Cytogenetic and molecular delineation of a region of chromosome 3q commonly gained in marginal zone B-cell lymphoma

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Background and Objectives. Whole or partial trisomy 3 represents the most recurrent chromosomal abnormality occurring in marginal zone B-cell lymphoma (MZBCL), a distinct subtype of B-cell non-Hodgkin's lymphoma (NHL). By conventional cytogenetic analysis, unbalanced translocations involving chromosome 3 and leading to a partial trisomy 3q were identified in a series of 14 MZBCL patients. Fluorescent *in situ* hybridization (FISH) experiments were then performed to characterize the breakpoints further and to delineate the extent of the 3q gained region more accurately.

Design and Methods. We studied 14 cases of MZBCL combining cytogenetics and FISH techniques using specific probes for the long arm of chromosome 3, including the chromosome 3 α satellite probe, a representative panel of yeast artificial chromosome (YAC) clones mapping the chromosomal 3q region (3q11.2 to 3q23) and the chromosome 3 subtelomeric (3q29) probe.

Results. In the 14 cases, additional chromosome 3q material was found to be involved in different unbalanced translocations with chromosomes 1, 6, 7, 8, 11, 13, 14, 15, 17, 19 and 21, leading to a derivative chromosome. None of the chromosomal abnormality juxtaposed the 3q regions with the heavy and/or light κ and λ immunoglobulin gene loci. Eight different breakpoints distributed between the 3q11.2 and the 3q13.32 regions were identified and a common 3q13.32–3q29 overrepresented region was delineated.

Interpretation and Conclusions. These results suggest that this critical region may be of importance in the pathogenesis of MZBCL and support the hypothesis that a gene dosage effect rather than a specific gene disruption may be involved in the development of this disease.

Key words: marginal zone B-cell lymphoma, cytogenetics, *in situ* hybridization, chromosome 3q gains, molecular delineation.

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In the recently presented World Health Organization (WHO) classification of lymphoid neoplasms, marginal zone B-cell lymphomas (MZBCL) are considered as well individualized lymphomas including extranodal mucosa-associated lymphoid tissue (MALT) lymphomas, splenic MZBCL with or without villous lymphocytes and nodal MZBCL.[1] Although cytogenetic studies on large series of MZBCL are still rare, recurrent chromosomal aberrations have been described, including trisomy 3, and less frequently chromosome 7q21-35 deletions, trisomy 12, and trisomy 18. Whole or partial trisomy 3 has been reported most consistently, occurring in 56-78% of cytogenetically abnormal cases.[2-4] Besides MZBCL, complete and partial trisomy 3q occur in a limited spectrum of B-cell lymphoproliferative malignancies, especially in diffuse large B-cell lymphomas and in mantle B-cell lymphomas. In these latter cases, the 3q25 and 3q27-3q29 regions are usually found to be gained.[5,6]

Despite the large occurrence of whole or partial trisomy 3 in MZBCL, little is known about the origin, the pathogenetic significance and the genetic mechanism by which this abnormality may contribute to malignant transformation and/or disease progression. In this respect, cases with partial trisomy 3q are of particular significance to delineate a pathogenetically important subregion of chromosome 3. Only a few cytogenetic studies, mainly focusing on comparative genomic hybridization (CGH) experiments, have tried to define this subregion. In all of the investigated cases, the relevant regions of overrepresentation were previously narrowed down to between the 3q21 and 3q29 regions.[7,8]

In the present study, we focused the cytogenetic analysis on rearrangements leading to a partial trisomy for the long arm of chromosome 3 (+3q). Fourteen MZBCL cases with unbalanced translocations leading to a chromosome 3q overrepresentation were then selected. To characterize the breakpoints further and to delineate the extent of the duplicated region more accurately, fluorescence *in situ* hybridization (FISH) experiments using representative yeast artificial chromosome (YAC) clones mapping to the 3q11-3q23 regions were performed.

Design and Methods

Patients

Between March 1993 and February 2000, one hundred and forty-one cases of MZBCL diagnosed by morphology, histology and immunology were referred to our institution. Among them, fourteen patients presented conventional karyotypes with unbalanced translocations leading to a trisomy 3q. All cases were reviewed by two

expert cyto-histopathologists and the diagnosis was established according to the WHO classification.[1]

Immunologic analysis

Immunologic characterization was performed on fresh frozen sections, paraffin sections, imprints and/or cell suspensions using a flow cytometer. On cell suspensions, the expression of CD3, CD5, CD10 CD23 and CD43 was evaluated on CD19 positive cells by double staining.

Cytogenetic analysis

Conventional studies were performed on peripheral blood (patients 1, 2, 4, 8-11 and 14), bone marrow (patients 3 and 7), lymph nodes (patient 6) and spleen samples (patients 5, 12 and 13). Cells were cultured for 18h-72h in RPMI 1640 medium (Techgen, les Ulis, France) supplemented with 10% fetal calf serum (Techgen), L-glutamine, antibiotics and phorbol 12-myristate 13-acetate (TPA, 0.1μg/mL, Sigma, St Louis, MO, USA). Chromosome analyses were carried out on RHG-banded metaphases as previously described[9] and evaluated according to the ISCN recommendations.[10]

Fluorescence in situ hybridization

FISH was performed in all cases with available methanol/acetic acid fixed cells previously processed for cytogenetic analysis. The following DNA probes were applied: two painting probes specific for chromosome 3 and chromosome 3q (Oncor, Gaithersburg, MD, USA and Eurodiagnostica, respectively), a chromosome 3-specific alpha-satellite probe (D3Z1, Oncor), a specific subtelomeric probe Tel 3q (3q29-qter, Oncor) and a panel of CEPH-GENETHON Mega YAC clones that map to chromosome 3q (Table 1). These probes were obtained from the United Kingdom Medical Research Council Human Genome Mapping Project Resource Centre (UK MRC HGMP), Cambridge, UK and selected based on data from URL *http://www-genome.wi.mit.edu/* at the Whitehead Institute. As shown in Table 1, these 24 YAC clones covered the whole region of interest.

Each individual YAC clone was hybridized on normal metaphase preparations to confirm the chromosome localization and to ensure that none of them was chimeric.

Yeast cells containing YACs were grown in SD medium (yeast nitrogen base supplemented with glucose and amino acids) and DNA was prepared as previously described.[11] All the YAC probes were labeled by nick translation with biotin-14-dATP (Life Technologies, Gaithersburg, MD, USA) or digoxigenin-11-dUTP (Boehringer Mannheim, Mannheim, Germany) and completed with a 100-150-fold excess of Cot-1 DNA, 5 μg of yeast tRNA and salmon sperm DNA.

FISH was performed as previously described[12] with minor modifications. Briefly, the slides were pretreated in 2× standard saline citrate (SSC)/RNAse A (100 μg/mL) at 37°C for 1 hour and rinsed in 2 X SSC at room temperature for 5 minutes. Slides were then immersed in a 0.1% pepsin/1% HCl solution at 37°C for 10 minutes and rinsed in PBS at room temperature for 5 minutes. After sequential ethanol dehydration (70, 80 and 100%) for 2 minutes each at room temperature, slides were denaturated on a hot plate (72°C) with 100 μL of 70% formamide/2× SSC, pH 7 for 2 minutes. After a brief wash in 2× SSC at room temperature, slides were transferred into ice-cold 70, 80 and 100% ethanol and air-dried. The YAC probes (300 ng in hybridization solution) were denaturated 10 minutes at 72°C, pre-annealed for 30 minutes at 37°C and hybridized overnight in a moist chamber at 37°C. Biotinylated-labeled probes were detected with (1) avidin-FITC (Vector Laboratories, Burlingame, CA, USA); (2) goat anti-avidin biotinylated (Vector Laboratories); and (3) avidin-FITC antibodies. Digoxigenin-labeled probes were visualized using: (1) sheep anti-digoxigenin-rhodamine (Boehringer); (2) anti-sera rabbit anti-sheep (Dako, Glostrup, Denmark); and (3) sheep anti-rabbit-rho-

Table 1. YAC probes.

YAC (STS)	Probe localization			
	YAC contig.	Cytogenetic[a]	Genetic[b] (cM)	RH (cR)[c]
750c7 (WI-9972, AFMA052YE5)	WC3.14	3q11.2	116	396-404
908e7 (WI-3277, WI-4028)	WC3.14	3q11.1-3q11.2		412-427
904d1 (D3S1271, WI-2277)	WC3.14	3q12.1	118	420-426
824a12 (CHLC.GATA11F06.724)	WC3.14	3q12.1		429.57
928a7 (D3S1291, AFMA281WA5)	WC3.16	3q12.3-3q13.11	122	
880e1 (D3S3638, WI-5881)	WC3.16	3q13.11		483.44
854h3 (WI-5968, CHLC.GATA84B12)	WC3.16	3q13.11		481-483
939a3 (D3S1616)	WC3.16	3q13.11	125	
948c2 (AFM211XF4P, CHLC.GATA112D03)	WC3.16			476-485
960d1 (D3S2436, D3S1572)	WC3.16	3q13.13	129	488-490
857e12 (D3S1215, D3S1572)	WC3.16	3q13.13	129	490.48
752h12 (D3S3675, D3S1610)	WC3.16	3q13.13		499.82
960f11 (D3S1310, WI-5821)	WC3.16	3q13.2-3q13.31	132	524-527
898g4 (WI-5821, WI-3547)	WC3.16	3q13.31		
882g2 (D3S1303, D3S3703)	WC3.16	3q13.31-3q13.32	138	
803d10 (D3S3515, D3S3664)	WC3.16	3q13.32	138-140	
937c5 (WI-6862, WI-824)	WC3.16	3q13.32		
951f5 (D3S3573, D3S1551)	WC3.16	3q21.1	142	542.80
895e10 (D3S1269, D3S3552)	WC3.16	3q21.1-3q21.2	141	
827d3 (D3S3646, D3S3573)	WC3.16	3q21.1	141-142	
924g9 (WI-9328, D3S2370)	WC3.16	3q21.1		
858b8 (D3S1273, D3S3684)	WC3.18	3q21.3-3q22.1	153	615.79
794h4 (AFMA048ZH9, D3S3684)	WC3.18	3q22.1	153	616-619
961d7 (D3S13.1, D3S1309)	WC3.18	3q23		587.18

[a]*Data from The Ensembl Human Genome Center: http://www.ensembl.org/Homo sapiens.* [b]*Data from Genethon: http://www.genethon.fr/* [c]*Data from the Whitehead Institute/MIT Center: http://www-genome.wi.mit.edu/. YAC probes are part of the Whitehead contig.*

damine (Boehringer) antibodies. To facilitate the identification of the chromosome partner involved in the translocations with the chromosome 3, centromeric-specific probes for chromosomes 6, 7, 8, 13/21, 15 and 17 (Vysis Inc., Downers Grove, IL, USA) were co-hybridized with the YAC probes. In some cases, because of the large number of YAC probes used, additional FISH experiments were successively performed on the same slides, as previously described[13] with minor modifications. In brief, between 2 hybridization experiments, slides were immersed in PBS to remove the coverslip. Then, slides were washed at room temperature in a new PBS bath for 5 minutes and transferred into 4× SSC, 2× SSC and 0.5× SSC at 37°C, for 3 minutes each. After sequential ethanol dehydration (70, 80 and 100%) for 2 minutes each at room temperature, slides were immersed overnight in 100% ethanol at 4°C before starting another hybridization experiment. Cells and chromosomes were counterstained with DAPI (0.1 μg/mL) in an antifade solution. Digital images were captured using a cooled CCD camera (Photometrics, Tucson, AZ, USA) mounted on an epifluorescence axioskop microscope (Zeiss, Oberkochen, Germany) equipped with selective filters for fluorescein, rhodamine and DAPI, and run by Leica QFISH software (Leica Microsystems Imaging Solutions Ltd, Cambridge, UK). Results of the FISH experiments are described according to ISCN (1995).[10]

Results

Morphologic data and immunologic analysis

The morphologic data and the results of the immunologic analysis are described in Table 2. According to the WHO classification, thirteen cases were diagnosed as splenic MZBL and in one case, the diagnosis of nodal MZBL was established (case #6). MZBL is described as a usually CD5⁻, CD23⁻, CD10⁻ and CD43⁻ proliferation and although no case displayed positivity for all antigens, isolated CD5 and CD23 expression was found in 5 (cases #1, 5, 11, 12 and 14) and in 1 (case #2) patients, respectively. Among the CD5 positive patients, one case displayed positivity for CD23 (case #5) whereas no case showed positivity for CD43.

Cytogenetic analysis

Cytogenetic data of the 14 patients are shown in Table 3. In all cases, additional material of the long arm of chromosome 3 was found to be involved in different unbalanced translocations leading to the presence of a derivative chromosome. In thirteen cases, the third copy of chromosome 3q was involved in a rearrangement involving the chromosomal regions 1q32 (case #3), 6q21 (cases #9), 6p21 (case #13), 7q31 (cases #1 and 6), 7q34-35 (case #7), 8p21 (case #10), 11q24 (case #4), 13p11 (case #2), 15p11 (case #14), 17q25 (case #8), 19q13 (case #5) and 21q22 (case #11). In the remaining case (case #12), two distinct derivative chromosomes were observed in the same clone, involving the chromosomal regions 14p11 and 21p11-12 and leading to a tetrasomy 3q. None of the derivative chromosome identified in this series resulted in rearrangements involving the immunoglobulin (Ig) loci on 14q32, 2p12 or 22q11. Over-represented regions of long arm of chromosome 3 were defined as 3q11-q29 (case #2), 3q12-q29 (case #1), 3q13-q29 (cases #4, 5, 7 and 11) and 3q21-q29 (cases #3, 6, 8, 9, 10, 12, 13, 14).

In 12 cases partial trisomy 3q occurred in the stemline while in the remaining cases (cases #2 and 8) the rearrangement was identified in an additional derivative subclone. It was found as the sole chromosomal abnormality in three cases (#7, 9 and 13) and was part of a complex karyotype in 11 of the 14 cases.

The most common associated abnormality was complete or partial trisomy 18 which occurred in 4 cases (cases #2, 3, 5 and 10). Regarding the additional structural aberrations, 4 patients (cases #1, 2, 6 and 7) presented rearrangements involving the long arm of chromosome 7, between the 7q31 to 7q34 regions, and leading to a chromosome 7q deletion. Among these latter cases, 3 (cases #1, 6 and 7) resulted from unbalanced translocations involving the chromosomal 3q12 and 3q13-21 regions. Three patients presented a t(11;14)(q13;q32) rearrangement (cases #11, 12 and 14), one patient displayed a t(9;14)(p13;q32) translocation (case #1) and one patient revealed a

Table 2. Morphologic and immunologic data of the 14 patients.

Case	Sample	Diagnosis (WHO classification)	Immunologic analysis				
			CD3	CD5	CD10	CD23	CD43
1	PB	Splenic MZBL	–	+	–	–	–
2	PB	Splenic MZBL	–	–	–	+	–
3	BM	Splenic MZBL	–	–	–	–	–
4	PB	Splenic MZBL	–	–	–	–	–
5	S	Splenic MZBL	–	+	–	+	–
6	LN	Nodal MZBCL	–	–	–	–	–
7	BM	Splenic MZBL	–	–	–	–	–
8	PB	Splenic MZBL	–	–	–	–	–
9	PB	Splenic MZBL	–	–	–	–	–
10	PB	Splenic MZBL	–	–	–	–	–
11	PB	Splenic MZBL	–	+	–	–	–
12	S	Splenic MZBL	–	+	–	–	–
13	S	Splenic MZBL	–	–	–	–	–
14	PB	Splenic MZBL	–	+	–	–	–

PB: peripheral blood; BM: bone marrow ; LN: lymph node; S: spleen; SMZL: splenic marginal zone lymphoma; NMZL: nodal marginal zone; +: positive;-: negative.

Table 3. Cytogenetic and fluorescence in situ hybridization data.

Case	Sample	Karyotype	FISH
1	PB	46,XX, del(6)(q14q25), der(7)t(3;7)(q12;q31), add(9)(p22-23), del(11)(q24) [5] / 46, idem, t(9;14)(p13;q32) [8] / 46,XX [7].	der(7)t(3;7)(q11.2;q31).ish der(7)t(3;7)(D3Z1-,D7Z1+,750c7+,tel3q+)
2	PB	47,XY, der(1)del(1)(p33)add(1)(q41), del(7)(q34), add(8)(p21), -16, +18, +mar [1] /47, idem, der(13)t(3;13)(q11;p11) [11] / 46,XY [88].	der(13)t(3;13)(q11.2;p11).ish der(13)t(3;13)(WCP3+, D3Z1-,D13Z1/D21Z1+, 750c7+,tel3q+)
3	BM	43-44,XY, der(1)t(?;1;3;2;?)(?::1p35-->cen-->1q32::3q21-->3qter::2 porq::?), -2, -4, t(6;8) (p22;q21), -8, add(9)(q33-34), -13, ?der(13)t(1;2;?13) (1porq::2porq::?13p11-->cen-->13qter).ish ?der(13) t(1;2;?13), -14, add(17) (p11), der(18)t(18;?)(q23;?) dup(18) (q11q22-23), +der(?)t(2;?)(p or q;?), + mar [6] / 43-44,idem, +der(22)t(22;?)(p11;?)del(22) (q12) [3] / 46,XY [9].	der(1)t(?;1;3;2;?)(?::1p35-->cen-->1q32::3q11.2-->3qter::2 porq::?).ish der(1)t(?;1;3;2;?)(WCP1+, WCP2+,WCP3+, D3Z1-,750c7+,tel3q+)
4	PB	46,XX, t(X;10)(q21;q21), der(11)t(3;11)(q13;q24), del(13)(q12-13), i(17)(q10) [5] / 46,XX [16].	der(11)t(3;11)(q12.3-q13.11;q24).ish der(11)t(3;11)(WCP3+,WCP11+, D3Z1-, 824a12-, 928a7+,tel3q+)
5	S	48,XY, +18, +der(19)t(3;19)(q13;q13) [17] / 46,XY[3].	+der(19)t(3;19)(q13.11;q13).ish der(19)t(3;19)(WCP19+,D3Z1-,880e1-, 854h3+, tel3q+)
6	LN	46,XY, del(1)(q43), der(7)t(3;7)(q13-21;q31) [18] / 46,XY [4].	der(7)t(3;7)(q13.1;q31).ish der(7)t(3;7)(WCP3q+,D3Z1-,D7Z1+,854h3-, 948c2+,tel3q+)
7	BM	46,XX, der(7)t(3;7)(q13;q34-35).ish der(7)t(3;7)([7] / 46, XX [12].	der(7)t(3;7)(q13.1;q34-35).ish der(7)t(3;7)(WCP3q+,WCP7+,D3Z1-,854h3-, 948c2+ tel3q+,)
8	PB	46,XY, i(17)(q10), der(?22)t(X;?22) (porq;?p11) [7] / 46, idem, -i(17)(q10), +der(17)i(17)(q10)t(3;17)(q21;q25) [6] / 46,idem, -i(17)(q10),+der(17) i(17)(q10)t(17;?)(q25;?) [2] / 46,XY [2].	+der(17)i(17)(q10)t(3;17)(q13.1;q25).ish der(17)i(17)(q10)t(3;17)(WCP3+, WCP17+,D3Z1-,854h3-,948c2+,tel3q+)
9	PB	46,XX, der(6)t(3;6)(q21;q21) [9] / 46,XX [11].	der(6)t(3;6)(q13.13;q21).ish der(6)t(3;6)(D3Z1-,D6Z1+,948c2-, 960d1+, tel3q+).
10	PB	49,XY, +der(8)t(3;8)(q21;p23), +18, +mar [2] / 50, idem, +Y [3] / 49, idem, t(14;19)(q32;q13.1) [10] / 46,XY [5].	+der(8)t(3;8)(q13.13;p21).ish der(8)t(3;8)(WCP3+,D3Z1-,D8Z1+,948c2-, 960d1+,tel3q+), +mar.ish der(3)(D3Z1+).
11	PB	46,XY, t(1;10)(q32;p14), del(6)(q23q24), i(8)(q10), 13ps+, t(11;14)(q13;q32), der(21)t(3;21)(q13;q22) [11] / 46,XY [11].	der(21)t(3;21)(q13.2-q13.31;q22).ish der(21)t(3;21)(WCP3+,D3Z1-, D13Z1/D21Z1+,752h12-, 960f11+,tel3q+).
12	S	45,X, -X, der(1)(1pter-->cen-->1q11::Xp10-->cen-->Xq11::1q12-->1q41:: 8q24.2-->8qter), add(6)(q23), der(8)t(X;8)(porq;q23), der(14)t(3;14)(q21;p11)t(11;14)(q13;q32), der(21)t(3;21)(q21;p11-12) [15] / 46,XX [7].	der(14)t(3;14)(q13.32;p11)t(11;14)(q13.2;q32).ish der(14)t(3;14)t(11;14) (WCP3q+, WCP11+,WCP14+,D3Z1-,882g2-,803d10+,937c5+,tel3q+), der(21)t(3;21) (q13.32;p11-12).ish der(21)t(3;21)(WCP3q+,D3Z1-, D13Z1/D21Z1+,21882g2-, 803d10-,937c5+,tel3q+).
13	S	46,XX, der(6)t(3;6)(q21;p25) [8] / 46,XX [4].	der(6)t(3;6)(q13.32;p21).ish der(6)t(3;6)(D3Z1-,D6Z1+,803d10-,937c5+, tel3q+).
14	PB	46,XX, t(11;14)(q13;q32), der(15)t(3;15) (q21;p11) [9] / 46,XX [11].	der(15)t(3;15) (q13.32;p11).ish der(15)t(3;15)(WCP3+, D3Z1-,D15Z+, 803d10-,937c5+,tel3q+)

+ presence of the hybridization signal; — absence of the hybridization signal.

t(14;19) (q32;q13.1) aberration (case #10). In those two later cases, PAX5/IgH and BCL3 rearrangements were not studied.

FISH studies

FISH results are shown in Table 3 and Figure 1.

In all cases, FISH experiments using the chromosome 3 specific paint, the chromosome 3q specific subpaint and the subtelomeric probe 3q confirmed the presence of an unbalanced translocation leading to a gain of the long arm of chromosome 3. In none of the cases was the hybridization signal from the chromosome 3 α-satellite probe (D3Z1) found to be present on the derivative chromosome with the 3q gain.

The panel of YACs distributed along 3q11-3q23 was especially useful in refining and correcting the cytogenetic description of the chromosomal break-

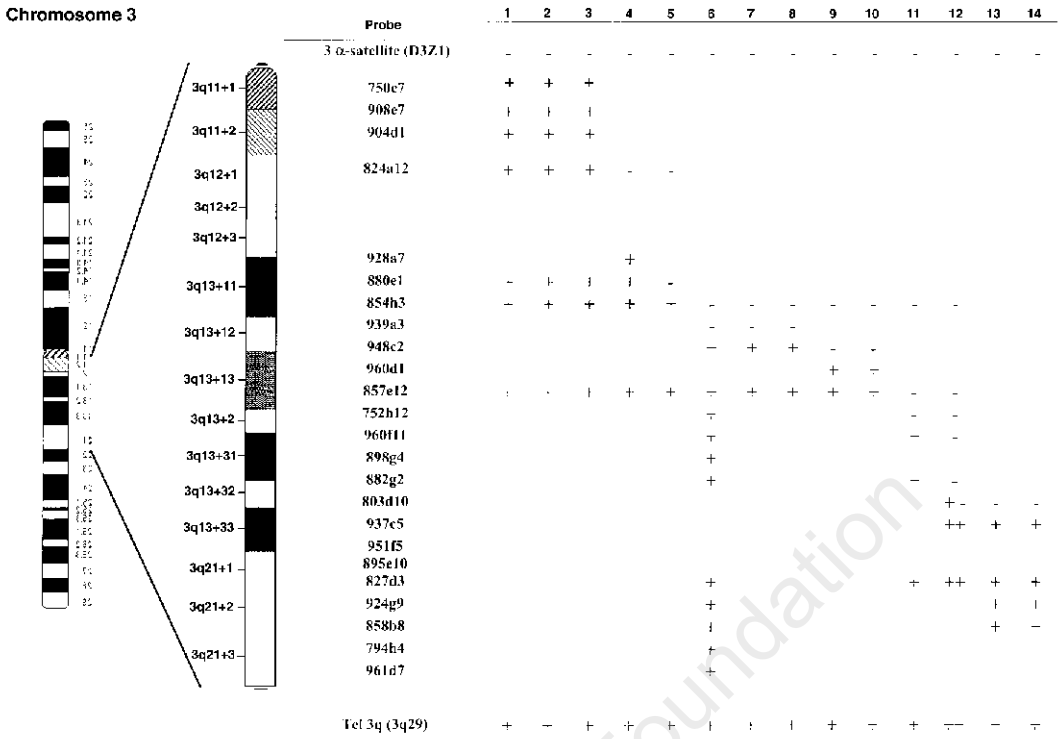

Figure 1. Mapping of duplications involving chromosome 3q in 14 patients with MZBCL by FISH analysis. +: signal present on gained 3q ;-: signal not present on gained 3q.

points on chromosome 3q. In cases #1 to 3, the hybridization signal from YAC 750c7 (mapped to band 3q11.2) was present on the gained chromosome 3q and the overrepresented region was found to be more centromeric (3q11.2) than assumed by banding analysis. In these cases, gains represented almost the entire long arm of chromosome 3. In cases #4 to 14, the duplicated regions could be delineated between bands 3q13 to 3q29. The overrepresented regions started with YAC probe 928a7 in case #4; 854h3 in case #5; 948c2 in cases #6, 7 and 8; 960d1 in cases #9 and 10; 960f11 in case #11 and 937c5 in cases #13 and 14. In case #12, two duplicated regions of the long arm of chromosome 3 were observed. The first one, including a translocation with chromosome 14, was delineated by YAC probe 803d10 whereas the second one, involving chromosome 21, started with probe 937c5. All the previously mentioned YACs were defined as the most proximal probes involved in the duplications while probe tel 3q determined the most distal one.

As a result, eight different breakpoints could be identified in the chromosome 3q unbalanced translocations and the delineation of a commonly duplicated region on chromosome 3 could be assigned proximally to the 3q13.32 region (YAC 937c5) and distally to the 3q29 region (Tel 3q probe) (Figure 2).

Discussion

This study presents cytogenetic and FISH data from a series of 14 patients with MZBCL cytogenetically characterized by partial trisomy 3. Although whole or partial trisomy 3 represents the most frequent chromosomal abnormality in MZBCL,[14-16] few data have so far been reported on the consensus regions of gains on chromosome 3.[7,8] In the present study, 14 distinct unbalanced rearrangements were found to result in partial trisomy 3, which consistently included the long arm of chromosome 3 or part of it. By conventional analysis, eight different breakpoints were identified on chromosome 3q, between the q11 and the q23 regions. A similar variation in the cytogenetic description of trisomy 3q, even within the same disease entity is also apparent from the literature.[4,14,17] These differences may reflect not only a variety of underlying chromosomal abnormalities

but may, at least in part, be due to different methods and quality of chromosomal banding leading to difficulties in the precise identification of the derivative chromosome and the breakpoint localization. Using a panel of YAC spanning the 3q11.2-3q23 region, chromosomal breakpoints were confirmed in 3 cases (cases #2, 6 and 11), whereas 10 cases were located by FISH more centromeric than assumed by cytogenetic analysis (cases #1, 3, 4, 7, 8, 9, 10, 12, 13 and 14). In one case (case #5), FISH experiments showed that the breakpoint was more distal than supposed from the conventional analysis. A total of eight different breakpoints were identified on chromosome 3q between the q11.1 and the q13.32 regions, allowing the delineation of a commonly overrepresented region between bands 3q13.32 to 3q29.

Using comparative genomic hybridization (CGH) technology, two recent studies focusing on the prevalence of trisomy 3q in MZBCL have demonstrated that the relevant regions of overrepresentation could be narrowed to 3q21-23, 3q25-29 and 3q23-25 regions.[7,8] Using a different methodological approach, we confirmed the previously reported studies, and, through this combined cytogenetic and FISH analysis, we were also able to demonstrate that multiple chromosomal breakpoints occurred on chromosome 3q and that various chromosome partners could be involved in the rearrangements leading to trisomy 3q. Such variability in the breakpoint localization has, to the best of our knowledge, not been reported before.

When considering the partner chromosomes identified in the unbalanced rearrangements, 3 cases involved chromosome 7. Interestingly, the 7q31 (cases #1 and 6) and the 7q34-35 (case #7) regions were involved and as a result, all three cases led to the loss of a part of the long arm of chromosome 7. Chromosome 7q deletions have already been reported as recurrent chromosomal abnormalities in MZBCL and particularly in patients with a spleen enlargement.[18-20] However the coexistence of both trisomy 3q and chromosome 7q deletions in MZBCL seems to be still rare in literature and some reports have hypothesized that two different pathways could contribute to the pathogenesis of MZBCL, one related to a 7q deletion and the other related to the chromosome 3q amplification.[20] However, in the present study, three cases of t(3;7) translocation were identified and one patient (case #2) presented a chromosome 7q deletion as well as trisomy 3q. This suggests that the coexistence of both abnormalities is not such a rare event in MZBCL and may deserve particular attention.

Although the t(11;14) translocation is considered characteristic of mantle cell lymphoma (MCL), this abnormality has been reported in multiple myeloma, plasma cell leukemia, B-prolymphocytic leukemia and B-chronic lymphocytic leukemia.

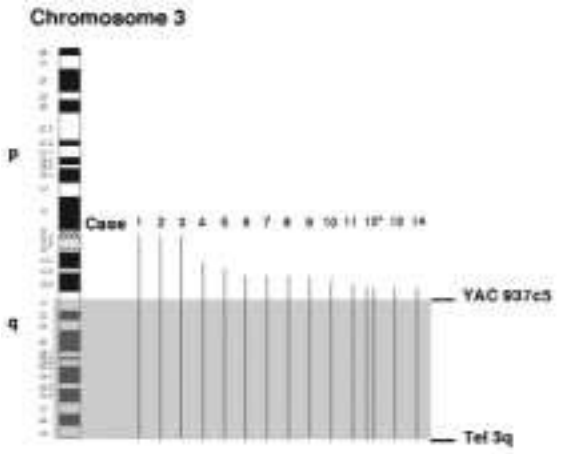

Figure 2. Delineation of the commonly duplicated region of chromosome 3 in 14 cases of MZBCL characterized by partial trisomy or tetrasomy* 3. The vertical lines indicate the over represented chromosomal region assessed by cytogenetic analysis and FISH investigations. The commonly duplicated region appears in gray and is delineated by YAC probe 937c5 (3q13.32) proximally and by the subtelomeric probe 3q (3q29) distally.

Moreover, cases histologically and cytologically classified as MZBL but exhibiting the t(11;14) translocation have been described.[18,21,22] However, when the t(11;14) is observed in MZBL, the possibility of a misdiagnosis must be checked closely and the immunologic profile of the tumor cells is an important diagnostic feature aiding differentaiation from other small B-cell lymphomas and particularly MCL. It is noteworthy that atypical immunophenotypes with $CD5^+$ or $CD23^+$ or $CD43^+$ expression have also been reported in MZBCL and rare *borderline* cases do exist.[23-25] In the present series, although the three t(11;14) cases displayed an isolated positive CD5 expression, none presented typical histomorphologic features observed in either MCL or lymphocytic lymphomas.

With regard to the possible biological significance of additional 3q material, a gene dosage effect can be suspected. Commonly overrepresented regions may include genes which are of particular importance in neoplastic transformation or in disease progression in MZBCL characterized by complete or partial trisomy 3. On the other hand, as a local consequence of the chromosome 3q rearrangements, expression of gene(s) may be altered. Thus, this could also constitute another mechanism relevant to pathogenesis.

In NHL, recurrent chromosomal aberrations have led to the identification of some important genes that play a role in lymphomagenesis, such as reciprocal chromosomal translocations involving the

immunoglobulin loci and resulting in dysregulated expression of oncogenes.[26] However, in the present study, none of the identified unbalanced translocations juxtaposed the chromosome 3q regions with the heavy and/or light κ and λ immunoglobulin gene loci, localized in the 14q32, 2p12 and 22q11 regions, respectively. Moreover, the identification of 8 different breakpoints dispersed along the long arm of chromosome 3 argues against the hypothesis of deregulation of a candidate gene located on this 3q region. Several genes have been mapped to the 3q region, including the gene coding for the surface antigen B7 (3q13.3-3q21), which can enhance CD28-mediated T-cell interactions and increase the production of various lymphokines, particularly interleukin 2 (Il-2);[27] the *PBX-2* (pre B-cell leukemia transcription factor 2) homebox gene (3q22-23), which corresponds to a transcriptional factor of the lymphocytic cell line (an homologous gene (*PBX-1*) is implicated in the t(1;19)(q23;p13) translocation in acute pre-B-cell lymphoid leukemia;[28] the gene coding for a putative subunit of interleukin 12;[29] the gene coding for TRAIL (TNF-related apoptosis inducing ligand) (3q26), a protein of the tumor necrosis factor (TNF) gene family;[30] the *hTR* (human telomerase RNA) gene (3q26.3), overexpressed in most solid tumors,[31] the *BCL6/LAZ 3* proto-oncogene (3q27-29),[32] frequently rearranged in diffuse large B-cell lymphomas (DLBCL) arising at extra-nodal sites,[33] and more recently, the *BAL* (B-aggressive lymphoma) gene (3q21), also described in DLBCL which promotes malignant B-cell migration.[34] However, the role of all these genes in the pathogenesis of MZBCL remains to be determined.

The genetic mechanisms by which additional whole or parts of chromosome may contribute to malignant transformation is less understood. This may be explained by the difficulty in analyzing gene expression in such huge chromosomal regions. Thus, the hypothesis of a gene dosage effect is currently proposed to explain the neoplastic transformation. The biological significance of the presence of additional chromosomal material has been recently demonstrated in acute myeloid leukemia (AML), in a study comparing the expression profiling of AML with isolated trisomy 8 versus AML without cytogenetic abnormalities. Fundamental biological differences were observed and clearly demonstrated an increased overexpression of genes localized on the additional chromosome.[35] As in AML with trisomy 8, the hypothesis of a gene dosage effect for genes localized on chromosome 3q could be suggested in MZBCL. Thus, better knowledge of transcriptional genes products and their effects would probably help better understanding of the significance of the chromosome 3q gains in MZBCL.

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Pre-publication Report & Outcomes of Peer Review

Contributions

SG was responsible for the conception of the study, its design and the recruitment of and contact with the participants. SG and ECB were responsible for drafting the manuscript. SG, CP and ECB were in charge of cytogenetic data. SG performed the FISH analyses. PF, DM, LB, and FB were reponsible for the morphologic, histologic and immunologic studies. BC and GS were involved in collection of the clinical data. LC and GS gave their critical contribution to the manuscript and GS and ECB approved its final version. Primary responsibility for the paper: ECB; Table 1: SG; Table 2: SG, PF, LB; Table 3: SG, ECB; Figures 1 and 2: SG, ECB.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Professor Cazzola and the Editors. Manuscript received September 24, 2002; accepted November 18, 2002. In the following paragraphs, the Editor-in-Chief summarizes the peer-review process and its outcomes.

What is already known on this topic

Whole or partial trisomy 3 represents the most recurrent chromosomal abnormality occurring in marginal zone B-cell lymphoma.

What this study adds

A common 3q13.32-3q29 chromosomal region was found to be overexpressed in 14 patients with marginal zone B-cell lymphoma, suggesting that a gene dosage effect rather than a specific gene disruption may be involved in the development of this disease.