

Brief report

Deregulation of the telomerase reverse transcriptase (*TERT*) gene by chromosomal translocations in B-cell malignancies

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Sequence variants at the *TERT-CLPTM1L* locus in chromosome 5p have been recently associated with disposition for various cancers. Here we show that this locus including the gene encoding the telomerase reverse-transcriptase *TERT* at 5p13.33 is rarely but recurrently targeted by somatic chromosomal translocations

to *IGH* and non-*IG* loci in B-cell neoplasms, including acute lymphoblastic leukemia, chronic lymphocytic leukemia, mantle cell lymphoma and splenic marginal zone lymphoma. In addition, cases with genomic amplification of *TERT* locus were identified. Tumors bearing chromosomal aberrations involving *TERT* showed

higher *TERT* transcriptional expression and increased telomerase activity. These data suggest that deregulation of *TERT* gene by chromosomal abnormalities leading to increased telomerase activity might contribute to B-cell lymphomagenesis. (*Blood*. 2010;116(8):1317-1320)

Introduction

Chromosomal translocations to the immunoglobulin heavy chain (*IGH*) or light chain (*IGL* or *IGK*) loci, and less frequently to non-*IG* loci represent well known mechanisms of oncogene activation in B-cell neoplasms.^{1,2} Many of the genes deregulated by chromosomal translocations in these neoplasms are involved in important cellular processes like cell-cycle control (*CCND1*, *CCND3*, *BCL6*, and *CDK6*), apoptosis (*BCL2*), proliferation (*MYC*), or signal transduction (*BCL3* and *MALT1*).¹

Telomere maintenance across cell divisions is essential for tumor cell growth and immortalization.³ Here, we provide evidence that the telomerase reverse-transcriptase (*TERT*) gene at 5p13.33, which encodes for the rate-limiting catalytic protein subunit of the telomerase,⁴⁻⁶ is deregulated by chromosomal translocations to *IG* and non-*IG* loci in precursor and mature B-cell malignancies.

described previously.⁷ Generation of FISH probes as well as FISH procedures are described in supplemental Methods. A list of FISH probes used is shown in supplemental Table 2.

Array CGH to custom-designed arrays

The microarrays were designed using the eArray software from Agilent (<https://earray.chem.agilent.com/earray/>) and the 4 × 44k format. The experimental procedures were performed according to the manufacturer's instructions. Arrays were scanned with the GenePix4000B Scanner (Axon Instruments) and log ratios obtained with the comparative genomic hybridization (CGH) Analytics Version 3.5.14 software (Agilent). All microarray data are available to be viewed at ArrayExpress under accession number E-MEXP-2675 (<http://www.ebi.ac.uk/miameexpress/>).

Quantitative reverse transcription PCR

RNA was extracted from tumor cell-containing samples and control tissue, using the RNeasy Mini Kit (QIAGEN) and transcribed into cDNA with the QuantiTect Rev. Transcription Kit (QIAGEN). *TERT* transcripts were amplified and detected as described elsewhere.⁸

TRAP assay

The PCR-based telomeric repeat amplification protocol (TRAP) assay was performed as described previously.^{9,10} Protein from tumor cell containing samples and control tissue was isolated according to Kim et al.¹¹

Additional information about materials and methods is available in supplemental Methods.

Methods

Patient samples

The features of B-cell malignancies showing aberrations in the *TERT* region (5p1) are listed in supplemental Table 1 (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). The study was performed as part of the "Molecular Mechanisms in Malignant Lymphoma" Network Project for which approval from the Institutional Review Board of the Medical Faculty of the Christian-Albrechts-University Kiel has been obtained.

FISH

Fluorescence in situ hybridization (FISH) was performed on fixed cells from bone marrow, peripheral blood, or lymph node cell suspensions as

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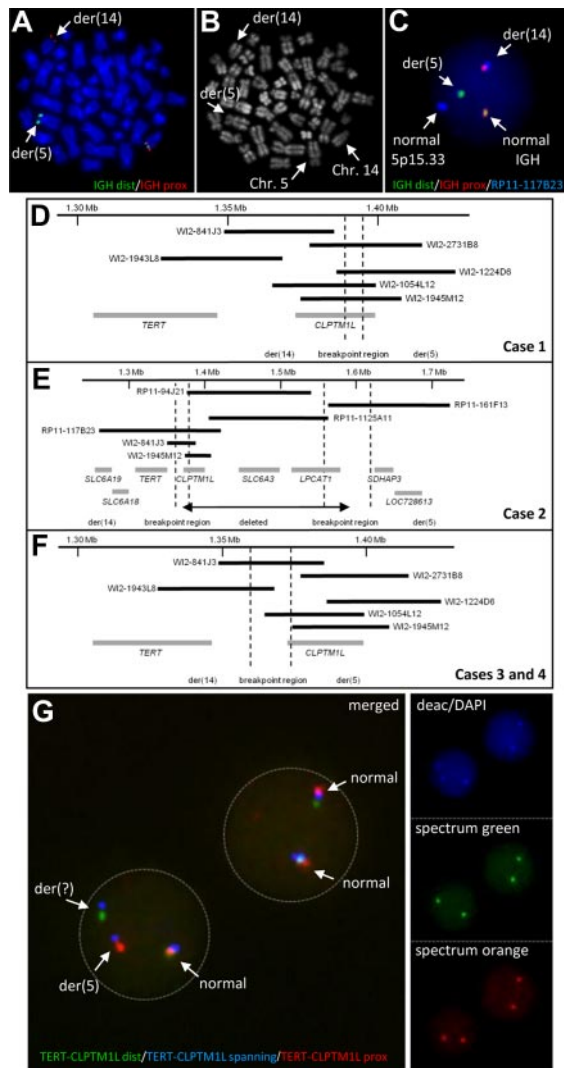


Figure 1. Characterization of chromosomal aberrations affecting the *TERT-CLPTM1L* locus. *IGH* FISH (A) and R-banding analysis (B) of the same metaphase of case 1 showing the cytogenetic cryptic t(5;14). (C) Interphase FISH showing a fusion of *IGH* and the *TERT-CLPTM1L* locus in case 1. (D-F) Schematic maps of the breakpoint regions in cases with t(5;14)(p15;q32) based on FISH mapping. Black bars indicate the hybridized BAC and Fosmid clones; gray bars represent the genes in the region. The breakpoint regions were determined by the respective FISH hybridization patterns. (G) FISH using the *TERT-CLPTM1L* 3-color break-apart assay on MCL case 10 showing break in the clone RP11-117B23 (blue signal). The R-banding image with Chromomycin A3 (C2659, Sigma-Aldrich) and Methyl green (M8884, Sigma-Aldrich) was obtained using a 63×/1.40 numeric aperture oil objective in a Zeiss Axioskop Imager M1 fluorescence microscope (Zeiss) with R-banding filter sets (Zeiss) and documented using the IKAROS imaging system version 5.2.11 (MetaSystems). FISH images were acquired using a 63×/1.40 numeric aperture oil objective in a Zeiss Axioskop 2 fluorescence microscope (Zeiss) equipped with the appropriate filter sets (AHF) and documented using a CV-M300 camera (JAI Corporation) and the ISIS imaging system Version 5.2.11 (MetaSystems). dist indicates distal; and prox, proximal.

Results and discussion

By FISH screening of B-cell neoplasms for translocations affecting the *IGH* locus we observed a cytogenetically cryptic translocation t(5;14)(p15;q32) involving the *IGH* locus in 3 cases of chronic lymphocytic leukemia (CLL) and one case of precursor B-cell acute lymphoblastic leukemia (ALL; Figure 1A-C). The breakpoints in 5p15.33 in all 4 cases mapped to a region containing the *TERT* (telomerase reverse transcriptase; chr5: 1.306 Mb to 1.348

Mb) and *CLPTM1L* (cleft lip and palate transmembrane 1-like; chr5: 1.371 Mb to 1.398 Mb) genes (Figure 1D-F, supplemental Table 1).

The *IGH* locus harbors strong transcriptional enhancers, which can activate oncogenes by translocation on both der(14) and der(5) chromosomes¹² (supplemental Figure 1). FISH mapping showed the *TERT* gene juxtaposed to the *IGH* locus indicating *TERT* translocation to der(14) in all 4 cases. The signal patterns indicated *CLPTM1L* to be disrupted by the translocation in case 1 (Figure 1D). In case 2, the breakpoint was associated with an interstitial deletion resulting in loss of *CLPTM1L* (Figure 1E). This deletion was confirmed by high-resolution array CGH, which also allowed fine-mapping the translocation breakpoint to a repeat-rich region centromeric of *TERT* (1.354 Mb to 1.366 Mb; supplemental Figure 2A). The 5p15.33 breakpoints of cases 3 and 4 were shown to be between *TERT* and *CLPTM1L* (Figure 1F).

To investigate the frequency of rearrangements in the *TERT-CLPTM1L* region we screened additional cases by FISH (supplemental Figure 3, supplemental Table 1). Among 34 lymphoid neoplasms, in which conventional cytogenetics revealed an aberration in 5p1, 5 cases showed breaks and one case showed amplification at the *TERT-CLPTM1L* region. Four of these 6 cases were diagnosed as mantle cell lymphomas (MCLs). Thus, we extended the screen to 123 primary MCLs without known 5p1 abnormality and identified 2 additional cases, one with break (Figure 1G) and one with amplification at the *TERT-CLPTM1L* region (supplemental Table 1).

None of the additional 6 cases with translocations detected by FISH showed juxtaposition to any of the 3 *IG* loci. Cytogenetically, in 4 of the 6 variant translocations (ie, non-*IG*), 7p11, 9q31~33, 10q25 and 19p13 were identified as partners of 5p15. In a splenic marginal zone lymphoma (SMZL, case 5) with t(5;7)(p15.33;p11), FISH and tiling array CGH again confirmed a breakpoint centromeric to *TERT* with loss of *CLPTM1L* similar to that in the t(5;14)-positive CLL mentioned above (Figure 1E, supplemental Figure 2B). Except for the SMZL case, the remaining 5 additional cases showed the same breakpoint in 5p15.33 as in cases 3 and 4 (Figure 1F).

The inactivation of *CLPTM1L* by deletion and disruption in 3/10 cases with break at the *TERT-CLPTM1L* region and also the fact that in precursor B-cell ALL with *IGH* translocation oncogene activation has always been assigned to the der14 chromosome¹³ (supplemental Figure 1) strongly supports *TERT* to be the gene targeted by these aberrations.

To investigate the effect of the identified chromosomal aberrations on *TERT* expression, we performed qRT-PCR in cases with t(5;14)/*TERT-IGH* juxtaposition ($n = 3$; Figure 2A for CLL, supplemental Figure 5 for ALL) and 5p15.33 variant translocations ($n = 5$; Figure 2A, supplemental Figure 4 for MCL) where suitable material was available. Significantly increased *TERT* mRNA expression was observed in all 3 CLLs and the SMZL with a break in the *TERT-CLPTM1L* region compared with eleven CLLs without aberration at the *TERT-CLPTM1L* region as detected by FISH (Figure 2A). Similarly, the 3 MCLs with break in 5p15.33 showed higher *TERT* mRNA expression compared with 5 MCLs without break in the *TERT-CLPTM1L* region and controls (supplemental Figure 4). The ALL with t(5;14) displayed high *TERT* expression but this was also detected in 6 ALL cases without such a change (supplemental Figure 5). These findings show that independent of the presence of centromeric (*CLPTM1L*) deletions or the translocation partner, rearrangements affecting the *TERT-CLPTM1L* region are associated with high *TERT* expression.

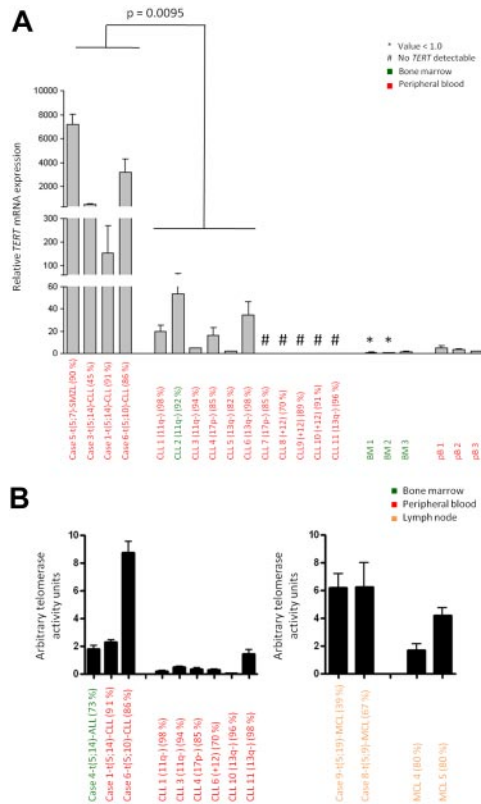


Figure 2. TERT expression and telomerase activity of B-cell malignancies with and without aberration in the TERT-CLPTMIL locus. (A) Relative TERT mRNA expression determined by qRT-PCR. Cases 1, 3, 5, and 6 with 5p15.33 break by FISH were compared with CLL 1-11 showing no 5p15.33 aberration by FISH. The P value was estimated using the Mann-Whitney test. Samples with undetectable TERT mRNA expression were excluded from statistical analysis. (B) Telomerase activity measured by TRAP assay. Cases 1, 4, and 6 and cases 8 and 9 with 5p15.33 aberration by FISH were compared with CLL cases 1, 3, 4, 6, 10 and 11 and MCL cases 4 and 5 without aberration in this region by FISH. The relevant cytogenetic aberrations and the tumor cell contents are given in brackets.

We next assessed whether the up-regulation of TERT mRNA was also associated with increased telomerase activity. Using the TRAP assay, cases of CLL (cases 1 and 6) and MCL (cases 8 and 9) with chromosomal breakpoints at the TERT-CLPTMIL locus showed markedly higher telomerase activity compared with appropriate disease and tissue controls (Figure 2B, supplemental Table 3).

Taken together, we show that the TERT-CLPTMIL region in 5p15 is recurrently targeted by chromosomal translocations in B-cell neoplasms. Based on the finding that CLPTMIL is recurrently lost or disrupted by the mentioned aberrations, it does not seem to contribute to the process of lymphomagenesis. Interestingly, germ line sequence variants in the TERT-CLPTMIL region were recently shown to be associated with predisposition for

several cancer types.¹⁴⁻¹⁷ All 6 additional translocations and both amplifications identified herein were of somatic origin as cells without the aberration were always present. The pattern of IGH translocations as well as the increased TERT expression and telomerase activation in cases with 5p15 translocations strongly suggest TERT to be the candidate gene involved in lymphomagenesis. Telomerase activity, which is clearly detectable in up to 90% of human tumors, including hematologic neoplasias, but not in most normal somatic cells, is in part explained by changes in chromatin structure or amplification of the TERT locus.^{11,18-20} Moreover activation of TERT transcription through viral integration into the TERT promoter has been shown to be associated with B-cell lymphoma and other tumors.^{21,22} Remarkably, on the cytogenetic level the t(5;14)/IGH-TERT was the sole abnormality in one case of CLL, suggesting TERT deregulation to be an early event in lymphomagenesis. This is in accordance with the recent observation that mice with constitutive expression of TERT in thymocytes and peripheral T cells are prone to develop T-cell lymphomas, which are more invasive than those of wild-type mice and may be explained by the recent finding that TERT acts, independent of telomerase activity, as a cofactor in the Wnt pathway.^{23,24} Remarkably, TERT translocations might not be restricted to lymphatic neoplasms as a recent study by Zhao et al also identified a translocation breakpoint within CLPTMIL in the breast cancer cell line HCC1954 similar to one case of CLL (case 1) with translocation t(5;14)(p15;q32) described herein.²⁵

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Authorship

Contribution: I.N. and R.S. designed experiments; L.H., E.C.B., R.D.G., D.H., A.M., J.A.M.C., S.S., and M.J.S.D. provided tumor samples; I.N., M.S., J.I.M.S., L.H., T.A., O.A., S.G., W.K., and H.T. generated and/or analyzed experimental data; and I.N., J.I.M.S., and R.S. wrote the paper.

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