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BRIEF COMMUNICATION

Amplification of *IGH/MYC* Fusion in Clinically Aggressive *IGH/BCL2*-Positive Germinal Center B-Cell Lymphomas

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Activation of an oncogene via its juxtaposition to the *IGH* locus by a chromosomal translocation or, less frequently, by genomic amplification is considered a major mechanism of B-cell lymphomagenesis. However, amplification of an *IGH*/oncogene fusion, coined a *complicon*, is a rare event in human cancers and has been associated with poor outcome and resistance to treatment. In this article are descriptions of two cases of germinal-center-derived B-cell lymphomas with *IGH/BCL2* fusion that additionally displayed amplification of an *IGH/MYC* fusion. As shown by fluorescence in situ hybridization, the first case contained a *IGH/MYC* complicon in double minutes, whereas the second case showed a *BCL2/IGH/MYC* complicon on a der(8)t(8;14)t(14;18). Additional molecular cytogenetic and mutation analyses revealed that the first case also contained a chromosomal translocation affecting the *BCL6* oncogene and a biallelic inactivation of *TP53*. The second case harbored a duplication of *REL* and acquired a translocation affecting *IGL* and a biallelic inactivation of *TP53* during progression. Complicons affecting *Igh/Myc* have been reported previously in lymphomas of mouse models simultaneously deficient in *Tp53* and in genes of the nonhomologous end–joining DNA repair pathway. To the best of our knowledge, this is the first time that *IGH/MYC* complicons have been reported in human lymphomas. Our findings imply that the two mechanisms resulting in *MYC* deregulation, that is, translocation and amplification, can occur simultaneously.

The translocation t(14;18)(q32;q21) is the cytogenetic hallmark of follicular lymphomas (FLs) but is also recurrent in 15%–20% of de novo germinal center–derived diffuse large B-cell lymphomas (Cigudosa et al., 1999; Akasaka et al., 2000; Nathwani et al., 2001; Huang et al., 2002; Rosenwald et al., 2002). This translocation juxtaposes the *BCL2* locus in 18q21 to regulatory elements of the *IGH* locus in 14q32, leading to deregulated expression of the former. However, a t(14;18) is not sufficient to induce malignant transformation, and the acquisition of secondary genetic changes is required for tumorigenesis (McDonnell and Korsmeyer, 1991; Hoglund et al., 2004).

The clinical behavior of FL is indolent, with a 5-year median survival of 72% (The Non-Hodgkin's Lymphoma Classification Project, 1997). About 30% of the FLs transform to a high-grade lymphoma. This histologic transformation, which usually is associated with rapidly progressing disease and a fatal outcome (Nathwani et al., 2001), is characterized by increased chromosomal instability and the accumulation of secondary chromosomal changes. Among these secondary changes, various rearrangements of the *MYC* locus, at 8q24, leading to deregulated expression of the MYC transcription factor have been reported recurrently in high-grade *IGH/BCL2*-positive lymphomas (Lee et al., 1989; Thangavelu et al., 1990; Yano et al., 1992; Farrugia et al., 1994; Macpherson et al., 1999; Lossos et al., 2002). These chromosomal changes include, on the one hand, chromosomal translocations juxtaposing *MYC* with immunoglobulin (*IG*) and non-*IG* loci, and on the other hand, genomic gains and amplifications of 8q24, leading to an increased

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gene dosage of *MYC*. Remarkably, mice double-transgenic for *Igh/Bcl2* and *Igh/Myc* rapidly develop lymphoid tumors, suggesting cooperation between Bcl2 and Myc in the process of lymphomagenesis (Strasser et al., 1990; Marin et al., 1995)

In this article, we describe two cases of clinically aggressive *IGH/BCL2*-positive germinal-center lymphomas with concurrent *IGH/MYC* fusion. Remarkably, both cases showed genomic amplifications of the fused *IGH/MYC* loci. Such complex rearrangements containing amplification of two loci juxtaposed by a chromosomal translocation have recently been designated as *complicons* by Zhu et al. (2002). These and other authors reported amplification of the *Igh/Myc* fusion in pro-B-cell lymphomas arising in transgenic mice deficient in the *Tp53* gene and members of the non-homologous end–joining (NHEJ) DNA repair pathway (Difilippantonio et al., 2002; Zhu et al., 2002; Gladdy et al., 2003; reviewed in Mills et al., 2003).

Mechanistically, complicons imply two alternative genomic means of *MYC* deregulation—translocation and amplification—to act simultaneously on the very same allele. To the best of our knowledge, such complicons containing *IGH/MYC* fusions have not yet been reported in human lymphomas.

Case 1. A 40-year-old man presented with a 3-week history of fever and night sweats. Physical examination revealed cervical lymphadenopathy, hepatosplenomegaly, and an epigastric mass. Morphologic analyses of bone marrow and gall bladder biopsy specimens were diagnostic for diffuse large B-cell lymphoma (DLBCL) according to the WHO classification. Immunophenotypic analyses of these biopsy specimens, performed with flow cytometry and immunohistochemistry, revealed the tumor clone to be positive for CD10, CD19, CD20, CD38, CD45, CD79a, HLA-DR, sIgKappa, Ki-67 (95%), BCL2, and BCL6 and negative for CD3, CD22, CD33, CD34, CD177, cMPO, FMC7, and sIglambda (Table 1). The bone marrow biopsy displayed two small paratrabecular foci with morphologic features characteristic of FL, suggesting the diagnosis of a secondary aggressive DLBCL transformed from clinically occult FL. The patient was treated with anthracycline-containing polychemotherapy (protocol BFM-86) and achieved a partial remission after the first treatment cycle. The clinical course was complicated by septicemia from gram-negative bacteria, from which the patient succumbed 2 months after the initial diagnosis. The family history was uneventful. Clinical and laboratory parameters are listed in Table 1.

Case 2. A 28-year-old man presented with general discomfort and multiple sites of lymphadenopathy on both sides of the diaphragm. The morphologic examination of a lymph node biopsy specimen was compatible with a diagnosis of FL, grade II. Immunohistochemical analyses from the same specimen revealed the tumor clone to be positive for CD10, CD20, CD45RA, Ki-67 (50–60%), BCL2, and BCL6 and negative for CD5, CD43, and CD45RO (Table 1). A simultaneous bone marrow biopsy showed a Burkitt-like lymphoma. The patient was treated with 8 cycles according to the CHOP protocol. Nine months after the initial diagnosis, still during evaluation of the treatment response, CT scan revealed a large abdominal mass, and a biopsy revealed an FL, grade III. Further chemotherapeutic regimens induced only limited responses. The patient died 16 months after the initial diagnosis as a result of progressive disease. The family history was unremarkable. Clinical and laboratory parameters are listed in Table 1.

Conventional cytogenetic analyses of G-banded chromosomes from bone marrow specimens revealed complex karyotypes in both cases. In case 1, the karyotype was near-triploid, with additions of unknown origin, marker chromosomes, and multiple double minutes (dmins). Spectral karyotyping (SKY) in this case, performed as described recently (Cigudosa et al., 2003), revealed a t(2;3)(q21;q27), a t(14;18)(q32;q21), and a der(17)t(4;8;17)(?;q24?;p11.2) as well as multiple double minutes and aneusomies (Table 1). In case 2, the G-banding karyotype at diagnosis was hyperdiploid, with several unresolved chromosomal changes (Table 1). After 9 and 14 months from the time of initial diagnosis, cytogenetic analysis of a second and third bone marrow sample, respectively, showed several additional chromosomal alterations, indicating clonal evolution (Table 1). SKY, performed in the sample at progression (14 months after diagnosis), identified t(3;22)(q27;q11.2), der(8)t(8;18)(q24;q?) hsr(8)(q24), der(14)t(14;16)(q32;q?), and der(17)t(7;16;17)(?;?;p11.2) and indicated the presence of complex rearrangements including homogeneously staining regions (hsrs) on chromosome arms 1q, 2p, and 8q (Table 1). The der(8)t(8;18)(q24;q?) hsr(8)(q24) was already present at diagnosis and was initially described as an add(8q24) by G-banding analysis.

Considering the chromosomal complexity in both cases, multiple metaphase and interphase FISH analyses were performed for further cytogenetic characterization (Tables 1 and 2), applying previously published protocols (Martin-Subero

TABLE 1. Clinical, Phenotypic, Cytogenetic, and Molecular Features of the Two Cases Reported in the Present Study

	Case 1	Case 2		
	At diagnosis	At diagnosis	At relapse (9 months after diagnosis)	At progression (14 months after diagnosis)
Patient characteristics				
Sex/age (years)	Male/40	Male/28	–	–
Clinical parameters				
Histopathologic diagnosis	Diffuse large B-cell lymphoma	Follicular lymphoma grade II	Follicular lymphoma grade III Burkitt–like lymphoma	
Clinical stage	IVB	IVB	IVB	–
Extranodal site	Peripheral blood, bone marrow, gall bladder, epiploic appendix, intestinal mesentery	Bone marrow, spleen	Bone marrow, right abdominal tumor including colon and duodenum	Bone marrow, abdominal tumor including both kidneys
ECOG performance status (1–4)	2	1	2	1
LDH (<210 U/L normal)	19,000	2,067	632	31,800
IPI (0–5)	4	3	4	3
Immunophenotype	CD3−, CD10+, CD19+, CD20+, CD22−, CD33−, CD34−, CD38+, CD45+, CD79a+, CD177−, cMPO−, FMC7−, HLA-DR+, sIgkappa+, sIglambda−, BCL2+, BCL6+, Ki-67 (95%)	CD5−, CD10+, CD20+, CD43−, CD45Ra+, CD45Ro−, BCL2+, BCL6+, Ki-67 (50–60%)	ND	CD3−, CD10+, CD19+, CD20+, CD22−, CD33−, CD34−, CD38+, CD45+, CD79a+, CD177−, cMPO−, HLA-DR+, sIgkappa−, sIglambda+, TdT−
Karyotype[a]	78, XXX, Y, +1, t(2;3)(q21;q27)×2, +2, +3×2, +4, +5×2, +6×2, +7, +9, +11, +12, +13×2, t(14;18)(q32;q21), +14, +15, +16, der(17)(t(4;8;17)(?;q24?;p11.2)×2, +17×2, +18×2, +19, +20×2, +21, +22×2, +dmin[28]/46, XY[2]	49, XY, +X, add(1)(q44), add(2)(p25), +7, add(8)(q24), +12[42]/46, XY[8]	48, XY, +X, add(1)(q44), add(2)(p25), der(3q), der(4p), +7, add(8)(q24), −10, +12, t(14;18)(q32;q21), add(19q), del(22)(q12) [18][b]/46, XY[107]	49, XY, +Y, der(1)hsr(1)(q31?), der(2)hsr(2)(p?), t(3;22)(q27;q11.2), +7, der(8)t(8;18)(q24;q?)hsr(8)(q24), +12, der(14)t(14;16)(q32;q?), der(17)t(7;16;17)(?;?, p11.2)
Fluorescence in situ hybridization[a]				
IGH (14q32)	nuc ish 14q32(*IGH*-cen × 4, *IGH*-const × amp, *IGH*-var × 2)	nuc ish 14q32(*IGH*-cen × 2, *IGH*-const × 4–5, *IGH*-var × 2) (*IGH*-cen/const sep *IGH*-var × 1)	ND	nuc ish 14q32(***IGH*-cen × 3, *IGH*-const × 5–6**, *IGH*-var × 2) (*IGH*-cen/const sep *IGH*-var × 1)
IGL (22q11)	nuc ish 22q11(*IGL*-cen × 4, *IGL*-tel × 4)	nuc ish 22q11(*IGL*-cen × 2, *IGL*-tel × 2)	ND	nuc ish 22q11(*IGL*-cen × 2, *IGL*-tel × 2) (***IGL*-cen sep *IGL*-tel × 1**)
IGK (2p12)	nuc ish 2p12(*IGK*-cen × 3, *IGK*-tel × 3)	nuc ish 2p12(*IGK*-cen × 3, *IGK*-tel × 3)	ND	nuc ish 2p12(*IGK*-cen × 3, *IGK*-tel × 3)

(Continued)

TABLE 1. Clinical, Phenotypic, Cytogenetic, and Molecular Features of the Two Cases Reported in the Present Study (Continued)

	Case 1	Case 2		
	At diagnosis	At diagnosis	At relapse (9 months after diagnosis)	At progression (14 months after diagnosis)
MYC (8q24)	nuc ish 8q24(*MYC*-cen × amp, *MYC*-tel × 3–4)	nuc ish 8q24(*MYC*-cen × 3–4, *MYC*-tel × 2)(*MYC*-cen sep *MYC*-tel × 1)	ND	nuc ish 8q24(*MYC*-cen × 3–4, **MYC-tel × 3)(MYC-cen sep MYC-tel × 2**)
BCL2 (18q21)	nuc ish 18q21(*BCL2*-cen × 4, *BCL2*-tel × 6)	nuc ish 18q21(*BCL2*-cen × 2, *BCL2*-tel × 4–5)(*BCL2*-cen sep *BCL2*-tel × 1)		nuc ish 18q21(*BCL2*-cen × 2, *BCL2*-tel × 4–5) (*BCL2*-cen sep *BCL2*-tel × 1)
MYC, IGH, CEP8	nuc ish 8(CEP8 × 2, *MYC* × amp), 14q32 (*IGH* × amp)(*MYC* con *IGH* × amp)	nuc ish 8(CEP8 × 2, *MYC* × 4), 14q32 (*IGH* × 5–6) (*MYC* con *IGH* × 3–4)	ND	nuc ish 8(CEP8 × 2, ***MYC* × 5), 14q32(*IGH* × 6–7)(*MYC* con *IGH* × 4–5**)
BCL2, IGH	nuc ish 14q32(*IGH* × amp), 18q21(*BCL2* × 4)(*IGH* con *BCL2* × 2)	nuc ish 14q32(*IGH* × 5–6), 18q21(*BCL2* × 4, (*IGH* con *BCL2* × 3–4)	ND	nuc ish **14q32(*IGH* × 6–7)**, 18q21(*BCL2* × 4) (*IGH* con *BCL2* × 3–4)
BCL2, IGH, MYC	nuc ish 8q24(*MYC* × amp), 14q32(*IGH* × amp), 18q21(*BCL2* × 4) (*MYC* con *IGH* × amp)(*IGH* con *BCL2* × 2)	nuc ish 8q24(*MYC* × 4), 14q32(*IGH* × 5–6), 18q21(*BCL2* × 3–4) (*MYC* con *IGH* × 3–4) (*BCL2* con *IGH* × 3–4) (*MYC* con *IGH* con *BCL2* × 2–3)	ND	nuc ish 8q24(*MYC* × 5), **14q32(*IGH* × 6–7)**, 18q21(*BCL2* × 4) **(*MYC* con *IGH* × 4–5**) (*BCL2* con *IGH* × 3–4)(*MYC* con *IGH* con *BCL2* × 2–3)
REL (2p13–16)	nuc ish 2p13∼16(*REL*-cen × 3, *REL*-tel × 3)	nuc ish 2p13∼16(*REL*-cen × 3, *REL*-tel × 3)[c]	ND	nuc ish 2p13∼16(*REL*-cen × 3, *REL*-tel × 3)[c]
BCL6 (3q27)	nuc ish 3q27(*BCL6*-cen × 4, *BCL6*-tel × 4)(*BCL6*-cen sep *BCL6*-tel × 2)	nuc ish 3q27(*BCL6*-cen × 2, *BCL6*-tel × 2)	ND	nuc ish 3q27(*BCL6*-cen × 2, *BCL6*-tel × 2)
ATM (11q22–23)	nuc ish 11q22–23(*ATM* × 3)	nuc ish 11q22∼23(*ATM* × 2)	ND	nuc ish 11q22∼23(*ATM* × 2)
TP53 (17p13), CEP17	nuc ish 17(*TP53* × 2, CEP17 × 4)	nuc ish 17(*TP53* × 2, CEP17 × 2)	ND	nuc ish 17(***TP53* × 1**, CEP17 × 2)
BCL3 (19q13)	ND	nuc ish 19q13(*BCL3*-cen × 3, *BCL3*-tel × 3)	ND	nuc ish 19q13(*BCL3*-cen × 3, *BCL3*-tel × 3)
PCR for *IGH/BCL2* juxtaposition	*IGH/BCL2* positive (MBR)	*IGH/BCL2* negative (MBR and MCR)	ND	ND
TP53 mutation analysis (exons 2–11)	625_626delAG	No inactivating mutations detected	ND	991C>T

[a]Described according to the ISCN-95 guidelines. The karyotypes of patients 1 and 2 (sample at progression) were resolved by SKY, whereas those of patient 2 at diagnosis and relapse are based on G banding.
[b]Aberrant clone in patient 2 at relapse detected only in a culture stimulated with TPA.
[c]Intrachromosomal duplication of the *REL* gene in patient 2 shown by FISH in metaphase cells; cen: probe centromeric to the gene; const: probe specific for the *IGH*-constant region; tel: probe telomeric to the gene; ND: not done. For patient 2, chromosomal changes found only in the sample at progression are shown in boldface.

TABLE 2. Composition of Complicons on the dmin in Case 1 and on the Derivative Chromosome der(8) in Case 2 (based on FISH to metaphase cells)

FISH probe	Source/reference of the FISH probes	Double minutes in patient 1	der(8) in patient 2
LSI *IGH*/*MYC*, CEP8	Vysis/Abbott, Downers Grove, IL	*IGH*/*MYC* fusion amplified	*IGH*/*MYC* fusion 2–3 copies
LSI *IGH*/*BCL2*	Vysis/Abbott, Downers Grove, IL	*IGH* amplified	*IGH*/*BCL2* fusion 2–3 copies
LSI *IGH*/*BCL2* + *MYC*	Vysis/Abbott, Downers Grove, IL *MYC* cosmids: Siebert et al., 1998	*IGH*/*MYC* fusion amplified	*BCL2*/*IGH*/*MYC* fusion 2–3 copies
LSI *MYC*	Vysis/Abbott, Downers Grove, IL	only centromeric signal amplified	only centromeric signal 2–3 copies
RP11-1136L8 (proximal to *MYC*)	RPCI11 BAC library*	amplified	2–3 copies
CTD-3056O22 (spanning *MYC*)	CTD BAC library*	amplified	2–3 copies
CTD-2267H22 (distal to *MYC*)	CTD BAC library*	not present	not present
Triple color *IGH* LSI *IGH* + RP11-417P24	Vysis/Abbott, DG, Il; RPCI11 BAC library*	only signals for the *IGH* constant region amplified	only signals for the *IGH* constant region 2–3 copies
BCL2 (RP11-13L22/RP11-215A20-centromeric + RP11-635N19/RP11-851B10-telomeric)	RPCI11 BAC library*	not present	only telomeric signal 2–3 copies
LSI *BCL6*	Vysis/Abbott, Downers Grove, IL	not present	not present
LSI *ATM*/*P53*/CEP17	Vysis/Abbott, Downers Grove, IL	not present	not present
IGK	Martin-Subero et al., 2002c	not present	not present
IGL	Martin-Subero et al., 2002c	not present	not present
REL (RP11-625B6 telomeric/ RP11-91218 centromeric)	RPCI11 BAC library*	not present	not present
BCL3	Martin-Subero et al., manuscript in preparation	not present	not present

*BAC clones were obtained from Research Genetics/Invitrogen (http://mp.invitrogen.com/), the German Resource Center for Genome Research (http://www.rzpd.de/), or the Sanger Centre (http://www.sanger.ac.uk/).

et al., 2002a, 2002c). FISH with the LSI *IGH*/*BCL2* probe indicated the presence of an *IGH*/*BCL2* juxtaposition in both patients. Interestingly, case 1 displayed massive amplification of the hybridization signals for the *IGH* locus in multiple dmins, whereas case 2 displayed triplication in tandem with the *IGH*/*BCL2* juxtaposed signals on the derivative chromosome der(8) (Table 2). Molecular genetic analyses based on PCR demonstrated the presence of an *IGH*/*BCL2* fusion (in the major breakpoint region of *BCL2*, MBR) in patient 1, whereas in case 2, this molecular analysis was negative for MBR and minor cluster region breakpoints, although the sample was *IGH*/*BCL2*-positive by FISH. This finding is not surprising because systematic studies comparing different methods of detecting the *IGH*/*BCL2* fusion have shown FISH to be more reliable than molecular genetic approaches (Poetsch et al., 1996; van Dongen et al., 2003).

In case 1, FISH with the LSI *IGH*/*MYC* probe revealed amplification of the *IGH*/*MYC* fusion signals in the dmins. To characterize the composition of the complicon, a number of DNA probes spanning or flanking the *MYC* locus were applied as well as a triple-color probe for *IGH* covering the variable and constant sequences and the *IGH* proximal region. The amplification in dmins was shown to contain signals for the probes spanning/proximal to *MYC* (Fig. 1A), and spanning the *IGH* constant region (labeled in blue; Fig. 1B, Table 2). Additional molecular cytogenetic analyses (summarized in Table 1) indicated the presence of a translocation affecting the *BCL6* locus (Fig. 1C) and a deletion of the *TP53* gene (Fig. 1D). Deletion of the *TP53* gene is a well-established marker of a poor prognosis in germinal center B-cell lymphoma (Farrugia et al., 1994; Tilly et al., 1994).

To determine whether the remaining allele also was inactivated, mutation screening of exons 2–11 of *TP53* was performed by direct sequencing using previously reported PCR conditions and primers (Agirre et al., 2002). This analysis indicated a 2-bp deletion in codon 209 of exon 6, leading to a stop

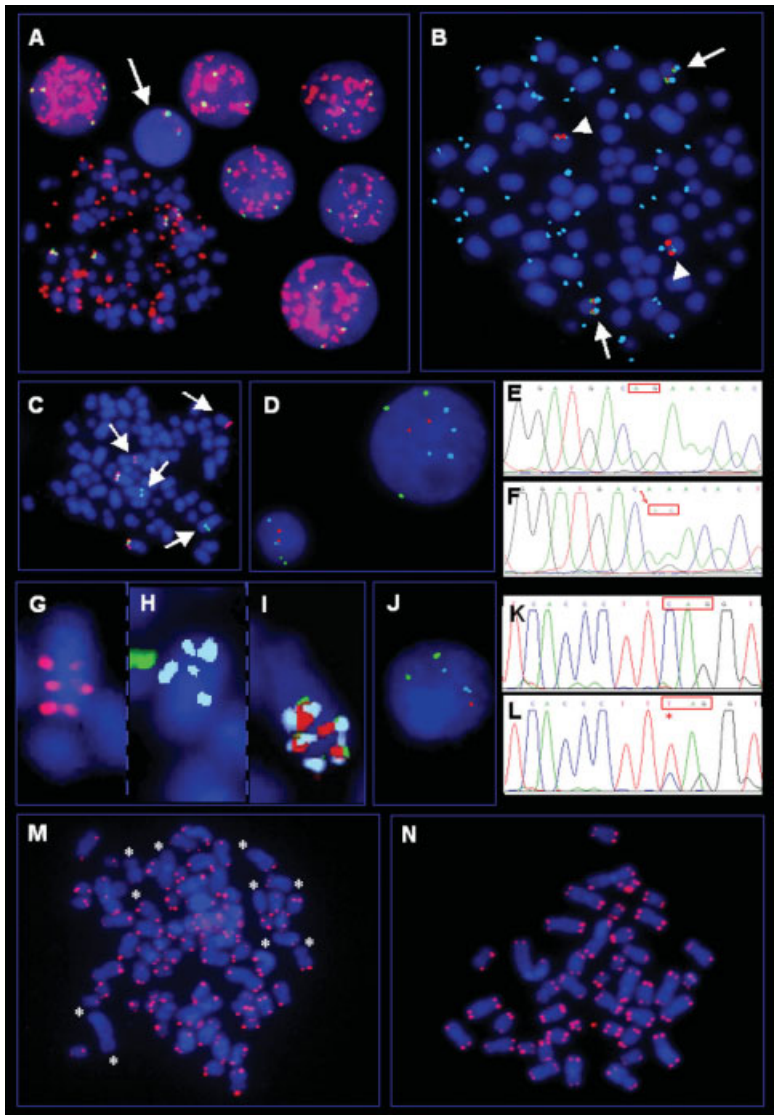

Figure 1. (A–D) FISH analyses of case 1. (A) Interphase and metaphase nuclei hybridized with the LSI *MYC* breakapart probe (Vysis, Downers Grove, IL). Six nuclei show high-level amplification of the centromeric *MYC* probe (labeled in red), whereas one nucleus displays a regular signal constellation, that is, two colocalized red/green signals (arrow). The metaphase shows the amplified *MYC* sequences to be located in double-minute (dmin) chromosomes. (B) Metaphase cell hybridized with the *IGH* triple-color probe. Chromosomes containing triple red/green/pale blue colocalizations indicate the presence of an intact *IGH* locus (arrows). Chromosomes with sequences centromeric to *IGH* (red) and spanning the *IGH* constant region (pale blue) point to der(14)(q32) from a translocation affecting *IGH* (arrowheads). Blue signals mapping to extrachromosomal elements indicate that the dmin contain sequences from the *IGH* constant region. (C) Metaphase cell hybridized with the LSI *BCL6* breakapart probe (Vysis, Downers Grove, IL), showing two each of isolated red and green signals (arrows), indicating a translocation affecting *BCL6* and two colocalized red/green signals pointing to an intact *BCL6* locus. Dmin chromosomes are not visible with DAPI staining. (D) Interphase nuclei hybridized with the three-color LSI *ATM* (green)/*TP53* (red)/CEP17 (chromosome enumeration probe, pale blue) probe (Vysis, Downers Grove, IL). The large nucleus on the right-hand side contains three copies of *ATM*, two copies of *TP53*, and four of the centromere of chromosome 17, pointing to a deletion of the *TP53* gene. In contrast, the small nucleus on the left-hand side displays the regular signal pattern, that is, two signals for each locus. (E–F) Sequence analysis of *TP53* exon 6 in case 1. (E) Wild-type sequence. (F) Sequence from case 1 showing a 2-bp deletion (AG) corresponding to the 209 codon (AGA) of the *TP53* gene. (G–J) FISH analyses of case 2. (G–I) Partial metaphase cells hybridized with (G) the LSI *MYC* breakapart probe (Vysis, Downers Grove, IL) and the triple-color probes for (H) *IGH* and (I) *BCL2* (red)/*IGH* (green)/*MYC* (pale blue). The derivative chromosome der(8) contains three copies in tandem of the signals centromeric to (G) *MYC* (red) and spanning the (H) *IGH* constant region (pale blue) and (I) a triple fusion of the *BCL2*, *IGH*, and *MYC* loci. (J) Interphase nucleus hybridized with the LSI *ATM*/*P53*/CEP17 probe (Vysis, Downers Grove, IL) showing two copies of *ATM*, one of *TP53*, and two of CEP17, indicating that one allele of the *TP53* gene is deleted. (K–L) Sequence analysis of *TP53* exon 9 in case 2. (K) Sequence from case 2 at diagnosis showing the wild-type sequence. (L) Sequence from case 2 in transformation showing a nonsense mutation in codon 331 (CAG→TAG) of the *TP53* gene. (M–N) Metaphase cells hybridized with a PNA probe for all human telomeres. (M) Metaphase cell from case 1 showing multiple chromosome ends lacking detectable hybridization signals (asterisks). Dmin chromosomes are not visible because of their faint DAPI staining, but they clearly lack telomeric signals. (N) Metaphase cell from case 2 showing all chromosome ends to contain telomeric hybridization signals.

six codons downstream (Fig. 1E and F). Consequently, the function of the *TP53* gene in this patient was shown to be abolished in the tumor cells. With regard to the translocation targeting *BCL6* detected by FISH, SKY pointed to chromosome band 2q21 as the translocation partner, a rearrangement that has been described previously (Chen et al., 1998a). This finding indicates a *BCL6* promoter substitution from a non-*IG* locus as a translocation partner of *BCL6*, a common phenomenon in DLBCL (Chen et al., 1998b).

In case 2, FISH with the LSI *IGH/MYC* probe at diagnosis revealed the presence of a triplication of the fused *IGH/MYC* allele on the derivative chromosome der(8). Considering the presence of an *IGH/BCL2* amplification in the same chromosome, this finding suggested a complicon involving a *BCL2/IGH/MYC* triple juxtaposition, which was confirmed by three-color FISH (Fig. 1I, Table 2).

Complex chromosomal translocations leading to concurrent translocation of the *BCL2* and *MYC* loci to the same *IGH* allele has been described previously in transforming FL. In those cases, the *BCL2* gene was shown to be juxtaposed next to *IGH* as a result of an illegitimate V(D)J rearrangement, whereas the *MYC* locus was fused to the already translocated *IGH/BCL2* allele because of a defective class switch rearrangement (Dyer et al., 1996). Similarly to patient 1, hybridization with probes flanking or spanning the *MYC* locus and the triple probe for *IGH* showed that only the signals proximal/spanning the *MYC* locus (Fig. 1G) and the signals spanning the *IGH* constant region (Fig. 1H) were present in der(8) (Table 2). Additional molecular cytogenetics in metaphase nuclei from the bone marrow sample at diagnosis from patient 2 (summarized in Table 1) indicated the presence of an intrachromosomal duplication in 2p, including the *IGK* and *REL* loci.

Chromosomal gain of 2p has been suggested to confer a poor prognosis in B-NHLs (Yunis et al., 1989) and is a common finding in transforming FL, germinal-center-type DLBCL, and Hodgkin lymphoma (Joos et al., 2002; Martin-Subero et al., 2002b; Rosenwald et al., 2002; Martínez-Climent et al., 2003). Amplification in tandem of the *BCL2/IGH/MYC* loci as well as duplication targeting the *REL* locus also were detected in the sample after transformation. Moreover, in the sample after transformation, FISH provided evidence of deletion of the *TP53* gene (Fig. 1J) and translocation with a breakpoint in the *IGL* locus, which were not present at diagnosis and, thus, were acquired during tumor progression. Results are shown in Table 1.

Mutation analysis of exons 2–11 of the *TP53* gene was performed in the samples at diagnosis and at transformation. This analysis revealed a nonsense mutation in codon 331 (CAG→TAG; Gln→Stop) in exon 9, but only in the sample at transformation. In addition, in the sample at diagnosis, two silent heterozygous polymorphisms were detected, in codon 213 (CGA→CGG; Arg→Arg) of exon 6 and in codon 264 (CTA→TTA; Leu→Leu) of exon 8. In the sample at transformation, only the CGA and CTA sequences were identified, indicating that the allele containing the CGG and TTA sequences was the one lost as a result of chromosomal deletion.

With regard to the translocation involving *IGL* in 22q11, cytogenetic analysis performed by SKY indicated band 3q27 as the translocation partner. However, although *BCL6* was the most likely candidate partner, FISH on metaphase cells with a breakapart probe (from Vysis/Abbott, Downers Grove, IL) demonstrated that the breakpoint was telomeric to *BCL6*. This might point to the presence of an alternative breakpoint region targeting *BCL6* that has been described as being 200–270 Kb telomeric to the gene (Chen et al., 1998a) and might therefore remain undetected with the applied FISH probe, which spans 300 Kb telomeric to the *BCL6* locus (http://www.vysis.com). Another hypothesis is that this translocation targets another oncogene locus telomeric to *BCL6*.

To the best of our knowledge, this is the first time that complicons affecting *IGH/MYC* have been described in human malignant lymphomas. Several recent reports have shown that transgenic mice deficient both in *Tp53* and in genes involved in the NHEJ pathway (*Ku70*, *Xrcc5* (*Ku80*), *Xrcc4*, *Dna-pk*, and *Dna Ligase IV*) developed pro-B-cell lymphomas that frequently displayed the nonreciprocal translocation C12;15 (*Igh* on mouse chromosome 12 and *Myc* on mouse chromosome 15; C indicates the chromosome containing the centromere) with genomic amplification of the *Igh/Myc* fusion (Difilippantonio et al., 2002; Zhu et al., 2002; Gladdy et al., 2003; reviewed in Mills et al., 2003). These studies proposed a model in which complicons arose from several cycles of chromosome breakage and fusion that eventually finished with telomere capture from a different chromosome, stabilizing the derivative chromosome. In support of this hypothesis, the authors provided cytogenetic evidence of both dicentric chromosomes and chromosome tips lacking telomeric sequences that rep-

TABLE 3. Overview of the 17p/*TP53* Status in Published Hematologic Tumors Containing Complicons

Case/Reference	Diagnosis	Organization of complicon	17p/*TP53* Status at the moment when the complicon was detected
Case 1 in present report	DLBCL	*MYC/IGH* in dmins	*TP53* inactive (deletion and mutation)
Case 2 in present report (FL)	FL	*BCL2/IGH/MYC* in hsr	*TP53* intact (but most likely inactivated by BCL6 expression)
Case 1 in Gruszka-Westwood et al., 2002	MCL	*CCND1/IGH* in hsr	*TP53* inactive (deletion and mutation)
Case 1 in Metzke-Heidemann et al., 2001	CML in BC	*BCR/ABL* in hsr	17p13 deletion by der(17)
Case 2 in Metzke-Heidemann et al., 2001	CML in BC	*BCR/ABL* in hsr	17p13 deletion by add(17p)
Case 1 in Morel et al., 2003 (CML)	CML in megakaryoblastic crisis	*BCR/ABL* in dmins	17p13 deletion by add(17p)
Case 1 in Campbell et al., 2002 (CML)	CML in myeloid BC	*BCR/ABL* in hsr	17p13 deletion by add(17p)
Case 2 in Campbell et al., 2002 (CML)	CML in myeloid BC	*BCR/ABL* in hsr	17p13 deletion by −17
Case 1 in Gargallo et al., 2003	CML in myeloid BC	*BCR/ABL* in hsr	Complex karyotype without cytogenetically detectable del(17p)
K-562 cell line in Rodley et al., 1997	CML in BC	*BCR/ABL* in hsr	*TP53* inactive (deletion and mutation)

DLBCL: diffuse large B-cell lymphoma; FL: follicular lymphoma; MCL: mantle cell lymphoma; CML: chronic myeloid leukemia; BC: blast crisis; dmins: double minutes; hsr: homogeneously staining region.

resent intermediates of the breakage-fusion-bridge model leading to complicons.

In light of these new findings and considering the striking cytogenetic similarity between the above-noted animal models and the two cases described in this article, it is tempting to speculate that a similar mechanism might have taken place in the patients reported. However, this hypothesis is difficult to prove because all clonal metaphase cells studied by G-banding in both patients already were very complex but without evidence of unstable dicentric chromosomes. Thus, the intermediate steps in the generation of the complicons could not be studied. Nevertheless, we applied a telomere sequence–specific peptide nucleic acid probe (PNA; DAKO, Glostrup, Denmark) to determine the status of the telomeres in the two cases. The cutoff for missing telomeres, calculated as the mean of chromosome tips lacking telomeric hybridization signals plus 3 times the standard deviation of five metaphase cells each from five healthy donors, was determined as 3.1%. Five tumor metaphase cells from each case (case 1 at diagnosis and case 2 at progression) were evaluated for the signals derived from the PNA-telomere probe. In the tumor metaphases of case 1, 6.4% (range: 4.0%–8.3%) of the chromosome tips lacked detectable hybridization signals (Fig. 1M), whereas the tumor metaphase cells of case 2 showed intact telomere sequences in all chromosomes (Fig. 1N).

Whether the lack of detectable telomeres in case 1 is directly or indirectly related to the generation of the *IGH/MYC* complicon itself is unknown, but it seems likely that it might promote increased chromosomal instability. The dmin chromosomes in case 1 did not show any detectable hybridization signals with the PNA-telomere probe.

In case 2, a three-step rearrangement might have taken place as an alternative to a process of iterative breakage-fusion-bridge. According to this second model, a defective V(D)J rearrangement leading to a t(14;18) with *IGH/BCL2* fusion would be followed by a defective switch rearrangement to 8q24, leading to a *BCL2/IGH/MYC* triple fusion. Finally, a sequential unequal sister chromatid exchange would occur, leading to the hsr on der(8). In support of this model, the der(8)t(8;14)t(14;18) telomere belonged to chromosome 18 according to the SKY analysis, and therefore a telomere capture event seems unlikely. Interestingly, *TP53* was shown to be completely inactivated (by mutation and deletion) in this case, but only in the sample in transformation. As the sample at diagnosis already contained the complicon, the *BCL2/IGH/MYC* amplification either arose independently of *TP53* inactivation, or *TP53* was inactivated by other means. Remarkably, recent evidence suggests that the BCL6 protein represses *TP53* transcription in germinal-center B cells (Phan and Dalla-Favera, 2004). Both cases in the present study expressed

BCL6 in the tumor cells at diagnosis, as shown by immunohistochemistry (Table 1). Thus, *TP53* expression is likely to be functionally inactivated in the tumor cells of both cases.

Complicons affecting genes other than *IGH/MYC* have been reported rarely in hematologic malignancies and are associated with a poor outcome and chromosomal complexity. Recently, Gruszka-Westwood et al. (2002) described a clinically aggressive case of leukemic mantle cell lymphoma with an *IGH/CCND1* complicon. In chronic myelogenous leukemia (CML), *BCR/ABL* complicons have been described repeatedly as a mechanism of disease progression and resistance to treatment with STI-571 (Gorre et al., 2001; Metzke-Heidemann et al., 2001; Morel et al., 2003). Most interestingly, when the complicon was detected in most hematologic disorders for which cytogenetic data have been published as well as in one of the cases presented here, a deletion of 17p13, the site of the tumor-suppressor gene *TP53*, was detected (Table 3). All the CMLs contained a classical t(9;22)(q34;q11) at diagnosis and acquired a *TP53* deletion and a *BCR/ABL* complicon in blastic crisis, indicating that *TP53* might also play an important role in the development of *BCR/ABL* complicons. Remarkably, NHEJ-deficient mice acquire complicons only in the context of *Tp53* inactivation, which supports the hypothesis that complicons in human hematologic cancers also might arise from defective NHEJ DNA repair.

In the two cases reported here, no apparent cytogenetic changes were detected in the chromosomal bands that are the sites of *KU70* (22q13), *KU80* (2q35), *XRCC4* (5q14), *DNAPK* (8q11), and *LIG4* (13q33) (Table 1).

Despite the inconclusive findings regarding the mechanics of generating *IGH/MYC* complicons in the two cases reported here, the marked similarity of the mouse models reported by Zhu et al. (2002), Difilippantonio et al. (2002), and Gladdy et al. (2003) with the two cases presented in this report warrants further investigation of the molecular status of genes involved in the NHEJ DNA repair pathway.

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