Case Report

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Fast Approach for Clarification of Chromosomal Aberrations by Using LM-PCR and FT-CGH in Leukaemic Sample

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Key Words
Chromosomal aberrations · Fine tiling comparative genomic hybridization · Ligation-mediated PCR · Lymphoblastic leukaemia

Abstract
Chromosomal abnormalities, like deletions, amplifications, inversions or translocations, are recurrent features in haematological malignancies. However, the precise molecular breakpoints are frequently not determined. Here we describe a rapid analysis of genetic imbalances combining fine tiling comparative genomic hybridization (FT-CGH) and ligation-mediated PCR (LM-PCR). We clarified an inv(14)(q11q32) in a case of T cell acute lymphoblastic leukaemia with a breakpoint in the *TRA/D* in 68% of cells detected by fluorescence in situ hybridization. FT-CGH showed several mono- and biallelic losses within *TRA/D*. LM-PCR disclosed a *TRA/D* rearrangement on one allele. The other allele revealed an inv(14)(q11q32), joining *TRDD2* at 21,977,000 of 14q11 together with the *IGH* locus at 105,948,000 and 3′-sequence of *TRAC* at 22,092,000 joined together with *IGHV4–61* at 106,166,000. This sensitive approach can unravel complex chromosomal abnormalities in patient samples with a limited amount of aberrant cells and may lead to better diagnostic and therapeutic options.

Introduction

Chromosomal aberrations play a key role in malignant transformation and are frequently found in cancer cells using cytogenetic approaches. These chromosomal changes may lead to alteration of gene function and thereby presumably play a key role in malignant transformation [1]. So far, only few of these aberrations have been characterized at the molecular level, particularly in solid tumours. Many of these genomic alterations like inversions or translocations are accompanied by some loss or gain of genetic material. The combination of fine tiling comparative genomic hybridization (FT-CGH) and ligation-mediated PCR (LM-PCR) allows the molecular characterization of such alterations. FT-CGH detects ge-

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nomic imbalances like deletions or amplifications and LM-PCR enables the precise molecular characterization of the molecular breakpoints. Using these techniques we characterized selected genomic areas, including the loci of the T cell receptor α/δ *(TRA/D)* and immunoglobulin heavy chain *(IGH),* and were able to clarify an inv(14)(q11q32) in a T cell acute lymphoblastic leukaemia (T-ALL) sample. This sensitive approach allows the clarification of even complex chromosomal abnormalities and can unravel involved genes. This knowledge may lead to a better understanding of pathogenetic aspects and may open new diagnostic and therapeutic options.

Material and Methods

Fine Tiling Comparative Genomic Hybridization

Aberrant genomic areas play a more and more important role in the clarification of alteration gene expressions. Since chromosomal translocations involving the *TRA/D* locus are usually accompanied by DNA losses due to V(D)J recombinations [1], we assumed that using FT-CGH and LM-PCR allows the detection of unknown translocation partners. For this analysis we used genomic DNA of a male patient (24 years of age) with a diagnosis of a mature T-ALL. Immunophenotyping of the peripheral blood cells revealed 49% of blast cells, which were positive for CD5, CD7, cyCD3, sCD3, CD8, TdT, CD52, TCRg/d. Chromosome R-banding analysis of the tumor cells at initial diagnosis revealed the karyotype: 46,t(X;5)(q26;q33),Y,del(1)(p3?4p3?5),add(11)(q13), der(12)t(12;18)(p13;q12)add(12)(q21),inv(14)(q11q32), add(17)(p11) [cp18]/46,XY. The patient was treated according to the ALL protocol, but died 8 weeks after diagnosis. In this study, the loci of *TRA/D* and *IGH* were analyzed by a custom-designed high-resolution fine-tiling oligonucleotid array of 385,000 oligonucleotids with a length of 45–60 bp by using maskless array synthesizer technology (NimbleGen Systems, Reykjavik, Iceland). In brief: 3 μg of genomic DNA of T-ALL sample and a human embryonic kidney cell line (HEK-293-T) (as control) were separately labelled with cyanine-3 and cyanine-5 dye. Both DNA samples were hybridized to a custom-designed FT-CGH array. The analysis of *TRA/D* on 14q11.2 (21,130,000–22,130,000; genomic build hg18, March 2006) with 19,500 probes and the *IGH* locus on 14q32 (105,080,586–106,360,585; genomic build hg18, March 2006) with 17,000 probes allowed the detection of genetic imbalances in these areas by comparing the signal intensity of the test and control DNA amount. The high probe density allows the statistic coverage of the genome with a hybridization probe of all 400 nucleotides.

LM-PCR and Sequencing

After detection of gains or losses of genetic material upon FT-CGH, LM-PCR was performed to precisely determine the putative breakpoint region. In brief, 1 μg of high-molecular-weight DNA was digested with blunt end restriction enzymes (*Eco*RV, *Dra*I, *Pvu*II, *Stu*I, *Sma*I and *Hin*dII) and 50 μM of an adaptor was ligated to both ends of the restriction fragments. The adaptor was prepared by hybridization of two oligonucleotides: sense: 5′-GTA ATA CGA CTC ACT ATA GGG CAC GCG TGG TCG ACG GCC CGG GCT GGT and reverse: 5′-ACC AGC CC-NH_2. The amine group blocks the extension of the 3′-end of the reverse oligonucleotide, thereby preventing formation of a primer-binding site on non-specific adaptor-ligated restriction fragments and adaptor duplexes. The ligation products were subjected to two rounds of PCR with nested adaptor-specific primers and two sets of *TRA/D*-specific primers. The specific primers were located at the borders of the deleted regions. After gel electrophoresis PCR bands differing in size from the germline configuration observed in HEK 293-T were excised from the gel, purified and directly sequenced. Sequence analyses of the regions of interest were performed via the University of California Santa Cruz Genome Bioinformatics database using BLAT.

Results

Here, we describe the precise molecular characterization of a T-ALL case with a split of the *TRA/D* found by fluorescence in situ hybridization (FISH*)* suggesting an inv(14)(q11q32). About 68% of cells of this sample showed a breakpoint affecting the *TRA/D* locus detectable by recently described *TRA/D* FISH probes RPCl11-242H9, RPCl11-447G18 and RPCl11-678M7 [2]. FT-CGH of the *TRA/D* locus was performed in order to precisely clarify the chromosomal aberration. Several mono- and biallelic deletions (fig. 1a) were identified between positions 21,635,000, 21,976,000, 21,989,000 and 22,093,000 within the *TRA/D* locus. The SignalMap image revealed a complete loss of genetic material of the T-ALL sample on 14q11.2 between 21,978,000 and 21,989,000. By using two round LM-PCR in the region of 21,635,000 with two sets of nested forward primers Vδ1-for(-275) and Vδ1-for(-69) (5′-ACT CAA GCC CAG TCA TCA GT and 5′-CGT CGC CTT AAC CAT TTC AG, respectively), we clarified a normal rearrangement between *TRDV1* and *TRDJ1* (online suppl. fig. 2A, for all online suppl. materials, see www.karger.com/doi/10.1159/000330519) on one allele. This breakpoint of *TRDJ1* at position 21,989K was also visible in the FT-CGH. This rearrangement was confirmed by PCR with the gene specific primers Vδ1-for(–69) and Jδ1(–189) on undigested genomic DNA (online suppl. fig. 2B). To clarify the remaining breakpoint in the area at 21,976,000 two sets of nested forward gene specific primers Dδ2-for(-73) and Dδ2-for(-41) were used (5′-GGC AGC GGG TGG TGA TGG CAA AGT G and 5′-AGA GGG TTT TTA TAC TGA TGT, respectively). Sequencing of atypical fragments of this LM-PCR revealed the inversion of chromosome 14 with the breakpoints at sequence 21,977,838 of 14q11 together with the sequence of 105,948,661 of 14q32 in the *IGH* locus (online suppl. fig. 3A). The joining region consisted of two fused recom-

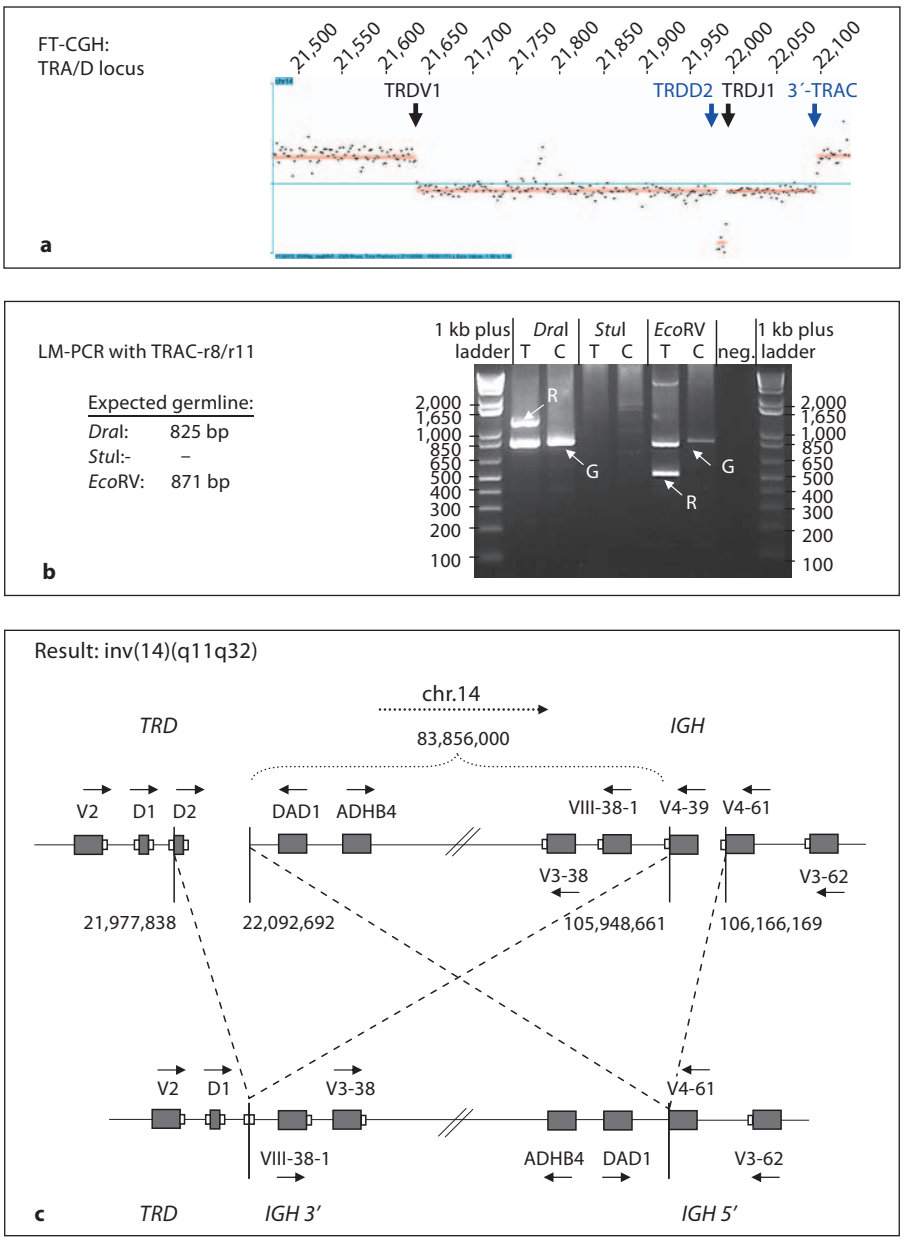

Fig. 1. a FT-CGH: TRA/D locus. FT-CGH analysis of the TRA/D locus on chromosome 14q11 revealed a large monoallelic deletion of 458,000 and located within it a small biallelic deletion of 11,000. The chromosomal localization (in kb) and the involved gene segments are indicated. Breakpoints of TRD rearrangement are indicating by black arrows. TRA/D breakpoints of inv(14)(q11q32) are shown by blue arrows. **b** LM-PCR with TRAC-r8/r11 LM-PCR with *Dra*I, *Eco*RV and *Stu*I restriction enzymes. G = Germline amplification products; R = rearranged fragments; T = T-ALL sample; C = HEK 293-T cell line used as a germline control. **c** Result: inv(14)(q11q32). Sequence analysis of the atypical rearrangement leading to the inv(14)(q11q32). By this inversion *IGHV4–61* is agminate to a sequence locating the 3′-end of the constant region of *TRA/D*.

bination signal sequences, indicating a signal joint typical for a VDJ recombinase-mediated rearrangement. This rearrangement was confirmed again using gene-specific primers IGHV4-39-f1 (5′-AAA TGA GGT ATA ATG GGC TGT GCA GGT AC) and Dδ2(-73) on undigested DNA (online suppl. fig. 3B). Analysis of the breakpoint, located downstream of the *TRA/D* locus at position of 22,093,000 by LM-PCR (fig. 1b) with reverse gene-specific primers TRAC-r8/r11 (5′-CAC AAG GCC GTT CTA ATT CCC TCT GAC ATA and 5′-CTC TGA GGT TCT TGG AGG GGT CTG TCT T, respectively) revealed an inversion of 14q11 at position 22,092,696 joined together with *IGHV4-61* at 106,166,169 of 14q32.33 (online suppl. fig. 4A). Here, the coding segment *IGHV4-61* was fused to an intron sequence without a recombination signal sequence. This rearrangement was confirmed by regular PCR using genomic DNA with primer TRAC-r11 and IGHV4-61-r1 (online suppl. fig. 4B). This inversion inv(14)(q11q32) deleted genomic regions of 114,858 bp within the *TRA/D* locus and 217,508 bp of the *IGH* locus (105,948,661–106,166,169) (fig. 1c). The deletion within the *IGH* locus was visible in the FT-CGH analysis (online suppl. fig. 5).

Discussion

The inv(14)(q11q32) is a known chromosomal event in lymphatic leukaemias such as T cell prolymphocytic leukaemia (T-PLL) [3], adult T cell leukaemia [4], T cell chronic lymphocytic leukaemia and ataxia-telangiectasia [5]. At the molecular level the inv(14)(q11q32) is very heterogeneous. It may involve a large genomic area where different genes located at 14q32: the *TCL1A/ TCL1B (T cell leukaemia/lymphoma)* in T cell prolymphocytic leukaemia [6], the *BCL11B (B cell CLL/lymphoma 11B)* gene in T-ALL [7] and the *IGH* locus in pre-B cell acute lymphoblastic leukaemia and T-ALL [4]. The molecular heterogeneity may reflect different biology and drug sensitivity of the leukaemic cells with the same translocation. Thus, molecular characterization of known chromosomal aberrations, including inv(14)(q11q32) may lead to better treatment of leukaemia patients. The precise molecular characterization may allow identification of sequences and therefore identification of genes involved in chromosomal aberration events and can be used for monitoring of minimal residual disease [8]. The combination of FT-CGH and LM-PCR allows not only the clarification of known breakpoints precharacterized by FISH but also unravels unknown breakpoints. Therefore, FT-CGH analysis reveals genomic imbalances (deletions, amplifications) indicating further chromosomal aberrations. These changes can be further clarified by LM-PCR and unravel so far missed alterations [9]. These combined techniques can be applied to cell lines as well as patient samples with limited percentage of malignant cells and may lead to better diagnostic and therapeutic options.

Acknowledgments

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