original paper

haematologica 2001; 86:64-70
http://www.haematologica.it/2001_01/0064.htm

Molecular cytogenetic characterization of marginal zone B-cell lymphoma: correlation with clinicopathologic findings in 14 cases

ANTONIO CUNEO, RENATO BIGONI, MARIA GRAZIA ROBERTI, RAFFAELLA MILANI, PAOLA AGOSTINI, FRANCESCO CAVAZZINI, CLAUDIA MINOTTO, CRISTIANO DE ANGELI, ANTONELLA BARDI, ELISA TAMMISO, MASSIMO NEGRINI,° PIERLUIGI CAVAZZINI,* GIANLUIGI CASTOLDI

Dipartimento di Scienze Biomediche e Terapie Avanzate, Sezione di Ematologia and Dipartimento di Medicina Sperimentale e Diagnostica; *Sezione di Anatomia Patologica, University of Ferrara, Italy; °Thomas Jefferson University, Kimmel Cancer Institute, Philadelphia, USA

Correspondence: Antonio Cuneo, M.D., Dipartimento di Scienze Biomediche, Sezione di Ematologia, Università di Ferrara, via Savonarola 9, 44100 Ferrara, Italy. Phone: international +39.0532.236978 Fax: international +39.0532.212142 – E-mail:sse@dns.unife.it

Background and Objectives. To improve the definition of the incidence and significance of chromosome lesions occurring in marginal zone B-cell lymphoma (MZBCL).

Design and methods. Fourteen cases of MZBCL diagnosed according to the REAL classification were studied by conventional chromosome analysis (CCA) and by interphase fluorescence *in situ* hybridization (FISH) using the following probes: 3q27/BCL6, 6q21, 7q31, 9p21/p16, 11q22/ATM, 13q14, 17p13, centromeres of #3, #7, #12. Pertinent clinical data were collected.

Results. Primary disease presentation consisted of histologically documented splenic MZBCL in 9 cases, nodal MZBCL in 3 cases and extra-nodal MZBCL in 2 cases. Four cases showed evolution into a high-grade lymphoma, due to the presence of a predominant large cell or blast cell component. Clonal karyotype anomalies were detected by CCA in 12 cases, 6 of which had a complex karyotype, including all 4 cases with high-grade histology. Interphase FISH confirmed cytogenetic data and revealed several cryptic chromosomal lesions. Overall, total/partial +12 was found in five cases; 13q14 and 17p13 deletion were found in four cases each; +3, 7q31 deletion and a BCL6 split signal were found in three cases; deletions at 6q21 and 11q22.3 in two cases each; +7 and a 9p21 deletion were found in one case each.

Interpretation and Conclusions. i) Besides +3 and 7q-, 13q14 deletion, total/partial +12, BCL6 rearrangement, and deletions at 6q21, 11q22-23, and 17p13.3 are relatively frequent events in MZBCL; ii) unlike in mantle cell lymphoma, 9p21 deletion occurred unfrequently in MZBCL; iii) a switch into high grade histology is usually associated with complex chromosome defects, including 6q-, 11q-, +12, and 17p.

Key words: non-Hodgkin's lymphoma; marginal zone B-cell lymphoma; chromosome deletions; BCL6 rearrangement

Marginal zone B-cell lymphoma (MZBCL) includes a primary extra-nodal form, namely lymphoma of the mucosa-associated lymphoid tissue (MALT), a nodal form, corresponding to monocytoid B-cell lymphoma[1] and a primary splenic form, with or without peripheral blood lymphocytosis, referred to as splenic marginal zone B-cell lymphoma, presenting overlapping features with splenic lymphoma with villous lymphocytes.[2] These three clinicopathologic forms share similar histologic and immunologic features,[3] they usually run a relatively indolent clinical course, though a switch to high grade lymphomas may occur.[4] Recent evidence was provided that, with the exception of MALT lymphomas carrying the t(11;18)(q21;q21) or the t(1;14) (p21;q32),[5-7] MZBCL may display a similar pattern of recurrent chromosomal lesions, irrespective of the primary site of involvement.[8] Recurring aberrations at disease presentation include total or partial trisomies of chromosomes 3, 18 and X in approximately 50%, 30% and 20% of the cases, structural aberrations of chromosome 1q21 and 1p34 in 20-30% of the cases, and partial or total deletions of chromosomes 17p and 9p in 10-20% of the cases.[8-11]

Fluorescence *in situ* hybridization (FISH) allows detection of cryptic chromosomal lesions, especially deletions, in a substantial fraction of lymphoid neoplasias. For instance, the incidence of cytogenetic anomalies of chromosomes 13q, 17p and 12 in non-Hodgkin's lymphoma (NHL) was previously thought to be relatively low, whereas it turned out to approach 20% in some studies using FISH in interphase nuclei,[12-14] with the 13q14 deletion reaching a surprisingly high incidence in some histologic subsets.[15-17] Because limited information is presently available on the cytogenetic profile of MZBCL, we elected to study 14 patients by conventional chromosome analysis (CCA) and FISH using a large panel of locus-specific probes. Results of this molecular-cytogenetic study are presented and discussed in correlation with the salient clinicopathologic features.

Design and Methods

Patients

Fourteen consecutive patients seen at our Institution over a ten-year period were included in this study. Inclusion criteria were the following: i) successful cytogenetic analysis on representative samples, ii) availability of a cytogenetic pellet for interphase FISH studies and, iii) histologically confirmed diagnosis of MZBCL according to the REAL classification.[3] Immunohistochemical studies were performed in 13 out of 14 cases (case no. 8 could not be studied because no material was available) using commercially available monoclonal antibodies detecting the following antigens: CD5, CD20, CD79a, CD10, BCL1, BCL2, CD103. Those cases with splenic lymphoma with villous lymphocytes lacking histologic diagnosis on a spleen specimen were excluded. The patients included in this report do not represent the whole population of MZBCL seen at our center, because it is our policy not to perform cytogenetic analysis on extra-nodal material to ensure accuracy of histologic diagnosis. Staging procedures included physical examination, a routine laboratory profile, a bone biopsy, a chest X-ray film, abdominal ultrasonography, CT scan and, when indicated, gastroduodenal endoscopy. Peripheral blood (PB) involvement was studied by light microscopy examination of smears stained by the May-Grünwald-Giemsa method and by immunophenotyping using the following panel of commercially available monoclonal antibodies: anti CD2, CD3, CD5, CD19, CD22, CD23, CD10, CD11c, CD25 and FMC7. Double labeling with anti CD5/CD19 was performed and the expression of surface immunoglobulins (sIg) was studied using rabbit antihuman antibodies against the Ig heavy and light chains as previously reported.[16]

Clinical parameters were surveyed for all cases. Complete remission was defined as the resolution of all pathologic symptoms attributable to the lymphoma for at least 3 months after the completion of chemotherapy.

Conventional chromosome analysis (CCA)

Cytogenetic investigations were performed at diagnosis or before therapy was given, using lymph node samples in 3 cases, spleen samples in 2 cases and PB/BM samples in 8 cases. In one case (no. 1) cytogenetic studies were performed on occasion of a lymph node relapse of gastric MALT lymphoma, treated by gastrectomy plus splenectomy and multiagent chemotherapy (COP regimen, consisting of cyclophosphamide, vincristine and prednisone). Methods for preparation of single cell suspensions from spleen or lymph node samples have been previously described.[18] These single cell suspensions and PB/BM samples were separated over a 1,077 density gradient and then cultured for 24-72 hours. The 72-hour cultures were stimulated by the following mitogens: phorbol myristate acetate (50 ng/mL) and lipopolysaccharide from *E. coli* (100 mg/mL). Whenever possible, 20 metaphases were studied and karyotypes described according to the ISCN (1995).

Interphase cytogenetics

Probe selection. We elected to use DNA probes targeting those chromosome bands found to be deleted and/or rearranged in at least two cases in our series plus a panel of probes recognizing chromosome regions which were previously shown to be involved in a significant fraction of B-cell NHL. Hence, probes recognizing DNA sequences at the following chromosome bands were used: the C21 cosmid, recognizing DNA sequences between the Rb gene and the D13S25 marker at 13q14;[16] two lambda EMBL3 clones, spanning an area of approximately 40 kb within the ATM gene at 11q22.3;[19] a 6q21 probe prepared by B. Schlegelberger (*Institute for Human Genetics, Kiel, Germany*) as previously described,[20] a commercially available 7q31 probe (Vysis); commercially available centromeric probes recognizing chromosomes 3, 7 and 12 (Oncor).

The following probes were also used which were made available by F. Birg (*Institut de Cancérologie e d'Immunologie de Marseille, INSERM 119, Marseille, France*) in the context of the Biomed I program, "E.U. concerted action for cytogenetic diagnosis of hematologic malignancies" (project leader: A. Hagemeijer, *Centre for Human Genetic, K.U.L., Leuven, Belgium*): a 17p13.3 cosmid recognizing p53 gene sequences isolated by H. Dohner (*Ruprecht-Karls-Universitat, Medizinische Klinik und Poliklinik V, Heidelberg Germany*); a 3q27 BCL6 probe prepared by Iwona Wlodarska (*Centre for Human Genetics, K.U.L., Leuven, Belgium*); a cosmid probe located at 9p21 band, recognizing p16 sequences, isolated by Berna Beverloo (*Centre for Human Genetics, Erasmus University, Rotterdam, The Netherlands*).

Hybridization conditions. FISH was performed using the same samples that had been submitted to cytogenetic analysis. Conditions for slide pre-treatment, probe preparation, hybridization, signal amplification and visualization, image capturing and screening have been described previously.[16] Signal screening was performed on at least 200 interphase cells with well-delineated signals. Dual color hybridization using the test probe and an irrelevant control probe was performed to rule out inefficient hybridization, as previously reported.[16] All probes were biotinylated, or digoxigenin-labeled and tested in 5 normal control samples: <1% cells showed 3 signals (false trisomy or false BCL6 rearrangement) and <3% cells showed 1 signal (false deletion) with each probe. The cut-off point for the recognition of trisomy or BCL6 rearrangement was set at >3% cells with three signals; >10% cells with 1 signal was a minimum requirement for a case to be classified as affected by deletion. To assess the efficiency of the BCL6 probe, one case with large cell lymphoma and a t(3;14)(q27;q32) was tested, producing three signals (one deriving from the normal BCL6 allele, two from the split allele) in 65% of the interphase cells.

Results

Hematologic features and outcome

Clinicopathologic and hematologic features in our patients are outlined in Table 1. All cases had the following immunohistologic profile on representative biopsy samples: CD5$^-$; CD10$^-$; CD79a$^+$; DBA44$^-$; CD20$^+$; BCL1$^-$, BCL2$^-$. Primary disease presentation was as follows: nine patients had splenic MZBCL, three had nodal MZBCL, 2 had a gastric MALT lymphoma. In most patients there was multi-organ involvement at presentation or at disease evolution: these data are detailed in Table 1, along with the outcome of induction therapy. PB involvement was detected in 10 cases and detailed immunologic findings on PB fresh cells in these patients are summarized in Table 2.

A predominent large and/or blast-like cell component suggestive of transformation into a high-grade NHL was observed in 4 patients: one (#14) had a primary gastric presentation with lymph node and spleen involvement, one (#13) suffered from nodal relapse of gastric MALT, one had splenic MZBCL (#6) and one had disseminated nodal and extra-nodal disease (#10).

Thirteen patients are alive after 3-120 months; one patient (#7) had progressive disease despite intensive treatment and died 84 months after diagnosis.

Cytogenetics and FISH

Results are detailed in Table 3. Three patients had a normal karyotype at diagnosis. Six out of twelve patients with clonal aberrations had a complex karyotype (i.e. more than 5 anomalies), including all 4 cases with a high-grade component. Recurrent chromosomal anomalies in the stemline were: structural anomalies of 7q and 3q anomalies (3 cases), trisomy 3, 6q21 anomalies and a 14q+ chromosome, in 2 cases each. As outlined in Table 3, interphase FISH confirmed karyotypic findings and showed 1-4 anomalies in all cases tested but two (cases #4 and 11): by combining karyotypes and FISH data, total/partial trisomy 12q was found in five cases, 13q14 and 17p13 deletion were found in four cases each, +3, 7q31 deletion and a BCL6 split signal in three cases, deletions at 6q21 and 11q22.3 in two cases each, +7 and a 9p21 deletion were found in one case each.

Discussion

The identification of secondary chromosome changes in specific forms of NHL permitted the identification of chromosome regions containing pathogenetically relevant genes/loci[21,22] and allowed stratification of patients for risk assessment.[14,23,24]

The recently recognized entity of MZBCL encompasses a spectrum of clinicopathologic manifestations, corresponding to three distinct forms,[3] having in common several biological features. It was noteworthy that clinical presentation and disease history in our patients (Table 1) were clearly indicative of a significant degree of overlapping between each entity (extra-nodal, nodal and splenic). Though further study is required to establish definitely that these forms all derive from margin-

Table 1. Hematologic and clinical features in 14 patients with MZBCL.

Patient	*Age/sex/stage diagnosis*	*Sites involved at diagnosis*	*Disease history*
1. B.R.I.	65 / m / IV splenic MZBCL	PB, BM,* S*	2 CHOP→persistent marrow involvement (50%) → chlorambucil + steroid (6 months) → limited marrow involvement (25%), survival 29+ months
2. B.G.	66 / m / IV splenic MZBCL	stomach* PB, BM*, spleen,* LN	gastrectomy + splenectomy → persistent disease →6 CEOP→CR → DFS 18 months → 1st relapse (BM) → chlorambucil + steroid 2 cycles PR, survival 93+ months
3. C.S.	56 / m / IV splenic MZBCL	PB, BM,* S,* LN,* gallbladder*	α–interferon PR → chlorambucil + steroid , PR→ splenectomy + 4 CNOP CR/survival 101+months
4. G.A.M.	69 / f / IV splenic MZBCL	PB, BM,* S	wait and watch, survival 57+ months
5. D.M.	65 / f : IV splenic MZBCL	S,* PB	splenectomy PR, chlorambucil → PR; duodenal involvement after 3 yrs, survival 37+ months
6. M.V. °	71 / f / IVB splenic MZBCL		PB, BM,* S,* splenectomy + 6 CNOP→CR , survival 36+ months
7. P.E.	73 / m / IV splenic MZL	PB, BM,* S,* lung, skin*	chlorambucil + steroid, PR→ fludarabine + steroid (6 cycles) PR→2 COP + 3 CNOP→PR →carboplatin+ steroid → splenectomy → vepeside + mitoxantrone + ara-c → persistent disease dead at 84 months
8. P.F.	75 / m / IV splenic MZBCL	PB, BM,* S*	splenectomy + COP, PR→ 6CHOP CR→RFS 8 yrs→ 1st relapse→6 CNOP→CR →survival 120+ months
9. T.A.	58 / m / IV splenic MZL	PB, BM,* S*	splenectomy→CR → survival 24+ months
10. P.M. °	34 / f / IV nodal MZBCL	stomach,* LN,* S,* ovary,* esophagus*	splenectomy + gastrectomy + salpingo-ovariectomy → MACOP-B→auto BMT → radiotherapy→CR, survival 24+ months
11. B.V.	77 / m / IV nodal MZBCL	PB, BM,* LN ,* S	watch and wait, survival 3+ months
12 6. M. L.	66 / m / IV nodal MZBCL	BM,* S, LN*	2 COP, survival 4+ months
13. B.L.°	61 / f / III gastric MALT lymphoma in Sjögren's syndrome	stomach,* S,* LN,* liver*	gastrectomy+splenectomy+ 6 COP→CR relapse after 2 yrs with LN involvement→6CHOP→CR, survival 48+ months
14. Gu.A.°	65 / f / II gastric MALT with LN involvement	stomach,* LN,* spleen*	gastrectomy + splenectomy + 6 CHOP→CR→survival 18+ months

**Histologically documented. Abbreviations: PR: partial remission; CR: complete remission; DFS: disease-free survival; S: spleen; LN: lymph node. °presence of a predominant large cell/blast cell component, consistent with evolution of MZBCL into high grade lymphoma.*

Table 2. Immunophenotype in 9 patients with PB involvement.*

Case	WBC (% lymphs)	CD19	CD5 and CD19	CD22	CD23	CD11c	CD25	FMC7	sIg (+/++)
1 BRI	5.6 (42%)	27%	–	23%	–	27%	–	20%	+
2. BG	58.8 (75%)	ND	–	78%	77%	71%	ND	–	++
3. CS	18.1 (61%)	ND	43%	57%	–	51%	52%	50%	++
4. GAM	18.1 (84%)	81%	94%	81%	–	–	77%	34%	++
5. DM	5.3 (24%)	31%	–	33%	–	–	–	–	+
6. MV	18.2 (39.9%)	63%	–	64%	56%	83%	65%	53%	++
7. PE	25.6 (71%)	80%	–	87%	85%	–	–	90%	+
8. PF	21.1 (26%)	26%	24%	27%	26%	6%	20%	–	+
9. TA	4.5 (48%)	12%	11%	13%	–	6%	7%	–	++
11. BV		21%	–	22%	ND	22%	–	20%	++

**Percentage of positive cells in the lymphocyte gate; — indicates <5% positive cells; (sIg:+ low intensity of fluorescence; ++ bright expression).*

al-zone lymphocytes,[25] the idea of their identical origin was reinforced by the observation that a similar pattern of chromosomal rearrangements is found in these neoplasias, irrespective of the primary site of involvement.[9,26,27]

The diagnosis of MZBCL in our series was supported by histologic and immunologic findings. All cases tested were CD5$^-$, CD10$^-$, CD79a$^+$ on biopsy specimen. In 10 cases with a monoclonal population of lymphoid cells in the PB, immunophenotyping showed a profile of reactivity (see Table 2) consistent with splenic lymphoma with circulating villous lymphocytes,[28] the leukemic counterpart of SMZBCL.[2] However, 4 cases were found with CD5$^+$ cells in the PB despite 3 of them being CD5$^-$ at immunohistochemical analysis (patient no. 7 could not be tested). This discrepant finding may be accounted for by the different sensitivity of immunohistochemistry on biopsy samples and immunophenotyping on fresh cells obtained from the PB. The notion that approximately 20% of splenic lymphoma with villous lymphocytes may express the CD5 antigen[28] supports this argument.

Using the sensitive interphase FISH technique, we were able to refine the profile of recurrent chromosome lesions marking the development of this lymphoma, showing that a similar pattern of chromosomal rearrangement may be found irrespective of the primary site of disease (spleen, lymph node). The association of trisomy 3 and 7q- with MZBCL, found in previous studies,[8,9] was confirmed by our analysis (see Table 3). Recently, attention was drawn to the possible role of 9p21/p16 deletions in B-NHL, especially mantle cell lymphoma (MCL), in which p16 deletion was associated with a high proliferation index by neoplastic cells.[29] We found one case carrying 9p21 deletion involving the p16 gene. This is consistent with the data by Dierlamm *et al.*,[26] who found that only 1 out of 25 cases of MZBCL investigated by comparative genomic hybridization carried a 9p21 deletion: more cases with a prevailing large/blast cell component need to be studied to rule out definitely a role for this gene in tumor progression of lymphomas of marginal-zone lineage.

FISH studies confirmed cytogenetic data in all cases; in addition they demonstrated the occurrence of BCL6 rearrangement in three patients carrying a 3q structural aberration. BCL6 involvement occurs in large cell lymphoma[30] and, less frequently, in follicle center cell lymphomas,[31] in chronic lymphocytic leukemia[32] and MZBCL.[33] It is noteworthy that two of our three patients with BCL6 involvement had a predominant large cell component and the other featured a relatively aggressive disease. In a previous study, a high frequency of BCL6 rearrangement was noted in primary high-grade gastric lymphomas[34] and disseminated disease with a prevailing blast-like component was described in 2/3 BCL6-rearranged cases in another study.[33]

In this analysis, cryptic chromosome anomalies were seen by FISH that were not recognized by CCA due to mitoses occurring in residual normal lymphocytes (two cases with normal karyotype) or due to the inadequacy of banding resolution (four cases with chromosome anomalies). The most frequent chromosome lesions were total/partial trisomy 12 and 13q14 deletion, found in 5 and 4 cases respectively, including two cases carrying both anomalies. The finding of 13q14 deletion in MZBCL is not surprising, since 13q14 deletion is known to occur at an incidence of approximately 50% in B-CLLs, small lymphocytic lymphomas and MCL[17,35] and at an incidence of 10% in other low-grade and high-grade lymphomas, in which it was predictive of a lower complete remission rate.[13] Likewise, the identifiction of extra chromosome-12 material as a recurrent anomaly in MZBCL is in line with previous studies that this anomaly was associated with evolutive forms of low grade NHL, including B-CLL, MCL and follicle center cell lymphoma.[14,36,37] It is noteworthy that 3 out of 4 patients with a large cell component in this series had total/partial +12: this finding is in keeping with a recent study using FISH on paraffin sections of gastric MALT lymphomas, where +12 was associated with histologic switch to aggressive disease.[38]

Three previously unreported chromosome lesions in

Table 3. Cytogenetic and interphase cytogenetic findings in 14 patients with MZBCL.

Patient	*Sample*	*Karyotype [No. abnormal / total]*	*Anomalies by FISH **
1. B.R.I.	S	46,XY [20]	7q-(45%); +12(40%)
2. B. G.	PB	46, XY [15]	+3(78%)
3. C.S.	PB	46, XY, del(13)(q22q32) [6/12]	+12(35%); 13q-(70%)
4. G.A.	PB	46, XX, t(11;14)(q13;q32)[13/13]	No lesion
5. D.M.	PB	46,XX, del(7)(q22q32) / 46, idem, t(11;14)(p11;q32) [12/20]	7q-(86%)
6 M. V. (°)	S	48, XX, dup(1)(q21q32), +3, -10, +der(12)t (12;?)(p13;?), +mar [18/20]	+3(65%);+7(50%);+12(55%)
7 P. E.	PB	45-46, XY, add(1)(q32), add(3)(q27), -10, add(14)(q32), add(18)(q22), +21 +mar [cp16] [16/21]	BCL6 split(70%); 11q-(60%); 13q-(80%); 17p-(70%)
8 P. F.	PB	47, XY, +3 [2/20]	+3 (20%)
9 T. A.	PB	46, XY [20]	13q-(72%)
10 P. M. (°)	LN	47-51,XX,der(1)t(1;10)(p22;q22), +del(1)(p11), del(4)(q27), del(6) (p13), add(6)(q21), +12, add (14)(q32), add(16) (p13), +21, + 2 mar [cp15] [15/22]	6q-(70%); 11q-(77%); +12(50%); 13q-(68%); 17p-(60%)
11 B. V.	LN+PB	46,XY, del(14)(q22)[5/29]	No lesion
12 M L	BM	45-46,XY,der(1)t(1;2)(q43;q14), del (2)(p14), dic(4;6)(p16;q27), -6, add(7)(q31), del(9)(p13), add(11)(q25), add(15)(p12), del(17)(p11) [cp7] [7/22]	17p-(44%); 9p-(41%)
13 B.L. (°)	LN	49-51, XX, add (3)(q27), add (3) (q27), +5, del (6)(q15q21), +del (7) (q22), -12, +19, +20, +2 mar [cp11] [11/20]	BCL6 split(75%); 6q-(60%); 7q-(50%);
14 Gu.A. (°)	LN	45-48,XX,del(2)(q33), t(3;14)(q27;q32), +5, -8, dup(12)(q13q22), del(17)(p11), add(22)(p12) [cp12] [12/18]	BCL6 split (75%); 17p-(67%)

*P.B: peripheral blood; B.M: bone marrow; S: spleen; N: node/nodal. *The 10-probe panel (see the Design and Methods section) was tested in all patients: the presence of each anomaly in interphase cells is indicated (% abnormal cells in parentheses); no indication means absence of the anomaly. °Patient with high-grade histology.*

MZBCL were found in this series which may be associated with evolving disease and/or with high-grade histology: deletions involving 17p13 (four cases) and/or 6q21, 11q22 (two cases each). In all cases a complex karyotype with many chromosomal rearrangements was observed and it is difficult to sort out the relative contribution of each of these anomalies to the observed clinicopathologic features. In this regard the following observations may be pertinent: a) 17p13 deletion involving the p53 gene is a marker of histologic transformation and clinically aggressive disease in several histologic subsets of NHL;[20,39] therefore a similar role for this anomaly in MZBCL would not be unexpected; b) there is a growing body of evidence linking 11q22-23 deletions involving the ATM locus and B-cell lymphomagenesis;[40-42] a recent study indicated an association of deletion at the ATM locus, karyotype complexity and severe outcome in indolent as well as in aggressive lymphomas;[43] c) 6q21 deletions occur frequently in other low-grade NHL,[21] and they have been associated with a large cell component in small lymphocytic lymphoma.[44]

In conclusion, we used conventional cytogenetics and a large panel of locus-specific probes to characterize MZBCL, showing that a) a similar pattern of chromosome lesions may occur irrespective of primary disease presentation, b) besides +3 and 7q$^-$, 13q14 deletion, total/partial +12, BCL6 rearrangement, and deletions at 6q21, 11q22-23, and 17p13.3 are relatively frequent events in this clinicopathologic entity; c) unlike in MCL, 9p21 deletion occurs unfrequently in MZBCL; d) a switch into high grade histology may be associated with complex chromosomal defects, including BCL6 rearrangement, 6q$^-$, 11q$^-$, +12, and 17p$^-$.

Contributions and Acknowledgments

GLC and AC were responsible for the design of the study and wrote the paper. RB, RM, FC, MN and CD performed the FISH studies; MGR, AB, PA and CM performed the cytogenetic studies, PC performed the histologic studies. All authors reviewed the manuscript for important intellectual content. The authors thank Professor Brigitte Schlegelberger, Institut fur Humangenetik, University of Kiel, Germany, for preparing the 6q21 probe.

Funding

This paper was supported by MURST ex 40% 1999 and 60%, by Biomed I CT 94-1703, by NCI grant 1-PO1-CA76259-01A.

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 Dr. Paolo G. Gobbi, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Dr. Gobbi and the Editors. Manuscript received June 1, 2000; accepted November 22, 2000.

Potential implications for clinical practice

The definition of the cytogenetic profile of MZBCL may be important for more accurate risk assessment.

References

1. Sheibani K, Burke JS, Swartz WG, Nademanee A, Winberg C. Monocytoid B-cell lymphoma. Clinicopatholgic study of 21 cases of a unique type of low-grade lymphoma. Cancer 1988; 62:1531-8.
2. Isaacson PG, Matutes E, Burke M, Catovsky D. The histopathology of splenic lymphoma with villous lymphocytes. Blood 1994; 84:3828-34.
3. Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms: a proposal From the International Lymphoma Study Group. Blood 1994; 84:1361-2.
4. Zucca E, Roggero E, Pileri A. B-cell lymphoma of MALT type: a review with special emphasis on diagnostic and management problems of low-grade gastric tumors. Br J Haematol 1998; 100:3-14
5. Ott G, Katzenberger T, Greiner A, et al. The t(11;18) (q21;q21) chromosome translocation is a frequent and specific aberration in low grade, but not high-grade, malignant non Hodgkin's lymphomas of the mucosa-associated lymphoid tissue (MALT) type. Cancer Res 1997; 57:3944-7.
6. Dierlamm J, Baens M, Wlodarska I, et al. The apoptosis inhibitor gene API2 and a novel 18q gene, MLT, are recurrently rearranged in the t(11;18)(q21;q21) associated with MALT lymphomas. Blood 1999; 93:3601-9.
7. Willis TG, Jadayel DM, Du MQ, et al. BCL10 is involved in t(1;14)(p22;q32) of MALT B-cell lymphoma and mutated in multiple tumor types. Cell 1999; 96:35-45.
8. Dierlamm J, Pittaluga S, Wlodarska I, et al. Marginal zone B-cell lymphomas of different sites share similar cytogenetic and morphologic features. Blood 1996; 87:299-307.
9. Wotherspoon AC, Finn TM, Isaacson PG. Trisomy 3 in low-grade B-cell lymphomas of mucosa-associated lymphoid tissue. Blood 1995; 85:2000-4.
10. Sole F, Woessner S, Florensa L, et al. Frequent involvement of chromosomes 1, 3, 7 and 8 in splenic marginal zone B-cell lymphoma. Br J Haematol 1997; 98:446-9.
11. Baldini L, Fracchiolla NS, Cro LM, et al. Frequent p53 gene involvement in splenic B-cell leukemia/lymphomas of possible marginal zone origin. Blood 1994; 84:270-8.
12. Clodi K, Younes A, Goodacre, et al. Analysis of p53 gene deletions in patients with non-Hodgkin's lymphoma by dual-colour fluorescence in-situ hybridation. Br J Haematol 1997; 98:913-21.
13. Cuneo A, Bigoni R, Rigolin GM, et al. 13q14 deletion in non-Hodgkin's lymphoma: correlation with clinicopathological features. Haematologica 1999; 84:589-93.
14. Cuneo A, Bigoni R, Rigolin GM, et al. Cytogenetic profile of lymphoma of follicle mantle lineage: correlation with clinicobiological features. Blood 1999; 93:1372-80.
15. Bullrich F, Veronese ML, Kitada S, et al. Minimal region of loss at 13q14 in B-CLL. Blood 1996; 88:3109-15.
16. Cuneo A, Bigoni R, Negrini M, et al. Cytogenetic and interphase cytogenetic characterization of atypical chronic lymphocytic leukemia carrying BCL1 translocation. Cancer Res 1997; 57:1144-50.
17. Stilgenbauer S, Nickolenko J, Wilhelm J, et al. Expressed sequences as candidates for a novel tumor suppressor gene at band 13q14 in B-cell chronic lymphocytic leukemia and mantle cell lymphoma. Oncogene 1998; 16:1891-6.
18. Leroux D, Le Marc' hardour F, Gressin R, et al. Non Hodgkin's lymphomas with t(11;14) (q13;q32): a subset of mantle zone/intermediate lymphocytic lymphoma. Br J Haematol 1991; 77:346-53.
19. Rasio D, Negrini M, Croce CM. Genomic organization of the ATM locus involved in ataxia–telangiectasia. Cancer Res 1995; 55:6053-7.
20. Zhang Y, Matthiesen P, Harder S, et al. A 3-cM commonly deleted region in 6q21 in leukemias and lymphomas delineated by fluorescence in situ hybridization. Genes Chromosomes Cancer 2000; 27:52-8.
21. Lo Coco F, Gaidano G, Louie DC, Offitt KA, Chaganti RSK, Dalla Favera R. p53 mutations are associated with histologic transformation of follicular lymphoma. Blood 1993; 82:2289-95.
22. Gaidano G, Hauptschein RS, Parsa NZ, et al. Deletions involving two distinct regions of 6q in B-cell non-Hodgkin's lymphoma. Blood 1994; 80:1781-7.
23. Offitt K, Wong G, Philippa DA, Tao Y, Chaganti RSK. Cytogenetic analysis of 434 consecutively ascertained specimens of non-Hodgkin's lymphoma: clinical correlations. Blood 1991; 77:1508-15.
24. Tilly H, Rossi A, Stamatoullas A, et al. Prognostic value of chromosomal abnormalities in follicular lymphoma. Blood 1994; 84:1043-9.
25. Isaacson PG. Splenic marginal zone lymphoma. Blood 1996; 88:751-2.
26. Dierlamm J, Rosenberg C, Stul M, et al. Characteristic pattern of chromosomal gains and losses in marginal zone B cell lymphoma detected by comparative genomic hybridization. Leukemia 1997;11:747-8.
27. Gruszka-Westwood AM, Matutes E, Coignet JA, Wotherspoon A, Catovsky D. The incidence of trisomy 3 in splenic lymphoma with villous lymphocytes: a study by FISH. Br J Haematol 1999; 104:600-4.
28. Matutes E, Owusu-Amkomah K, Morilla R, et al. The immunological profile of B-cell disorders and proposal of a scoring system for the diagnosis of CLL. Leukemia 1994; 10:1640-5.
29. Dreyling MH, Bullinger L, Ott G, et al. Alterations of the cyclin D1/p16-pRB pathway in mantle cell lymphoma. Cancer Res 1997; 57:4608-14.
30. Offitt K, Lo Coco F, Louie DC, et al. Rearrangement of BCL-6 gene as a prognostic marker in diffuse large-cell lymphoma. N Engl J Med 1994; 331:74-80.
31. Chaganti SR, Chen W, Para N, et al. Involvement of BCL6 in chromosomal aberrations affecting band 3q27 in B-cell non-Hodgkin lymphoma. Genes Chromosomes Cancer 1999; 23:323-7.
32. Horsman DE, McNeil BK, Anderson M, Shenkier T, Gascoyne RD. Frequent association of t(3;14) or variant with other lymphoma-specific translocations. Br J Haematol 1995; 89:569-75.
33. Dierlamm J, Pittaluga S, Stul M, et al. BCL6 gene rearrangements also occur in marginal zone B-cell lymphoma. Br J Haematol 1997; 98:719-25.
34. Gaidano G, Capello D, Gloghini A, et al. Frequent mutation of bcl-6 proto-oncogene in high grade, but not low grade, MALT lymphomas of the gastrointestinal tract. Haematologica; 1999; 84:582-8.
35. Corcoran MM, Rasool O, Liu Y, et al. Detailed molecular delineation of 13q14.3 loss in B-cell chronic lymphocytic leukemia. Blood 1998; 91:1382-90.
36. Juliusson G, Oscier DG, Fitchett M, et al. Prognostic subgroups in B-cell chronic lymphocytic leukemia defined

by specific chromosomal abnormalities. N Engl J Med 1990; 323:720-4.
37. Que TH, Garcia Marco J, Ellis J, et al. Trisomy 12 in chronic lymphocytic leukemia detected by fluorescence in situ hybridization: analysis by stage, immunophenotype, and morphology. Blood 1993; 82:571-5.
38. Hoeve MA, Gisbertz IAM, Schuuring E, et. Gastric low grade lymphoma, high grade MALT lymphoma and diffuse large B cell lymphoma show different frequencies of trisomy. Leukemia 1999;13:799-807.
39. Doehner H, Fisher K, Bentz M, et al. p53 gene deletion predicts for poor survival and non-response to therapy with purine analogs in chronic B-cell leukemias. Blood 1995; 85:1580-9.
40. Vandenberghe E, De Wolf-Peeters C, Wlodarska I, et al. Chromosome 11q rearrangements in B non Hodgkin's lymphoma. Br J Haematol 1992; 81:212-7.
41. Stankovic T, Weber P, Stewart G, et al. Inactivation of ataxia telangiectasia mutated gene in B-cell chronic lymphocytic leukemia. Lancet 1999; 353:26-9.
42. Dohner H, Stilgenbauer S, James MR, et al. 11q deletions define a new subset of B-cell chronic lymphocytic leukemia characterized by extensive nodal involvement and inferior prognosis. Blood 1997; 89:2516-22.
43. Cuneo A, Bigoni R, Rigolin GM, et al. Acquired deletion of the ataxia teleangiectasia (ATM) locus in B-cell non-Hodgkin's lymphoma: correlation with clinicobiologic features. J Clin Oncol 18:2607-14.
44. Offitt K, Gaidano G, Filippa DA, et al. 6q deletions define distinct clinico-pathologic subsets of non-Hodgkin's lymphoma. Blood 1994; 82:2157-62.