Novel Genomic Imbalances in B-Cell Splenic Marginal Zone Lymphomas Revealed by Comparative Genomic Hybridization and Cytogenetics

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Splenic marginal zone lymphoma (SMZL) has recently been recognized in the World Health Organization classification of hematological diseases as distinct type of non-Hodgkin's lymphoma. In contrast to the well-established chromosomal changes associated with other B-cell non-Hodgkin's lymphoma, few genetic alterations have been found associated with SMZL. The aim of our study was to analyze by comparative genomic hybridization (CGH) the chromosomal imbalances in 29 patients with SMZL and to correlate these findings with clinical and biological characteristics and patient outcome. In 21 cases, cytogenetic studies were simultaneously performed. Most of the patients (83%) displayed genomic imbalances. A total of 111 DNA copy number changes were detected with a median of four abnormalities per case (range, 1 to 12). Gains (*n* = 92) were more frequent than losses (*n* = 16), while three high-level amplifications (3q26-q29, 5p11-p15, and 17q22-q25) were observed. The most frequent gains involved 3q (31%), 5q (28%), 12q and 20q (24% each), 9q (21%), and 4q (17%). Losses were observed in 7q (14%) and 17p (10%). SMZL patients with genetic losses had a shorter survival than the remaining SMZL patients (*P* < 0.05). In summary, chromosomal imbalances in regions 3q, 4q, 5q, 7q, 9q, 12q, and 20q have been detected by CGH in SMZL. Patients with SMZL displaying genetic losses by CGH had a short survival. (*Am J Pathol 2001, 158:1843–1850*)

Splenic marginal zone lymphoma (SMZL) has been considered a subtype of B-cell non-Hodgkin's lymphoma (NHL) in the recent classification of hematological diseases.[1] SMZL has been recognized as a separate entity on the basis of its morphological, phenotypic, and clinical characteristics.[2] Several studies have been performed to assess the genetic abnormalities in marginal lymphomas. Recently, two translocations, t(1;14)(p22;q32) involving *BCL10*/IgH[3] and t(11;18)(q21;q21) *-API2/MLT-*[4] have been described, associated with high-grade and low-grade MALT lymphomas, respectively. However, genetic information of SMZL is scanty. Cytogenetic analyses have shown involvement of chromosomes 1, 3, 7, and 8.[5,6] Fluorescence *in situ* hybridization studies on interphase nuclei have demonstrated the presence of trisomy 3 in a proportion of cases ranging from 18 to 47% of SMZL.[6–9] In previous studies we have reported that in some SMZL cases, the only cytogenetic abnormality displayed was del(7q) that could suggest that del(7q) is associated with SMZL.[6,10] Recently, loss of heterozygosity studies demonstrated that the frequency of allelic loss in SMZL (40%) is higher than that observed in other B-cell lymphoproliferative syndromes. The most frequently deleted microsatellite was D7S487.[11] These results are in accordance with our fluorescence *in situ* hybridization studies that mapped the commonly deleted region in chronic B-cell lymphoproliferative disorders at 7q31.3.[12]

Comparative genomic hybridization (CGH) is a double-color hybridization procedure that provides, in a single experiment, a general view of genomic imbalances, including partial or complete trisomies, monosomies, or amplifications within the tumor genome.[13] This technique can be used to identify previously unexpected genetic abnormalities. Several groups have studied chromosomal changes by CGH in different subtypes of B-cell

NHL.[14–16] Thus, in marginal zone lymphomas, gains on chromosomes 3 and 18 are frequent.[17] In extranodal lymphomas such as gastrointestinal lymphomas, total or partial gains of chromosomes 11, 12, and 1q are frequently observed.[18] However, little is known about the genomic imbalances in SMZL.

The present study was designed to screen DNA copy changes by CGH in a series of 29 SMZL and to correlate the results obtained from CGH with the most relevant clinical, biological, and cytogenetic characteristics, including disease outcome.

Materials and Methods

Patients

Tumor specimens from 29 patients with SMZL were included in the study. All cases were classified according to the criteria proposed by Isaacson and colleagues[19] and Mollejo and colleagues.[2] All samples were studied at diagnosis and were reviewed and classified by three of the authors (MM, TF, and MAP).

Cytogenetics

Cytogenetic studies were available in 21 patients. Most chromosome analyses were performed on the spleen (11 cases), whereas in six cases studies were performed on peripheral blood, in three cases on the lymph nodes, and in the remaining case, on the bone marrow. Cytogenetic analysis of the splenic tissue was performed after 24 and 48 hours of culture without stimulating agent, and 48 hours culture stimulated with phorbol 12-myristate 13-acetate. Peripheral blood and bone marrow cells were cultured for 3 days in presence of phorbol 12-myristate 13-acetate. Metaphases were G-banded and karyotypes were described according to the International System of Human Cytogenetic Nomenclature.[20] At least 20 mitoses were analyzed in each patient.

CGH

Tumor DNA was isolated from the spleen (25 cases), peripheral blood (2 cases), or bone marrow (2 cases). Reference DNA was obtained from peripheral blood lymphocytes of healthy donors (same sex as patients). The phenol-chloroform method was used for DNA extraction according to standard procedure.[21] CGH analysis was performed according to the method described by Lichter and Ried[22] Briefly, tumor DNA (test DNA) was labeled with biotin-16-dUTP (Boehringer Mannheim, Mannheim, Germany) and normal DNA (reference DNA) was labeled with digoxigenin-11-dUTP (Boehringer Mannheim) by a standard nick translation reaction. The size of the nick-translated fragments ranged from 300 to 1,000 bp. Equal amounts (1 μg) of labeled tumor and normal DNAs, and 70 μg of unlabeled human Cot-1 DNA (Life Technologies, Inc., Gaithersburg, MD) were co-hybridized to slides with human metaphase chromosome spreads prepared from phytohemagglutinin-stimulated lymphocytes from normal

individuals. After hybridization for 1 to 2 days in a moist chamber at 37°C, posthybridization washes were performed to a stringency of 0.1× standard saline citrate at 42°C. Tumor and normal DNA were detected by avidin-fluorescein isothiocyanate and rhodamine-conjugated anti-digoxigenin, respectively. The slides were counterstained with 4,6-diamidino-2-phenylindole and mounted with an antifade solution. Image acquisition was performed with an epifluorescence microscope (Olympus BX60) equipped with a cooled charge-coupled device camera. Calculation of the tumor DNA to normal DNA fluorescent ratios along the length of each chromosome was performed by means of an automated CGH software package (Cytovision; Applied Imaging, Sunderland, UK). Ratio values obtained from at least 10 metaphase cells for each case were averaged. Ratio values >1.25 and <0.75 were considered to represent chromosomal gain and loss, respectively. Overrepresentation was defined as high-level amplification when the profile exceeded the cut-off value of 1.5. Chromosomal gains exceeding 1.5 involving the whole chromosome or large areas of a chromosomal arm were not considered as high-level DNA amplification. Negative control experiments were performed using differentially labeled male *versus* male and female *versus* female DNA. Additional control experiments included the interchange of the digoxigenin-dUTP and biotin-dUTP labels between normal and tumor DNA. According to previous recommendations, we measured the average number of copy alterations (ANCA) index.[23] This index represents the average number of copy alterations in a tumor type and is calculated by dividing the total number of copy alterations by the number of tumors analyzed.

Results

Patients

The most relevant clinical data from the 29 patients is shown in Table 1. No sex predominance was observed (male to female,14 to 15) and the median age was 64 years (range, 39 to 79 years). According to the Ann-Arbor classification, all patients presented with advanced stage because of bone marrow infiltration. However, only 20% of patients displayed high levels of serum lactic dehydrogenase (normal value, 460 IU/L). In 43% of cases, a lymph node involvement was present. Lymphocytosis was present in 55% of patients. All but two patients (cases 26 and 27) received a splenectomy and in 10 cases, chemotherapy was administrated. Overall survival of the whole series was 72 months (95% CI, 44 to 100 months). At the time of the study, 18 patients remained alive (Table 1).

Cytogenetics

In 16 of the 21 patients (76%) in which cytogenetic studies were available, an abnormal karyotype was present. Most cases were pseudodiploid. In two cases (cases 6 and 25) the modal number of chromosomes was more

Table 1. Clinical Characteristics and Genomic Imbalances in 29 Patients with Splenic Marginal Zone Lymphoma (SMZL)

Case	Reference	Age/sex	LDH (IU/L)	Organ involvement	Treatment	Follow-up (months)	Chromosomal changes		
							Losses	Gains	Amplifications
1	P1	67/F	499	Spleen, BM, PB	Splenectomy	53+	1	10	1
2	P2	62/M	935	Spleen, BM	Splenectomy	59+	0	0	0
3	P5	72/F	503	Spleen, LN, BM	Splenectomy, CT	72	0	5	0
4	P10	56/M	350	Spleen, BM, PB	Splenectomy, CT	77+	0	0	0
5	P12	65/F	350	Spleen, BM, PB	Splenectomy, CT	53+	0	5	0
6	P13	58/M	350	Spleen, BM, PB	Splenectomy, CT	17	2	0	0
7	P33	61/M	688	Spleen, LN, BM, PB	Splenectomy	42+	0	5	0
8	P74	63/M	350	Spleen, LN, BM, PB	Splenectomy, CT	57	2	0	0
9	P149	66/F	703	Spleen, BM, PB	Splenectomy, CT	29	0	0	0
10	P162	53/M	368	Spleen, BM, Liver	Splenectomy, CT	38+	0	4	0
11	P202	72/F	398	Spleen, BM, PB	Splenectomy	36+	2	5	0
12	P206	69/F	401	Spleen, BM, PB	Splenectomy	13+	0	7	0
13	P220	74/M	350	Spleen, LN, BM, PB	Splenectomy	3	2	0	0
14	1034	66/F	635	Spleen, LN, BM	Splenectomy	22	1	0	0
15	P222	72/M	NA	Spleen, BM	Splenectomy	36	0	7	0
16	1443	67/M	312	Spleen, LN, BM, PB	Splenectomy	2	1	8	0
17	P254	51/M	287	Spleen, BM, PB	Splenectomy, CT	6+	1	0	1
18	P256	69/F	350	Spleen, BM	Splenectomy	4	0	2	0
19	1020	70/F	444	Spleen, BM, PB	Splenectomy	34+	0	0	0
20	2184	68/M	388	Spleen, LN, BM, PB	Splenectomy	16+	0	7	0
21	2299	72/M	250	Spleen, LN, BM, PB	Splenectomy	15+	3	9	0
22	2760	60/F	397	Spleen, LN, BM, PB	Splenectomy	8+	0	2	0
23	4066	39/M	432	Spleen, BM	Splenectomy	28+	0	1	0
25	4722	45/F	415	Spleen	Splenectomy, CT	136	0	4	0
25	4340	51/F	450	Spleen, LN, BM	Splenectomy	24+	1	2	0
26	4941	55/M	428	Spleen, LN, BM	Not treatment	15+	0	3	1
27	5787	79/M	397	Spleen, BM	No treatment	26+	0	5	0
28	5388	48/F	317	Spleen, LN, BM	Splenectomy	21	0	2	0
29	1742	52/F	310	Spleen, LN, BM	Splenectomy, CT	113+	0	0	0

F, female; M, male; BM, bone marrow; PB, peripheral blood; LN, lymph node; CT, chemotherapy; NA, not available; +, patient alive at time of analysis.

than 49. Only one case (case 8) had a hypodiploid karyotype. Seven out of the 16 patients (44%) had a complex karyotype. The most frequent cytogenetic abnormality was deletion of 7q (six cases) whereas a trisomy of chromosome 3 was observed in only three cases. Trisomies of chromosomes 5 and 12 were detected in two cases, respectively. Several chromosomal breakpoints were found to be repetitively involved in SMZL patients. Thus, abnormalities involving 7q22-q33 were detected in seven cases; whereas rearrangements in 1p22, 1q21, and 5q31, were found in two patients each.

CGH

Twenty-four of the 29 patients with SMZL (83%) showed DNA sequence copy number changes and five cases displayed normal profiles. A total of 111 DNA copy number changes were detected with a median of four abnormalities per case (range, 1 to 12): 92 gains, 16 losses, and three high-level amplifications (Figure 1). All abnormal cases except cases 14, 22, and 23 showed more than one chromosomal imbalance (Table 2). In 19 patients, overrepresentations of chromosomal material were detected. Gains of chromosomal material involved 3q (31%), 5q (28%), 12q and 20q (24% each), 9q (21%), 4q (17%), and 11q and 12p (14% each). The consensus regions of gains were located at 3q23-q25, 4q25-q28, 5q13-q15, 9q31, 12q15-q21, and 20q. In 10 cases (35%), a genetic loss was present. In four of the 10 cases,

the losses of chromosomal material were the only changes detected. Losses were located in 7q (14%) with a commonly deleted region at 7q31-q32, 17p (10%), and 8p, 13q, and 15q (7% each) (Figure 1). Only three cases showed high-level amplifications located at 3q26-q29, 5p11-p15, and 17q22-q25. These amplifications were located in chromosomal regions in which known fragile
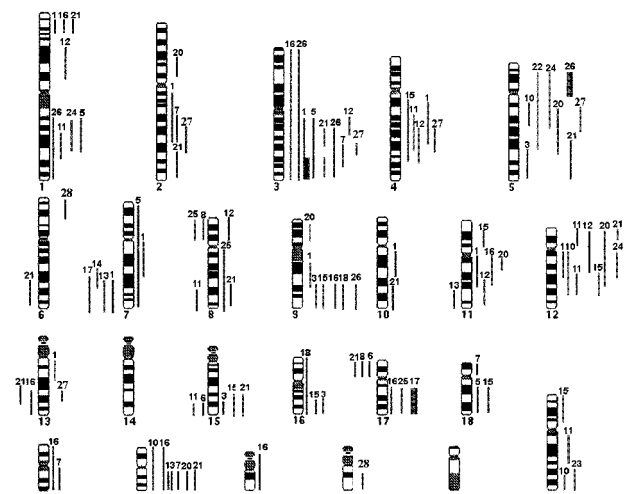

Figure 1. Summary of the genomic imbalances in 29 patients with SMZL. **Left:** Lines indicate loss of chromosomal material. **Right:** Lines indicate gain of chromosomal material. High-level DNA amplifications are represented as **solid squares**. Each line represents a gained or lost region in a single tumor. The numbers on top of each line refer to the patient analyzed.

Table 2. Results of CGH and G-Banding in Patients with SMZL

		CGH			Cytogenetics		
Case	Sample	Gains	Losses	Amplifications	Sample	Karyotype	
1	Spleen	1p34–p36,2q12–q32, 3q12–q26,4q12–q31,7q11–q21, 9q21–q22,10q21–q22,11q12– q21,12q13–q15, 13q12–q14,20q11–q13	7q31–q36	3q26–q29	LN	46,XX,t(1;5)(p11;q11),del(7) (q22q33)del(8)(q12),del(14) (q21),del(18)(q13)	[14]
2	Spleen	–	–	–	PB	46,XY	[23]
3	Spleen	5q31–q35,9q22–q34,15q24–q26, 16q21–q24,20q11–q13	–	–	–	ND	
4	Spleen	–	–	–	–	ND	
5	Spleen	1q21–q32,3q12–q29,4q12q26, 7,18q11–q21	–	–	–	ND	
6	Spleen	–	15q24–q26,17p11p13	–	LN	85–86,XXY,del(1)(q21q33),t(1;2) (p31;q22),del(3)(p12p24), add(4)(q31),del(5)(p12p15), del(6)(q21q25),del(9)(p13p23), dup(10)(q21q25),add(14)(q32), add(17)(q23),der(20)(q13)	[12]
7	Spleen	2q22–q31,3q24–q26,18p11, 19q13,20q11–q13	–	–	ND		
8	Spleen	–	8p12–p23,17p11–p13	–	Spleen	44,XY,t(1;3)(q21;q26),del(8)(q22), del(7)(q32),−7,+der(7)t(1;7) (p12;p12),−20,−21	[16]
9	Spleen	–	–	–	Spleen	46,XX,del(6)(q21q24)	[4]
						46,XX	[16]
10	Spleen	5q11–q14,12q12–q24,20,Xq27– q28	–	–	ND		
11	Spleen	1q31–q32,4q22–q28,12p11–p13, 12q21–q24,Xq13–q25	8q22q24,15q24–q26	–	Spleen	46,XX,t(2;17)(p13;q21)	[5]
						46,XX	[10]
12	Spleen	1p21–p31,3q13–q21,4q26–q31, 8p12–p22,11q22–q25,12p11– p13,12q12–q14	–	–	ND		
13	Spleen	–	7q31–q36,11q22–q25	–	Spleen	46,XY,t(1;2)(p22;q23),del(7)(q21), add(17)(p13)	[7]
						46,XY	[8]
14	Spleen	–	7q22–q32	–	Spleen	46,XX,del(7)(q21q31)	[12]
15	Spleen	9q32–q34,11p12–p15,12q22–q24, 15q22–q26,16q22–24q, 18q21–q23,Xp11–p22	–	–	ND		
16	Spleen	1p34–p36,3,9q31–q34,11q12–q21, 17q23q25,19,20,21	13q14–q31	–	Spleen	46,X,−Y,+3	[10]
						46,XY	[5]
17	Spleen	–	7q31–q36	17q22–25	Spleen	46,XY,del(7)(q31)	[9]
						46,XY	[7]
18	Spleen	9q32–q34,16	–	–	ND		
19	Spleen	–	–	–	Spleen	46,XX,add(9)(p12),add(16)(p12)	[6]
						46,XX	[5]
20	Spleen	2p12–p14,5q11–q23,10q21–q23, 11q12–q14,12p13–q24, 20q12–q13	–	–	Spleen	47,XY,+del(3)(q21q26)	[6]
						46,XY	[9]
21	Spleen	1p31–p36,2q32–q26,3q21–q25, 3q26–q29,5q23–q35,8q22–q24, 12p11–p13,15q21–q25,20q11– q13	6q21–q24 13q21–q31 17p11–p13	–	LN	47,XY,+1,add(5)(q31)	[9]
						47,XY	[8]
22	Spleen	5p15–q31	–	–	Spleen	47,XX,+del(5)(q31)	[10]
						46,XX	[4]
23	Spleen	Xq27	–	–	PB	46,XY	[23]
24	PB	1q21–q31,5p15–q21,12q12–q21	–	–	PB	46,XX,del(7)(q31q34),der(16)t(12; 16)(q13;q24)	[10]
						46,XX	[19]
25	Spleen	3q13–q29,8q11–q24,17q22–q24	8p11–p23	–	Spleen	50,XX,+5,+der(7)t(3;7)(p21;q22) x3,i(8)(q10)	[2]
						46,XX	[6]
26	PB	1q21–q43,3,9q32–q34	–	5p11–p15	PB	48,XY,del(1)(q42),+del(1)(p22), +3,+i(5)(p10),−9	[4]
						46,XY	[19]
27	BM	2q24–q32,3q25,4q24–q28,5q12– 5q21,13q22	–	–	PB	46,XX	[22]
28	Spleen	6p22–p25,22q12–q13	–	–	PB	46,XX	[18]
29	BM	–	–	–	BM	46,XX	[21]

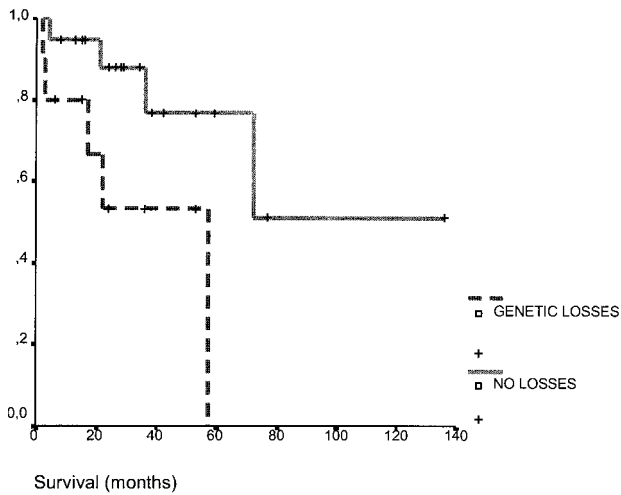PB, peripheral blood; BM, bone marrow; LN, lymph node; ND, not done.

Figure 2. Survival curves of patients with SMZL according to the presence of losses assessed by CGH (0 *versus* ≥ 1 loss; *P* = 0.02).

sites have been identified (3q26-29 and FRA3C, 5p11-p15 and FRA-5A, and 17q22-25 and FRA17B). As indicated in Material and Methods, ANCA index represents the average number of copy alterations in a tumor type. In the present study, the ANCA index was 3.8.

CGH provided more genomic imbalances than cytogenetic studies in the 21 cases analyzed with both techniques (Table 2). A correlation between both methodologies was seen in 12 cases: two patients (cases 2 and 29) without any change in both cytogenetics and CGH; two patients (cases 14 and 22) with the same results; and the remaining eight patients (cases 9, 13, 17, 19, 23, 25, 26, and 28) with minor changes. In four patients (cases 1, 8, 16, and 21) some changes observed by cytogenetics were also showed by CGH. In the remaining five cases (cases 6, 11, 20, 24, and 27) the results were different. It should be noted that in some of these cases CGH was performed in a different tissues than cytogenetics. Interestingly losses of 7q were observed in four out of the six patients with both methodologies and in two cases only by cytogenetics.

Correlation between CGH Results and Outcome

A correlation between losses and a shorter survival was found. Thus, the patients displaying a loss of chromosomal material had a survival of 36 months while the patients without losses had a longer survival (101 months, *P* = 0.02) (Figure 2). By contrast, patients with deletions assessed by conventional cytogenetics did not show differences in survival in relation with patients without deletions (76 *versus* 46 months, *P* = 0.78). Patients with SMZL and more than four changes detected by CGH did not present differences in survival from the cases of SMZL with less than four changes (60 months *versus* 80 months, respectively, *P* = 0.8).

Discussion

In the present study, we have identified chromosomal imbalances by CGH in 83% of patients with SMZL. This incidence is similar to that previously reported in the overall setting of marginal lymphomas,[17] and higher than the overall frequency reported in indolent lymphomas in which the number of changes ranged between 48 and 68%.[14,18,24,25]

Previous studies using conventional cytogenetics and fluorescence *in situ* hybridization have shown that interstitial deletion of the long arm of chromosome 7 is a recurrent cytogenetic abnormality in SMZL.[6,10,12,26] By CGH we have confirmed that partial loss of the 7q region is the most frequent loss in SMZL and the commonly deleted region is located at 7q31-q32. Recently, Corcoran and colleagues[27] found a deregulation of the *CDK6* gene that could be involved in the pathogenesis of the small group of SMZL with translocations at 7q21. However, partial losses in 7q are more frequent than translocations in SMZL. These deletions of 7q are infrequent in other NHL[14,25,28,29] including marginal zone lymphomas.[17] Moreover, studies by loss of heterozygosity have confirmed that 7q31-q32 loss is a relatively specific genetic marker of SMZL.[11] All of the cytogenetic, fluorescence *in situ* hybridization, loss of heterozygosity, and CGH data support the hypothesis that a loss in 7q31-q32 plays a key role in SMZL.

In our study, gains mostly involved 3q, 5q, 12q, and 20q. Chromosomal changes in 3q have been reported in several types of B-NHL. Half of the cases of mantle cell lymphoma have gains of 3q.[30,31] This incidence is lower in patients with diffuse large cell lymphoma (24%)[15] or extranodal lymphoma (7 to 13%).[18,25] The reported overall incidence of gains of chromosome 3 in marginal zone lymphomas, including MALT (nodal and extranodal) and SMZL, is 52% and only two of the 11 cases of SMZL studied by Dierlamm and colleagues[17] showed gains on 3q. In our series of 29 SMZL patients, the incidence was 31%. This frequency of imbalances on chromosome 3 is similar to that observed using fluorescence *in situ* hybridization.[6–9] It should be noted that, in contrast to other marginal cell lymphomas in which trisomy is the most frequent gain, using CGH we have observed that in SMZL the gain specifically occurs at the 3q arm.

In the present series, 28% of patients with SMZL had gains in chromosome 5q, with a consensus region in 5q13-q15. Gains of 5q have only been reported by CGH in NHL sporadically,[16,18,30,32] and in MALT lymphomas the incidence is 8%.[17] Only one case of diffuse large cell lymphoma of the gastrointestinal tract has been described with an amplification of 5q33-q35.[18] Moreover, by conventional cytogenetics abnormalities of 5q are also unusual in NHL and only two chromosome translocations, t(5;14)(q11;q32) and a t(5;7)(q13;q35) have been reported, in B and T NHL, respectively.[33] In 5q13, a gene involved in CDK-activating kinase complex of cyclin H *MAT1*[34] and in 5q31, a gene coding a phosphatase Cdc25 associated with regulation of cell cycle have both been recently located.[34] However, the possible relationship of these genes with SMZL still needs to be clarified. Although 5q13 abnormalities have not previously been related to SMZL, the region has been involved in hairy cell leukemia.[35,36] The demonstration in the present series of a high incidence of gains on 5q in SMZL, with a

consensus region located at 5q13-q15, suggests that this region could be related to SMZL.

Using conventional cytogenetics, trisomy 12 or duplication 12q are frequently observed as a secondary genomic change in lymphoid disorders.[37,38] By contrast, in marginal lymphomas, abnormalities of chromosome 12 have rarely been reported.[17] By CGH, numerical abnormalities of chromosome 12 have been detected in primary mediastinal B-cell lymphoma,[32] primary gastrointestinal large-cell lymphoma,[25] and chronic lymphocytic leukemia.[14] In our study, no cases with trisomy 12 were found. However we observed gains in the long arm of chromosome 12 in seven cases of SMZL with a consensus region in the bands 12q15-q21. In the long arm of chromosome 12 the region 12q24 has frequently been involved in mediastinal lymphomas[32] and in primary large B-cell lymphoma of the gastrointestinal tract,[18] whereas in mantle cell lymphomas, the consensus region was located at 12q13.[31] In the region 12q13-q21 several genes such as *GLI*, *MDM2*, and *CDK4* have been mapped. Amplification of these genes have been observed in diffuse large B-cell lymphoma, and associated with advanced state of disease.[39] Recently, our group has observed *CDK4* gene amplifications in mantle cell lymphoma.[31] However, their role in the pathogenesis of SMZL needs to be determined.

In the present study, gains of the long arm of chromosomes 9 and 20 were particularly frequent, 21 and 24%, respectively. Whereas changes observed in 9q have been reported in other lymphoma subtypes,[17,31] gains in 20q have been observed in only one case of mantle lymphoma.[30] Moreover, by conventional cytogenetics, the changes in this region are also uncommon in NHL.[40] However gains of 20q have been observed in solid tumors such as colorectal carcinoma,[41,42] breast,[43] bladder,[44] ovarian,[45] and pancreatic cancer.[46] Several candidate genes have been identified on 20q: the cellular apoptosis susceptibility (*CAS*) gene;[47] *BTAK*, a putative serine/threonine kinase gene;[48] *AIB1*, a steroid receptor co-activator gene;[49] *PTPN1*, a nonreceptor tyrosine phosphatase involved in growth regulation;[50] as well as *MYB12*, which encodes a transcription factor and plays an important role in cell cycle progression.[51]

Regarding gene amplifications, CGH studies are detecting an increased number of high-level DNA amplifications in lymphomas, which were relatively rare in NHL by cytogenetic studies.[52] These results suggest that gene amplifications may be more frequent in NHL than initially thought.[16] We found three high-level DNA amplifications in 29 SMZL involving 3q26-q29, 5p11-p15, and 17q22-q25. Amplifications of 3q26-q29 have been observed in NHL.[16,18,30,31] Abnormalities of 5p have been reported in B-cell disorders.[17,18,31,32,39] However, to the best of our knowledge, no cases of NHL with high-level amplifications of 5p have been described. Amplifications in 17q23-25 have been previously reported in one case of mantle cell lymphoma.[31] A relationship between high-level DNA amplifications and the location of chromosome fragile sites have been suggested.[53] In fact, the three amplified regions in SMZL were located in chromosomal regions where fragile sites have been identified. These data are according to the hypothesis that fragile sites may be implicated in the amplifications of certain chromosomal regions during tumor progression.[53] The ANCA index is a measure for the number of chromosomal copy alterations in a tumor. The correlation of the ANCA index with tumor progression may reflect the tumor aggressiveness.[23] The ANCA index of SMZL in the present series was 3.8 whereas this value ranged from 4.8 in the squamous cell carcinomas of the anal canal to the 8.3 in the high-grade astrocytomas.[23]

Concerning the outcome, in the present series, we have observed that the presence of genetic losses was associated with a shorter survival than in other patients without deletions (36 *versus* 101 months). Little data about the impact of CGH on the outcome of the NHL are available. Recently, a relationship between the presence of more than four abnormalities by CGH or losses on 9p have been related to a poor outcome in mantle cell lymphoma.[31] Our results suggest that in so-called indolent SMZL, two groups of patients based on CGH losses are present. Thus, patients with loss of chromosomal material in CGH showed a significantly poorer outcome as compared to patients without deletion or with few chromosomal changes, and therefore could be candidates for a more intensive therapeutic approach. Recently, Mateo and colleagues[11] associated the loss of 7q, assessed by loss of heterozygosity analysis, with a poor outcome.

In summary, the CGH data reported describes new regions related to SMZL (5q, 9q, 12q, and 20q). Although the high-level amplifications seem to be an uncommon mechanism in these lymphomas, three regions (3q26-q29, 5p11-p15, and 17q22-q25) were found to be amplified in SMZL. Moreover, our data confirmed that deletions of 7q31-q32 are closely associated with SMZL and showed that deletions in SMZL lead to a poor clinical outcome.

Acknowledgments

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