML1– ALL

The results of this analysis are summarised in Table 1. The number of patients with at least one detectable clonal (i.e. only mono- or bi-clonal/allelic) rearrangement is comparable within both selected cohorts (94% versus 95% for TEL/AML1-positive and TEL/AML1-negative groups, respectively; $p = 1.0$). However, we found a significantly higher total number of clonal rearrangements in the TEL/AML1-positive patients ($p = 0.0006$). The most significant difference between the two groups was identified in Igκ segment (72% versus 26%; $p < 0.0001$) and also the TCRγ rearrangements were significantly more frequent in the TEL/AML1-positive group (81% versus 56%; $p = 0.0128$). The frequency of clonal TCRδ and IgH rearrangements did not differ significantly in the TEL/AML1-positive compared to -negative patients (53% versus 56%; $p = 0.8349$ and 79% versus 65%; $p = 0.1658$, respectively).

3.2. Ig/TCR rearrangements at diagnosis and relapse

Summary of Ig/TCR rearrangements found in diagnostic and corresponding relapse samples of 11 patients with relapsed TEL/AML1-positive ALL is shown in Table 2. In four patients the pattern of rearrangements was the same at diagnosis and relapse (2, 3, 4 and 5 rearrangements, respectively). In five patients we found loss of some markers at relapse,

Table 1
Number of patients with clonal (mono- or bi-clonal/allelic) rearrangements

	IgH[a]	Igκ[a]	TCRδ[a]	TCRγ[a]	≥1[b]	Overall[c]
TEL/AML1+ ($n = 47$)	79% (37/47)	72% (34/47)	53% (25/47)	81% (38/47)	94% (44/47)	186 (4.0/pt.)
TEL/AML1– ($n = 43$)	65% (28/43)	26% (11/43)	56% (24/43)	56% (24/43)	95% (41/43)	116 (2.7/pt.)
p	0.17[d]	<0.0001[d]	0.83[d]	0.0128[d]	1.0[d]	0.0006[e]

[a] Number of patients with at least one clonal (mono- or bi-clonal/allelic) rearrangement of particular gene.
[b] Number of patients with at least one clonal rearrangement.
[c] Total number of clonal rearrangements within the group (number of rearrangements per patient).
[d] Statistical significance (*p*) calculated using Fisher's exact test.
[e] Statistical significance (*p*) calculated using Mann–Whitney test.