Identification of the gene encoding cyclin E1 (*CCNE1*) as a novel *IGH* translocation partner in t(14;19)(q32;q12) in diffuse large B-cell lymphoma

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ABSTRACT

In a subset of B-cell malignancies, the genes encoding members of the cyclin D familiy are juxtaposed to immunoglobulin loci through recurrent chromosomal translocations. Here, we identified the gene encoding cyclin E1 as novel translocation partner of the immunoglobulin heavy chain (*IGH*) locus involved in a t(14;19)(q32;q12) in a case of t(8;14)(q24;q32) *IGH-MYC*-positive leukemic diffuse large B-cell lymphoma. The translocation breakpoints were cloned and mapped to the switch region Sα1 of *IGH* in 14q32 and approximately 60kb centromeric to *CCNE1* in 19q12. Immunohistochemical analysis revealed overexpression of the cyclin E1 protein in this case, which to a comparable extent was observed in 3/41 independent DLBCL. These data indicate that cyclin E1 may act as a novel oncogene in B-cell lymphomagenesis.

Key words: cyclin E1, diffuse large B-cell lymphoma, *IGH*, *MYC*, translocation.

Citation: Nagel I, Akasaka T, Klapper W, Gesk S, Böttcher S, Ritgen M, Harder L, Kneba M, Dyer MJS, and Siebert R. Identification of the gene encoding cyclin E1 (CCNE1) as a novel IGH translocation partner in t(14;19)(q32;q12) in diffuse large B-cell lymphoma. Haematologica 2009;94:1020-1023. doi: 10.3324/haematol.2008.000968

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Introduction

Translocations juxtaposing oncogenes next to the immunoglobulin heavy chain (*IGH*) locus in chromosomal region 14q32 are the hallmark of various B-cell malignancies. As a result of these translocations, oncogenes are placed under the control of *IGH* regulatory elements leading to their deregulated expression, and subsequent cell proliferation and transformation. Some *IGH* translocations are closely associated with certain lymphoma subtypes, e.g. the t(14;18)(q32;q21) (*IGH/BCL2*) with follicular lymphoma and the t(11;14)(q13;q32) (*CCND1/IGH*) with mantle cell lymphoma (MCL).[1] In contrast, *IGH* translocations in diffuse large B-cell lymphoma (*DLBCL*) affect various known partners, *e.g. BCL6* (3q27), *BCL2* (18q21) or *MYC* (8q24), and other genes that remain to be identified.[2,3]

Several members of the cyclin gene family are involved in *IG* translocations. For example, MCL is characterized by *CCND1* translocations, and we recently described the presence of fusions of *CCND2* to *IGK* in cyclin D1-negative MCL.[4] Also, *CCND1* and *CCND3* are recurrently involved in 15-20% and 5% of multiple myelomas, respectively.[5] Here, we characterise a novel translocation t(14;19)(q32;q12) juxtaposing *CCNE1* with the *IGH* locus in a case of *DLBCL*.

Design and Methods

A 64-year old male presented with B-symptoms, hepatosplenomegaly, enlarged cervical, axillar and inguinal lymph nodes, increased LDH (3010 U/L) and lymphocytosis (165×10^9/L). A bone marrow trephine revealed almost complete infiltration by medium sized CD20-positive and IgM-positive lymphoid cells with basophilic cytoplasm showing a diffuse growth pattern. The proliferation rate determined by immunohistochemistry for Ki-S5 was above 90%. Flow cytometric analysis of peripheral blood revealed a light chain restricted B-cell population comprising 90% of total leukocytes. Those B-cells exhibited strong and homogeneous expression of BCL2, CD19, CD20, CD38, IgM, Igκ, partial positivity for CD5, BCL6 and IRF4, but no CD23, CD43 or CD10 expression. EBER *in situ* hybridization and immunhistochemistry for LMP1 were both negative. PCR-based analyses using BIOMED-primers[6] revealed a clonal *VH4-34DH3-9JH2*

IN and TA contributed equally to this work.

Funding: supported by the Deutsche Krebshilfe Grant 70-3173-Tr3. Acknowledgments: the authors gratefully thank Dorit Schuster, Claudia Becher, Magret Ratjen and Reina Zühlke-Jenisch for their technical assistance. We also thank Dr. rer. nat. Jose Ignacio Martin-Subero for his helpful discussions.
Manuscript received on September 24, 2008. Revised version arrived on February 2, 2009. Manuscript accepted on February 5, 2009.
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rearrangement and an *IGHV* mutation rate of 5%. Additionally we identified a *VH1-3DH3-3JH4* rearrangement with an *IGHV* mutation rate of 7.7% by long-distance inverse PCR (LDI-PCR). According to the clinical and laboratory parameters summarized in Table 1, the diagnosis of leukemic DLBCL was established. Applying the classifier proposed by Hans *et al.*[10] the case has to be classified as germinal center (GC) DLBCL, though the IRF4-immunhistophenotype is in the range of the cut-off (20%). The patient was treated with immunochemotherapy but died four weeks after diagnosis due to progressive disease. The following experimental investigations were performed according to the guidelines of the Network Project of the Deutsche Krebshilfe *Molecular Mechanisms in Malignant Lymphomas*, for which central and local ethics committee approval was obtained (D403/05).

Conventional cytogenetic analysis of the leukemic cells from the bone marrow at diagnosis was performed by R-banding according to routine methods and identified the presence of a complex aberrant clone with the karyotype: 50,XY,dic(1;1)(q32;p11),+3, add(3)(q13)x2, t(8;14)(q24;q32),+12,add(12)(q23),t(14;19)(q32;q12),+18, +mar[20] (Figure 1A).

Table 1. Clinical, histopathological, immunophenotypical, cytogenetic and molecular features of the DLBCL reported here.

Gender/age	M/64
Diagnosis	Diffuse large B-cell lymphoma
Clinical stage	IVB
Histology	Medium sized lymphoid cells with basophil cytoplasm
Immunophenotype (Flow cytometry)	BCL2^{*+}, BCL6$^{+/-}$*, CD5$^{+/-}$, CD10^{-}*, CD19^{+}, CD20^{+}, CD23^{-}, CD38^{+}, CD43^{-}, Igκ^{+}, Igλ^{-}, IgM$^{*+/-}$, IRF4$^{*+/-}$, Ki-S5* (90%) and LMP1^{*-}
Karyotype	50,XY,dic(1;1)(q32;p11),+3,add(3)(q13)x2,t(8;14)(q24;q32),+12, add(12)(q23),t(14;19)(q32;q12),+18,+mar
IGHV mutation status	VH4-34, DH3-9, JH2, 5% mutation/VH1-3, DH3-3, JH4, 7.7% mutation
EBER *in situ* hybridization	Negative

FISH probe (genomic location)	FISH results	Source/Reference
CCND3 bap (6p21)	Normal	(5)
CCNE2 bap (8q22)	Normal	RP11-905H12, RP11-320N21
LSI IGH/MYC (14q32/8q24)	1 Fusion, IGH x 4, MYC x 2	Abbott/Vysis
MYC centromeric (1) (8q24)	Break	(2)
MYC BAP (8q24)	Normal	Abbott/Vysis
MYC far centromeric (2) (8q24)	Normal	(2)
MYC far telomeric (3) (8q24)	Normal	(2)
LSI IGH/CCND1 (14q32/11q13)	Normal	Abbott/Vysis
CCND1 BAP (11q13)	Normal	Abbott/Vysis
ATM/FDX (11q22~23)	Normal	RP11-241D13, RP11-420H22
CCND2 (12p13)	x 3	(4)
CEP12 (12p11-12q11)	x 3	Abbott/Vysis
RB (13q14)	Normal	Abbott/Vysis
LSI IGH (14q32)	Break affecting both alleles	Abbott/Vysis
p53 (17p13)	Normal	(7)
CCNE1 (19q12)	Break within BAC RP11-345J21	RP11-17N20, RP11-345J21
CCNE1 (19q12)	Break	RP11-13D7, RP11-108P14
IGH/CCNE1 (14q32/19q12)	2 Fusions, IGH x 4, CCNE1 x 3	RP11-17N20, RP11-345J21,
		RP11-150I16, RP11-817G24,
		RP11-937M13, RP11-683L4,
		CTD-2011A5, RP11-141I7
IGH/CCNE1 (14q32/19q12)	2 Fusions, IGH x 4, CCNE1 x 3	LSI IGH, RP11-17N20, RP11-345J21
CEBPA (19q13)	Normal	(8)
BCL3 (19q13)	Normal	(9)
SPIB (19q13)	Normal	(3)

**detected by immunohistochemistry; M: male; x n: number of signals (including those in colocalizations).*

Both translocations t(8;14) and t(14;19) were shown to involve the *IGH* locus by FISH using the LSI IGH Break Apart Rearrangement Probe (Abbott/Vysis, Downers Grove, USA). Applying the LSI IGH/MYC Dual Fusion Translocation FISH probe (Abbott/Vysis) for detection of the t(8;14)(q24;q32), only one fusion signal was observed in 99% of the scored interphase nuclei. FISH with the LSI MYC Break Apart Rearrangement Probe (Abbott/Vysis) lacked an aberrant signal pattern. However, use of a FISH break apart assay for the region centromeric to *MYC* (differentially labeled BAC clones RP11-495D4/ RP11-697B24 and RP11-1136L8/CTD-3056O22) indicated a translocation.[2] These findings demonstrated the presence of a colocalization of the *MYC* and *IGH* loci but suggested that the chromosomal breakage occurred centromeric to the breakpoint regions in the *MYC* locus frequently affected in sporadic Burkitt's lymphoma. In line with these FISH findings, cloning of the translocation t(8;14)(q24;q32) by LDI-PCR[12] showed the breakpoint on chromosome 8 to be located approximately 500kb centromeric to *MYC* whereas in the *IGH* locus in 14q32 the switch region Sγ2 was affected (Figure 1E).

Concerning the t(14;19)(q32;q12), the breakpoint in 19q12 was mapped by FISH to a region of approximately 50kb and was shown to be located within BAC RP11-345J21 (Figure 1B). By applying a double-color FISH probe spanning the *IGH* locus and the breakpoint in 19q12, we confirmed the presence of the translocation t(14;19)(q32;q12) (Figure 1C). The breakpoints of the translocation t(14;19)(q32;q12) were also cloned by LDI-PCR and mapped approximately 60kb centromeric to the *CCNE1* locus in 19q12 (Figure 1F) and to the switch region Sα1 of *IGH* in 14q32.

Results and Discussion

The *CCNE1* gene encodes for the cell cycle regulator cyclin E1. In order to study whether the cyclin E1 protein was deregulated as a result of the translocation t(14;19), expression analysis was performed by immunohistochemistry with an antibody against cyclin E1 (Novocastra, Newcastle, UK) (Figure 1D). The majority of DLBCL cells (50-75%) stained strongly positive for cyclin E1 as compared to non-malignant lymphoid tissue, suggesting that cyclin E1 is indeed the target of the t(14;19). Cyclin E1 is the regulatory subunit of the cyclin E1/Cdk2 (cyclin-dependent kinase 2)-complex enhancing transition from G1 to S-phase of the cell cycle. In many human tumors, cyclin E1 is over-expressed and deregulated relative to the cell cycle[13] which leads to high cell proliferation[14] and chromosomal instability.[15] Moreover, high expression of cyclin E1 is related to a poor clinical outcome in DLBCL.[16]

We performed immunohistochemical analysis on a tissue microarray containing 67 DLBCLs with the cyclin E1 antibody described above; 41 of them could be evaluated. No cyclin E1 was expressed in 24 cases (59%), 5 cases (12%) showed expression in 1-25% of the cells and 9 cases (22%) in 26-50% of the cells. Only 3 cases (7%) showed cyclin E1 expression in 51-75% of the cells and, thus, comparably to the index case. To test whether the *CCNE1* locus is recurrently involved in translocations in DLBCL, we performed a FISH-screening with a double color break apart assay containing the differentially labeled BAC-clones RP11-345J21 and RP11-17N20 on the DLBCL evaluated by immunohistochemistry. The FISH analysis was successful in 35 of the 41 cases. We could not detect any translocation affecting the *CCNE1* locus, accordingly it seems to be a rare event in DLBCL. A gain of the *CCNE1* locus with 3-6 copies was detected in 10 cases (29%). Five of those DLBCL showed expression of cyclin E1 by immunohistochemistry, including 2 of the 3

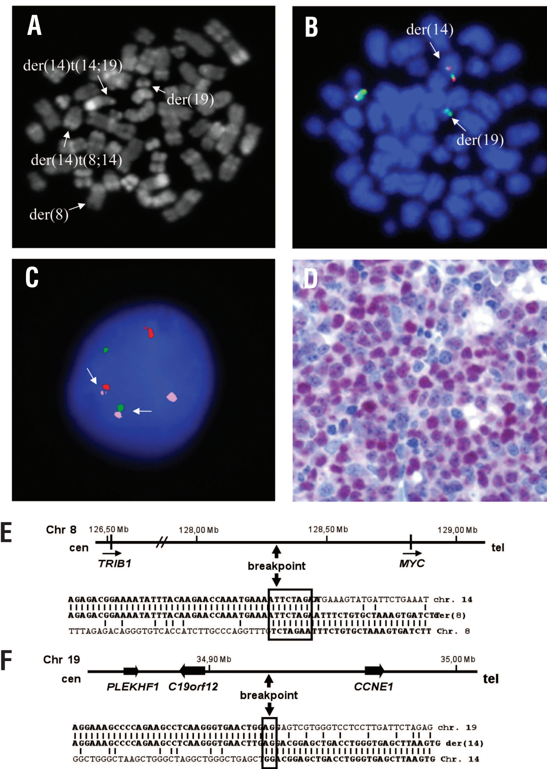

Figure 1. Conventional cytogenetics, FISH, protein expression analyses and IGH breakpoint cloning by LDI-PCR. (A) R-Banding metaphase from a bone marrow sample of the patient. Arrows point to the derivative chromosomes involved in the translocations t(8;14) and t(14;19) (B-C) Results of the double-color FISH assays for the characterization of the translocation t(14;19)(q32;q12). Fluorescence *in situ* hybridization (FISH) was performed as described previously (16). (B) Metaphase hybridized with BAC RP11-345J21 (green, centromeric) and BAC RP11-17N20 (red, telomeric) in 19q12 showing a split within BAC RP11-345J21. Arrows point to the derivative chromosomes involved in the t(14;19). (C) Interphase nucleus hybridized with an *IGH* double color, break apart probe (LSI *IGH* Break Apart Rearrangement Probe, Abbott/Vysis) in red (proximal) and green (distal) together with 4 pooled BAC clones (labeled with DEAC, displayed in pink) for the *CCNE1* locus in 19q12 (RP11-108P14, RP11-345J21, RP11-13D7 and RP11-17N20). The juxtaposition of the *CCNE1* locus to the *IGH* locus is confirmed by the red/pink and the green/pink fusion signals. (D) Immunohistochemical analysis using a mouse monoclonal antibody against human cyclin E1 (Novocastra, Newcastle, UK), high temperature antigen unmasking technique and detection by the APAAP method showing that cyclin E1 was expressed in the majority of tumor nuclei. (E) DNA sequence analysis of the translocation breakpoint from the t(8;14)(q24;q32) revealed the breakpoint to be located 500 kb centromeric to *MYC* in 8q24. (F) Sequence alignment of the translocation breakpoint from the t(14;19)(q32;q12) showing the breakpoint to be located 60 kb centromeric to *CCNE1* in 19q12. Cen: centromeric; chr: chromosome; tel: telomeric.

cases with expression comparable to the index case.

In the t(14;19)(q32;q12)-positive DLBCL, biallelic rearrangement affecting 14q32 was identified, i.e. t(8;14)(q24;q32) and t(14;19)(q32;q12). Both translocations were present in all studied metaphases. Therefore, it is not possible to determine which of the two rearrangements occurred first. Both t(8;14) and t(14;19) translocations involved *IGH* switch regions, which suggests that they were originated by errors in class switch recombination.[3] Although both *IGH* alleles were involved in chromosomal translocations, immunohistochemical analysis revealed expression of IgM heavy chain. This is possible because both breakpoints in *IGH* map centromeric of the *Igµ* constant region. Biallelic translocations involving the *IGH* locus have been previously reported in the literature. *MYC* seems to be one of the translocation partners in the great majority of these cases and often emerges as a secondary event. The primary rearrangement frequently affects *BCL2*, *CCND1* or *BCL6*.[17] Therefore, we speculate that in the case reported here, the *MYC* translocation probably also occured secondary to the *IGH-CCNE1* fusion.

Of diagnostic importance, our findings demonstrate that the use of the LSI MYC break apart probe alone is not sufficient to exclude a *MYC* translocation. Moreover, several other cytogenetically similar t(14;19) translocations have been described in hematolologic malignancies. In the more common t(14;19)(q32;q13), *IGH* is fused to *BCL3* in B-cell chronic lymphocytic leukemia and other B-cell malignancies.[9] In another t(14;19)(q32;q13) the *SPIB* gene is fused to the *IGH* locus in DLBCL.[3] Additionally, a recurrent t(14;19)(q32;q13) involves the *CEBPA* gene in acute lymphoblastic leukemia.[8] Thus, it is possible that some translocations t(14;19) involving *CCNE1* have hitherto remained undetected, because they have been cytogenetically interpreted as one of the other translocations. Therefore, screening by FISH is required to discern the different kinds of t(14;19), which seem to be associated with different subtypes of lymphatic neoplasms.

Authorship and Disclosures

IN: FISH analysis, collected and analyzed data, wrote the manuscript; TA: Breakpoint cloning, detection of clonal IGH rearrangement, mutation analysis, analyzed data, critical revision of the article; SG: Collected and analyzed clinical data, wrote the manuscript; WK: Immunhistochemistry, pathological evaluations; SB, MR, MK: Flow cytometry, detection of clonal IGH gene rearrangement, mutation analysis, analyzed data; LH: Cytogenetics; MJSD, RS: Conception of the study and experimental design, analysis and interpretation of data, wrote the manuscript.

The authors reported no potential conflict of interest.

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