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Short communication

Recurrent loss, but lack of mutations, of the *SMARCB1* tumor suppressor gene in T-cell prolymphocytic leukemia with *TCL1A–TCRAD* juxtaposition

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Abstract In T-cell prolymphocytic leukemia (T-PLL), chromosomal imbalances affecting the long arm of chromosome 22 are regarded as typical chromosomal aberrations secondary to a *TCRAD–TCL1A* fusion due to inv(14) or t(14;14). We analyzed recently obtained data from conventional karyotyping, SNP-chip array copy number mapping, genome-wide expression profiling, and interphase fluorescence in situ hybridization (FISH) of inv(14)-positive T-PLL with respect to structural aberrations on chromosome 22. Combined gene chip and interphase FISH analyses revealed interstitial deletions on 22q in 4 of 12 cases, with one case additionally showing a terminal copy number gain. A minimally deleted region of ~9.1 Mb was delineated, from 16.2 Mb (22cen) to 25.3 Mb (22q12.1). The distal borders of copy number alterations spread over a region of ~8.8 Mb, from 25.2 Mb (22q12.1) to 34 Mb (22q12.3). Mutation screening of candidate tumor suppressor genes *SMARCB1* and *CHEK2* mapping to the minimally deleted and the breakpoint regions, respectively, in cases with hemizygous deletion, revealed no inactivating mutations. With gene expression profiling, no significantly downregulated genes were identified in the minimally deleted region. We therefore assume that haploinsufficiency or alternative pathomechanisms underlie chromosome 22 aberrations in T-PLL.

1. Introduction

T-cell prolymphocytic leukemia (T-PLL) is a rare, post-thymic T-cell malignancy with an aggressive clinical course. In the majority of cases, tumor cells are cytogenetically characterized by chromosomal inversion inv(14)(q11q32) or its variant, the translocation t(14;14)(q11;q32). These lesions are regarded as primary events in development of T-PLL [1]. In addition, T-PLL displays a highly characteristic pattern of secondary aberrations. Besides isochromosome 8q, and losses in 11q and 6q, structural aberrations affecting chromosome 22 are the most prominent [2].

Of the 67 cytogenetically characterized T-PLLs with inv(14) or t(14;14) in the Mitelman database, 14 showed a loss of chromosomal material in 22q [3]. Considering published comparative genomic hybridization data of 31 T-PLL cases from the Progenetix database [4,5] gives a similar picture, with proximal deletions on 22q present in 11 cases and adjacent terminal gains in 9 cases. These data suggest the presence of a tumor suppressor gene in the centromeric part of 22q, involved in the pathogenesis of T-PLL.

With the goal of identifying such a tumor suppressor gene, we performed an in-depth analysis of recently obtained gene chip (Affymetrix, Santa Rosa, CA) mapping data for 11 T-PLL cases for chromosome 22 aberrations,

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corroborated by interphase fluorescence in situ hybridization (FISH) analyses and mutation screening of candidate tumor suppressor genes.

2. Patients and methods

Data from recently described cytogenetic analyses, genome-wide gene expression profiling, gene chip mapping, and interphase FISH analysis of 12 cases of T-PLL with FISH proven *TCL1A*-break were mined [3].

All nine exons of the *SMARCB1* gene with their flanking intron regions were screened for mutations by polymerase chain reaction amplification and direct sequencing, as previously described [6]. Screening for mutations in the *CHEK2* gene was done on DNA derived from leukemic blood samples used in the gene chip experiments. Mutation analyses were done on a WAVE 4500 DNA fragment analysis system (Transgenomic, Omaha, NE) according to screening methods developed by Dufault et al. [7] and Sodha et al. [8]. All exons were screened. Sequencing of amplicons displaying aberrant chromatograms was performed on a ABI 310 genetic analyzer according to standard procedures with a BigDye Terminator v1.1 cycle sequencing kit (Applied Biosciences, Foster City, CA).

3. Results and discussion

A total of 12 cases of T-PLL with cytogenetically proven inv(14) or t(14;14) or its molecular cytogenetic correlate, *TCRAD* and *TCL1A* break, were included. Of the 10 T-PLLs with clonally aberrant metaphases by R-banding analysis, 6 displayed aberrations of chromosome 22. The whole chromosome 22 was lost in two cases (cases 10 and 12, numbered according to supplementary Table S1 in Dürig et al. [3]). Additionally, case 8 displayed a dicentric chromosome dic(20;22)(p12;p11), with loss of a normal chromosome 22, and cases 3 and 9 showed translocations in the cytogenetic analysis described as der(21;22)(q10;q10) and der(22)t(?12;22)(q14;q11), respectively. T-PLL case 1 showed an add(22)(p13), but no imbalances in 22q.

FISH with a panel of seven probes distributed over the whole long arm of chromosome 22 in all 12 cases revealed that 4 of the T-PLL cases carried a partial loss in the proximal part of 22q (cases 1, 3, 5, and 12). A gain of the chromosomal region 22q12.1~q13.33 was seen in case 12, in addition to a hemizygous loss spanning 22q11~q12.1.

The 100 k gene chip analysis revealed clear interstitial deletions in 2 of 11 cases (cases 3 and 5), based on the recently determined evaluation criteria, whereas in T-PLL cases 1 and 12 the deletion events were detectable by FISH only. Apart from that, gene chip analyses confirmed the terminal gain of 22q in T-PLL case 12 (Fig. 1). No partial uniparental disomy affecting chromosome 22 could be detected in any of the T-PLL cases analyzed.

Because of poor single-nucleotide polymorphism tag density and variations in single-nucleotide polymorphism quality on chromosome 22 using the 100 k gene chip technology, the accurate determination of breakpoints associated with copy number changes was not possible. Therefore, cases with clear or suggestive imbalances were further analyzed by interphase FISH with 20 additional probes for chromosome 22 (Fig. 1).

The minimally deleted region was determined to extend from the centromere to 22q12.1 (22cen, 25.30 Mb, mapped according to the hg18 assembly of the human genome). It comprises the gene *SMARCB1* (alias *hSNF5*, *INI1*), located in 22.46–22.51 Mb, encoding for the SWI/SNF related protein, which is involved in chromatin remodeling, chromosomal stability, and checkpoint control [9]. The *SMARCB1* gene also acts as a tumor suppressor in rhabdoid tumors [10]. We therefore performed a mutation screening in the four cases in which hemizygous loss of *SMARCB1* was established by the FISH analysis. However, this revealed no inactivating mutations in the screened exons 1–9 on the remaining allele.

The distal borders of the deletion were not consistent in the cases showing imbalances, but were scattered over a breakpoint region of ~8.8 Mb within 22q12.1~22q12.3 (25.13–34.00 Mb). This chromosomal region includes the tumor suppressor gene *CHEK2*, located at 27.41–27.47 Mb, which encodes a protein kinase that is involved in DNA damage repair and cell cycle progression. A mutation screening of the 14 exons of *CHEK2* in the four T-PLL with 22q-deletion by denaturing high performance liquid chromatography and direct sequencing revealed no mutations.

A comparative analysis of gene expression profiles of five T-PLL (cases 1–5) and eight controls ($CD3^+$ sorted T-cells) did not show any significant differentially expressed genes in the minimally deleted region ($P > 0.05$; two-sided *t*-test with Bonferroni correction and false discovery rate correction for multiple testing). Comparison of the T-PLL cases with deletion in 22q (cases 1, 3, and 5) with T-PLL cases with normal chromosomes 22 (cases 2 and 4), as well as of T-PLL cases with deletion in 22q with controls, did not identify genes in the minimally deleted region that were significantly deregulated in their expression levels.

Based on the present results, we conclude that the well-known tumor suppressor genes *SMARCB1* and *CHEK2* in 22q11~q12 are not inactivated by biallelic mutation during T-PLL progression. We therefore postulate alternative pathomechanisms leading to chromosome 22 aberrations, probably caused by haploinsufficiency, or the existence of other tumor suppressor genes in this chromosomal region. Within the minimally deleted region, the antiproliferative factor meningioma 1 gene (*MN1*) is associated with myeloproliferative disorders through an involvement in balanced translocations and the formation of a MN1–Tel fusion protein [11,12]. Further, the seizure related 6 homolog (mouse)-like gene (*SEZ6L*) was demonstrated to be homozygously

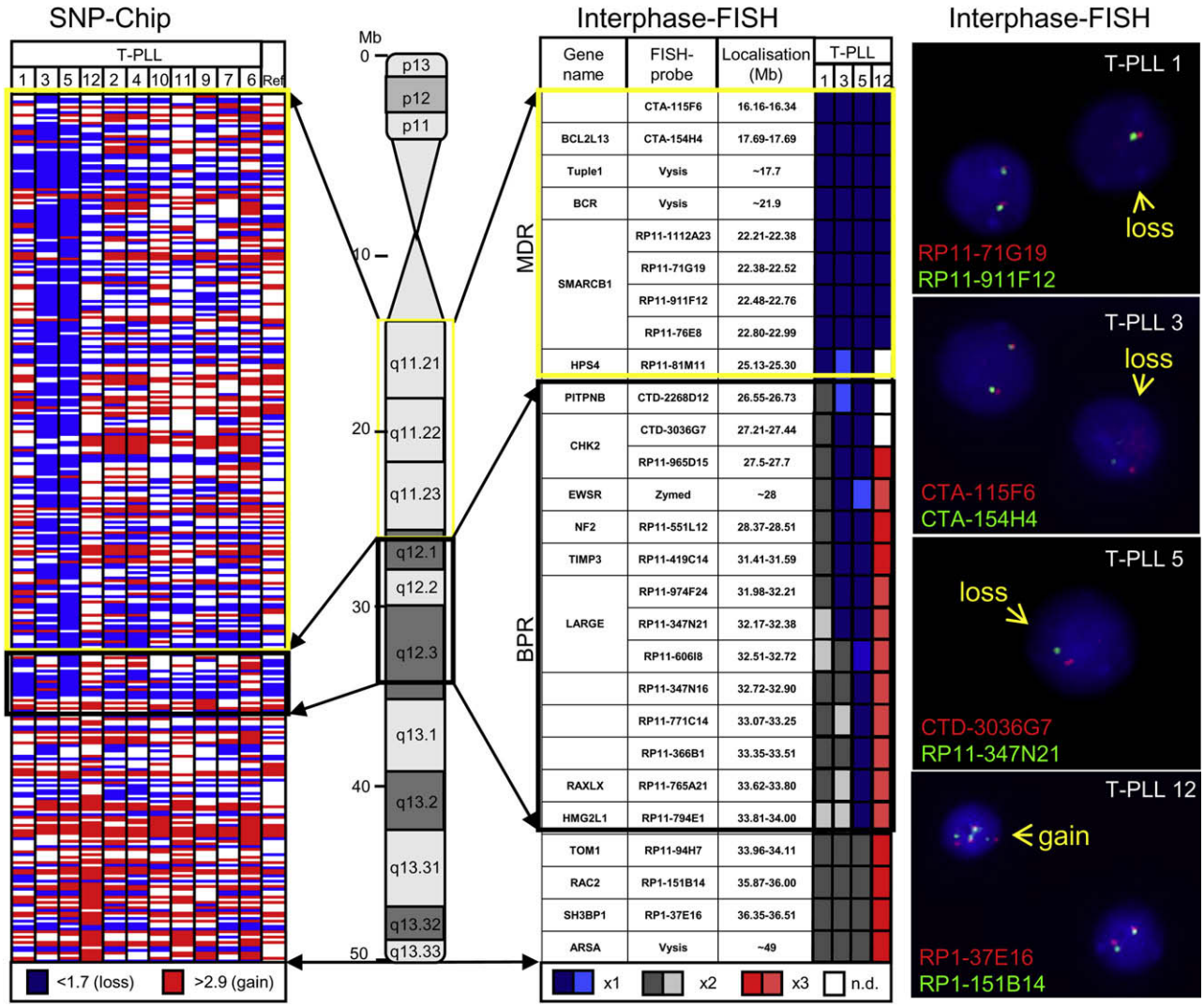

Fig. 1. Genomic characterizations of chromosome 22 in T-cell prolymphocytic leukemia (T—PLL). At the left of the ideogram is a graphical display of single-nucleotide polymorphism (SNP) chip analyses; at the right, interphase FISH results. Four interphase FISH images are at the far right. For details on evaluation, see Dürig et al. [3]. Yellow boxes outline the minimally deleted region (MDR); black boxes outline the breakpoint region (BPR). *SNP graph:* Each column corresponds to a patient sample, each line to a SNP. Data are displayed using a smoothing window of 10 consecutive SNPs. Copy number counts are lined up from the centromere to the long-arm telomere. *Interphase FISH results:* Each column corresponds to a patient sample, each line to a FISH probe. FISH probes are listed in order of their chromosomal location. Clone mapping was done according to the hg18 genome assembly (March 2006, NCBI Build 36.1). The respective copy numbers are marked blue for one copy, gray for two copies, red for three copies, and white for not determined (n.d.) due to loss of material. Lighter shades of respective colors indicate not investigated but putative copy numbers. Arrows indicate approximate chromosomal location of tags. *Interphase FISH images:* The probes named in green type were labeled with SpectrumGreen; the probes named in red type were labeled with SpectrumOrange. Tumor cells showing aberrations are indicated by yellow arrows.

deleted in small cell lung cancer cell lines [13]. The actual involvement of *MN1* or *SEZ6L* in the tumor progression of T-PLL remains to be elucidated.

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