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Malignant Lymphomas

BCL-1 rearrangements and p53 mutations in atypical chronic lymphocytic leukemia with t(11;14)(q13;q32)

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ABSTRACT

Background and Objectives. **The translocation t(11;14) (q13;q32), typically described in mantle cell lymphomas (MCL), has also been found in some cases of non-MCL lymphoproliferative disorders, such as splenic lymphoma with villous lymphocytes (SLVL), multiple myeloma (MM), prolymphocytic leukemia (PLL), typical and atypical chronic lymphocytic leukemia (CLL and aCLL). In order to define better the genetic features of aCLL with t(11;14), which could represent a distinct disease subset, we looked for genetic lesions in the BCL-1 locus and in BCL-2, BCL-6, c-myc and p53 genes.**

Design and Methods. **We investigated a panel of B-lymphoproliferative disorders with translocation t(11;14)(q13;q32) including nine aCLL, six MCL and one MM. Southern and Northern blot analyses were used to investigate DNA structure and RNA expression; SSCP and direct sequencing were used to detect and characterize p53 point mutations; cytofluorimetric analysis was used to quantify p53 protein.**

Results. **Alterations of BCL-2, BCL-6 and c-myc were not detected. Conversely, BCL-1 rearrangements were present in 4 out of 7 aCLL and in 2 out of 4 MCL. A high incidence of p53 gene alterations was found, almost equivalent in aCLL and MCL.**

Interpretation and Conclusions. **Our results indicate that the occurrence of BCL-1 locus lesions in aCLL selected for t(11;14) is as high as in MCL. Interestingly, rearrangements in the mTC1 (minor translocation cluster 1) were only found in aCLL. Therefore, the two B-cell chronic lymphoproliferative disorders share similar molecular rearrangements and the t(11;14) identifies a subset of B-CLL sharing molecular features with MCL and characterized by aggressive clinical evolution.**

Key words: t(11;14), atypical CLL, mantle cell lymphoma, BCL-1, p53.

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During the last decade, the molecular characterization of the most frequent cytogenetic abnormalities associated with non-Hodgkin's lymphomas (NHL) has led to the identification of genes that are altered in these diseases and that represent specific markers with a possible diagnostic significance.[1] In some cases, the association between a given histologic subtype and these abnormalities is not as strong as initially described.[2-5] In addition, a number of non-random secondary lesions play an important role in determining the tumor phenotype and its clinical behavior,[6] the best example being 17p abnormalities leading to p53 gene loss of fucntion.[7,8]

The t(11;14)(q13;q32) is strongly associated with mantle cell lymphoma (MCL)[9,10] and causes the deregulation of the BCL-1 (synonyms D11S287E, PRAD1, CCND1) proto-oncogene, a member of the cyclin G1 gene family (cyclin D1).[11,12] Chromosomal breakpoints are widely scattered on chromosome 11q13 in an area that may span more than 200 kb. Preferential breakpoints have been identified in MCL in the major translocation cluster (MTC),[13] 120 kb centromeric to the BCL-1/CCND1 gene and, to a lesser extent, in the minor translocation cluster 1 (mTC1, 20 kb telomeric to MTC).[14] Besides MCL, the t(11;14) has been found in many types of chronic B-cell lymphoproliferative disorders, including typical and atypical chronic lymphocytic leukemia (aCLL and CLL),[15-17] prolymphocytic leukemia (PLL),[18-19] multiple myeloma (MM)[20-22] and splenic lymphoma with villous lymphocytes (SLVL).[23-24]

Previous studies[19,25-28] have described cases of chronic B-cell lymphoproliferative disorders carrying the t(11;14), showing blood and bone marrow involvement without lymphadenopathy with morphology consistent with aCLL according to the FAB classification.[29] Because these patients had immunophenotypic profiles partially overlapping with MCL, the suggestion was that they may represent the leukemic presentation of a spectrum of diseases of follicle mantle lineage. While detailed hematologic, immunologic and cytogenetic data were described in these cases,[17] molecular genetic characterization was never reported. To characterize the genetic events underlying so-called atypical CLL with the t(11;14) in comparison with those occurring in MCL, we ana-

lyzed the configuration of BCL-1, BCL-2, BCL-6, c-myc, and p53 in 16 cases (9 atypical CLL, 6 MCL, and 1 MM).

Design and Methods

Patient selection and clinical parameters

Sixteen patients (9 atypical CLL, 6 MCL and 1MM) were included in this study. They were identified from among a group of more than 250 B-cell chronic lymphocytic leukemia and 150 non-Hodgkin's lymphoma cytogenetically analyzed cases, seen at our Institution between 1988 and 1998. Because selection criteria for this study included the availability of material for molecular genetic analysis, the patients do not reflect the incidence of the corresponding diseases at our Institution. In fact, criteria for inclusion in this study were the following: a) unequivocal diagnosis of MCL on histologic sections, according to the REAL classification,[1] and diagnosis of atypical CLL according to the FAB criteria,[29] for those cases presenting blood and marrow involvement in the absence of lymphadenopathy, b) presence of the t(11;14)(q31;q32) in metaphase cells and/or of the corresponding BCL-1 rearrangement as detected by interphase fluorescent *in situ* hybridization (see below); c) availability of material for molecular genetic studies.

Hematologic studies

Histologic diagnosis of MCL was performed according to recently summarized criteria[1,30] on lymph node specimens and/or bone-biopsy sections. Staging procedures included physical examination, a routine laboratory profile, chest X-ray film, abdominal ultrasonography and computed tomography (CT) scan. Bone biopsy and bone marrow (BM) aspiration were performed in all cases. Peripheral blood (PB) involvement was assessed by morphologic examination of PB smears and by immunophenotyping using the above described panel of reagents.

Diagnosis of aCLL was made according to standard clinical, cytological and immunologic criteria[29] on the basis of the presence of more than 10% large lymphocytes (i.e. cells usually greater than 14 µm in diameter having inconspicuous nucleoli), and/or prolymphocytes (PL) (i.e. cells usually greater than 14 µm in diameter with a prominent central nucleolus) in blood and marrow, and in the absence of lymphoadenopathies. Transformation of aCLL into prolymphocytic leukemia (PLL) was not an exclusion criteria.

Cytofluorimetric study was performed as previously reported,[19] gating primarily on lymphocytes on a FACScan analyzer (Becton Dickinson). The expression of the following surface markers was tested using commercially available monoclonal antibodies (MoAbs): CD2, CD3, CD5, CD19, CD22, CD23, CD10, CD11c and HLA-DR. Double labeling with monoclonal antibodies detecting the CD19 and CD5 antigens was performed. The cut-off point for positivity was set at 30% cells showing fluorescence above controls. An FMC7 MoAb (Silenius Lab Hauthorn, Australia),[31] and the PAb-1801 MoAb, detecting P53 protein by recognizing an epitope between amino acids 32 and 79,[32] were also used. Expression of surface immunoglobulins (sIg) (heavy and light chains) was tested using rabbit antibodies, and was interpreted as *weak* if the mean intensity of fluorescence was <512 and *bright* if >512 (logarithmic acquisition, 0-1024 channel range).

Staging procedures according to Rai's classification included physical examination, a routine laboratory profile, chest X-ray film and abdominal ultrasonography.[33]

Clinical records were reviewed in all patients and the salient parameters are summarized in Table 1.

Table 1. Clinical and immunologic findings of investigated cases.

Case/sex/age	Diagnosis	Stage	Involved organs			WBC	CD5/CD19	CD23	CD22	FCM7	sIgf
			BM	LN	Others	x 10^9/L					
1/F/69	CLL/PL[a]	IV[b]	>90%	no	spleen	81 (85)[e]	+	+	+	_	++
2/F/65	CLL/PL[a]	II[b]	>90%	no	no	26 (80)[e]	+	_	+	ND	++
3/M/62	CLL-MT[a]	II[b]	15%	no	liver	15 (74)[e]	+	_	+	_	++
4/M/57	CLL/PL[a]	II[b]	60%	no	spleen	45 (84)[e]	+	+	+	+	+
5/M/49	CLL/PL[a]	0[b]	25%	no	no	29 (90)[e]	+	_	+	_	++
6/M/76	CLL/PL[a]	0[b]	>90%	no	no	19 (44)[e]	+	+	+	+	++
7/M/63	CLL-MT[a]	0[b]	15%	no	no	11.9 (52)[e]	+/-	_	+	+	++
8/M/61	CLL-MT[a]	III[b]	>90%	no	spleen	15 (58)[e]	+/-	+	+	ND	++
9/F/39	CLL/PL[a]	0[b]	15%	no	no	34 (60)[e]	+	_	+	_	++
10/M/70	MCL	IVA[c]	yes	no	yes	93	+	_	+	+	++
11/M/71	MCL	IVB[c]	yes	no	yes	900	+	_	+	_	++
12/F/59	MCL	IIA[c]	no	yes	no	5.2	+	_	_	ND	++
13/M/49	MCL	IVB[c]	yes	yes	yes	32	+	_	+	_	++
14/M/46	MCL	IIIA[c]	yes	yes	yes	25	_	+	+	_	++
15/M/76	MCL	IVB[c]	yes	yes	yes	6.4	+	_	+	ND	++
16/F/59	MM	IIIB[d]	70% PC								

[a-d]: FAB, RAI, Ann-Arbor and Salmon-Durie classifications, respectively; [e]% of lymphocytes; f: (+) weak expression, (++) bright expression; CLL/PL: chronic lymphocytic leukemia/prolymphocytic leukemia; CLL-MT: chronic lymphocytic leukemia-mixed type; MCL: mantle cell lymphoma; MM: multiple myeloma; ND: not determined.

Cytogenetic and fluorescence in situ hybridization (FISH)

Peripheral blood samples were obtained from those patients with *atypical CLL*, whereas chromosome studies were performed from fresh lymph node samples in patients with MCL. Cytogenetic techniques in use at our laboratory were described previously.[34,35] Whenever possible, a minimum of 10 metaphases were studied and karyotypes described according to the ISCN.[36]

FISH was carried out on the same specimens as those that were used for conventional cytogenetic analysis. The demonstration of the t(11;14) in interphase cells was obtained by dual color FISH analysis, using an IgH probe and the YAC 214-D-11,17 spanning a 390 kb region encompassing the major translocation cluster and the minor translocation cluster of the BCL-1 locus at 11q13.[37] As previously reported,[38] the BCL-1 probe splitting, along with colocalization of IgH and BCL-1 signals, was considered as indicative of the presence of BCL-1 rearrangement.

Molecular genetic analysis

Samples for molecular studies were obtained from representative sites of disease involvement at diagnosis or before treatment in six patients and at relapse in ten patients.

DNA, isolated from lymphocytes using proteinase K digestion, was phenol extracted and analyzed as previously reported.[39] Ten micrograms of DNA were digested with BamHI, EcoRI and HindIII, separated on agarose gel and Southern blotted. The configuration of BCL-1 locus was investigated using the following probes: a BCL-1/CCND1 cDNA (pPL8),[40] a major translocation cluster (MTC) BCL-1 "b",[13] and a minor translocation cluster 1 (mTC1) p94PS.[41] The organization of the P53, c-MYC and BCL-2 genes was analyzed by hybridation with a p53 cDNA fragment of 1.8 Kb encompassing 5' and 3' untranslated sequences (pC53-SN)[42] a c-myc cDNA (Ryc 7.4)[43] and a 2.7 Kb Bcl-2 mbr region fragment (Oncogene Science), respectively. The configuration of the BCL-6 locus was investigated using a Sac 4.0 genomic fragment containing the 5' portion of BCL-6 gene and a Sac 0.8 probe derived from the BCL-6 first intron.[44]

p53 lesions were investigated using polymerase chain reaction/single-strand conformation polymorphism (PCR-SSCP) analysis. Exons 5-9, the most frequently mutated in lymphoid tumors, were amplified in the presence of α-^{32}PdATP, as previously described.[8] Fragments displaying an altered electrophoretic mobility were subsequently reamplified and studied by direct sequencing (Sequenase version 2.0 kit-United States Biochemical Corp., Cleveland, Ohio, USA).

Total RNA was extracted from lymphocytes lysed in guanidium isothiocyanate, isolated by phenol-chloroform extraction and isopropanol-ethanol precipitation (RNAzol B solution, Biotec Laboratory Inc.), separated on denaturing agarose gel, transferred to a Gene Screen Plus filter (NEN) and hybridized, essentially as previously described,[45] using the BCL-1/CCND1, BCL-2, p53, c-myc, and GAPDH cDNA probes.

Results

Hematologic features

Immunologic findings and clinical features are shown in Table 1. Patients #1 through #9 presented with lymphocytosis and BM involvement, with or without splenomegaly. These patients did not have lymphadenopathy. Morphology (a majority of small lymphocytes with prolymphocytes and/or large lymphocytes) was consistent with a diagnosis of CLL/PL in 6 cases, whereas a diagnosis of CLL mixed-cell type was considered more appropriate in 3 cases;[29] immunophenotype showed CD23 positivity in 4/9 cases and low expression of surface Ig (sIg) in one (Table 1). Because of the absence of adenopathy and typical morphologic features of MCL with PB involvement (heterogeneity of cell size and morphology with irregular nuclear outline), in these patients leukemic MCL appeared not to be an appropriate diagnosis. Patients #10 through #15 had histologically documented MCL on a lymph node biopsy. PB involvement was detected by morphologic examination of a PB smear or by the more sensitive immunologic techniques in all cases. Irregularity of nuclear outline, heterogeneity of cell size and, morphology, as well as cleaved cells, were seen in PB films in these cases. In the majority of MCL examined, we observed the classical MCL phenotype (pan-B markers positivity, $CD5^+$, CD23- and strong surface Ig expression).

Cytogenetic and molecular genetic findings

The t(11;14)(q13;q32) was found as the sole aberration in one case (case #12), whereas additional chromosome changes were detected in 13 patients. BCL-1 involvement was documented by FISH in all 16 cases,[17] including two patients with an apparently normal karyotype (cases #8 and 14). The percentage of cells with BCL-1 involvement ranged between 65% and 80%. Abnormalities of chromosome 17p were observed in 5 cases (cases #1, 2, 3, 11 and 13); 13q deletions were observed in 5 cases; 6q deletions, structural anomalies of 10q or 11q and trisomy 12 were detected in two cases each.

Alterations of BCL-1, BCL-2, BCL-6, c-Myc and p53 genes, were initially investigated by Southern blots of DNA isolated from PB lymphocytes (cases #1-7 and 10-13) and BM mononuclear cells (case #13) after restriction with EcoRI, BamHI and HindIII. No rearrangements of BCL-2, BCL-6, or c-Myc were detected in any of the samples tested (data not shown).

BCL-1 analysis

Results of BCL-1 analysis are summarized in Table 2. All cases included in the present study were previously shown to have a breakpoint in the BCL-1 locus, by interphase FISH using a 390 kb probe, centromeric to the BCL-1/CCND1 gene, spanning a region where the MTC, the mTC1 and the mTC2 were previously located.[17,35]

In twelve patients, BCL-1 locus was examined by Southern blotting using three separate probes (MTC, p94PS and CCND1 cDNA) localized on chromosome 11. No rearrangements were detected with the BCL-1/CCND1 cDNA probe. As shown in Table 2,

Table 2. Summary of genotypic findings of investigated cases.

Case	Disease	Bcl1		p53							Survival (months)
		DNA	RNA	DNA	RNA	TP53 (%)	PCR-SSCP	Mutated codon	Nucleotide substitution	Amino acid substitution	
1	aCLL	R (MTC)	+++	G	++	24	exon VII	234	TAC to TGC	Tyr to Cys	65+
2	aCLL	R (MTC)	ND	R	ND	ND	N	/	/	/	70+
3	aCLL	G	++	H	+	15	exon V	181	CGC to CCC	Arg to Pro	160+
4	aCLL	R (mTC1)	+/-	D	+	ND	N	/	/	/	39+
5	aCLL	R (mTC1)	+/-	G	+/-	8	N	/	/	/	137
6	aCLL	G	++	R	-	<1	N	/	/	/	53+
7	aCLL	G	+/-	G	+	8	N	/	/	/	83
8	aCLL	ND	ND	ND	+	15	ND	/	/	/	138+
9	aCLL	ND	ND	ND	+	17	N	/	/	/	48+
10	MCL	D (MTC)	++	G	+	ND	N	/	/	/	17+
11	MCL	G	+++	G	+	ND	N	/	/	/	14+
12	MCL	G	++	G	+	ND	N	/	/	/	38
13	MCL	R (MTC)	+	G	+	ND	exon V	175	CGC to CAC	Arg to His	24+
							exon VI	206	TTG to TAG	Leu to Stop	
14	MCL	ND	+	ND	+/-	<1	exon V	167	CAG to TAG	Gln to Stop	12+
15	MCL	ND	ND	ND	ND	37	exon V	158	CGC to CAC	Arg to His	19+
16	MM	G	ND	G	ND	ND	exon V	163	TAC to TGC	Tyr to Cys	28

R: rearranged; G: germline; H: hemizygous; D: duplicated; N: normal; ND: not determined; +: dead patient; aCLL: atypical chronic lymphocytic leukemia; MCL: mantle cell lymphoma; MM: multiple myeloma.

abnormalities were identified in 6 cases (50%): 4 were detected with the MTC probe (two aCLLs - cases #1, 2; two MCL - cases #10 and 13) and 2 with the p94PS probe (two aCLLs - cases #4 and 5). The MTC probe showed, in addition to the EcoRI normal band of 12 Kb in size, an additional band of 18, 13 and 21 Kb in cases #1, 2 and 13, respectively (Figure 1A). A similar pattern was observed after BamHI and HindIII digestions (data not shown), indicating that a rearrangement of the region had occurred. Case #10 showed a normal size band, but with double intensity, indicating DNA duplication. The p94PS probe for the mTC1, located about 20 kb 3' of the MTC, showed, in addition to the BamHI normal band of 16 Kb in size, bands of 9.5 and 21 Kb in cases #4 and 5, respectively (Figure 1B). Accordingly, a similar pattern was detected with the other restriction enzymes. RNA was available for Northern blot analysis in 6

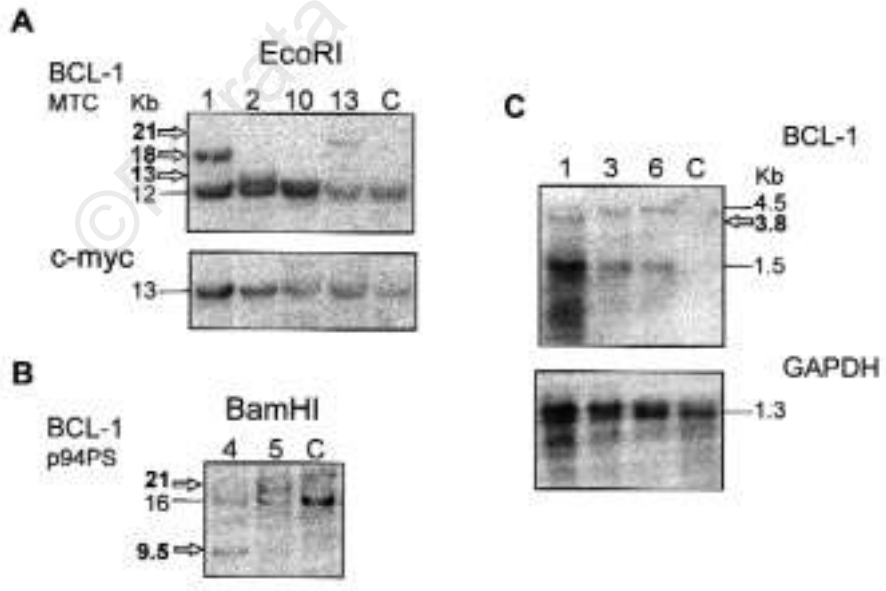

Figure 1. Analysis of structure and expression of BCL-1 locus. Southern blot analysis of BCL-1 DNA with the major translocation cluster (MTC) is shown in A and with the minor tranlocation cluster 1 (mTC1) in B. Abnormal DNA fragments are indicated by arrows. The BCL-1 DNA duplication of case #10 was assessed by comparing the optical density of BCL-1 autoradiographic signals with that of c-myc DNA; the ratio of optical densities (O.D.), expressed in arbitrary units, is 3.7 in case #10 and 1.8 in a control subject (C). Northern blot analysis of BCL-1 mRNA expression in three aCLL cases and a control subject is shown in section C. The filter was subsequently hybridized with CCND1 and GAPDH cDNA probes.

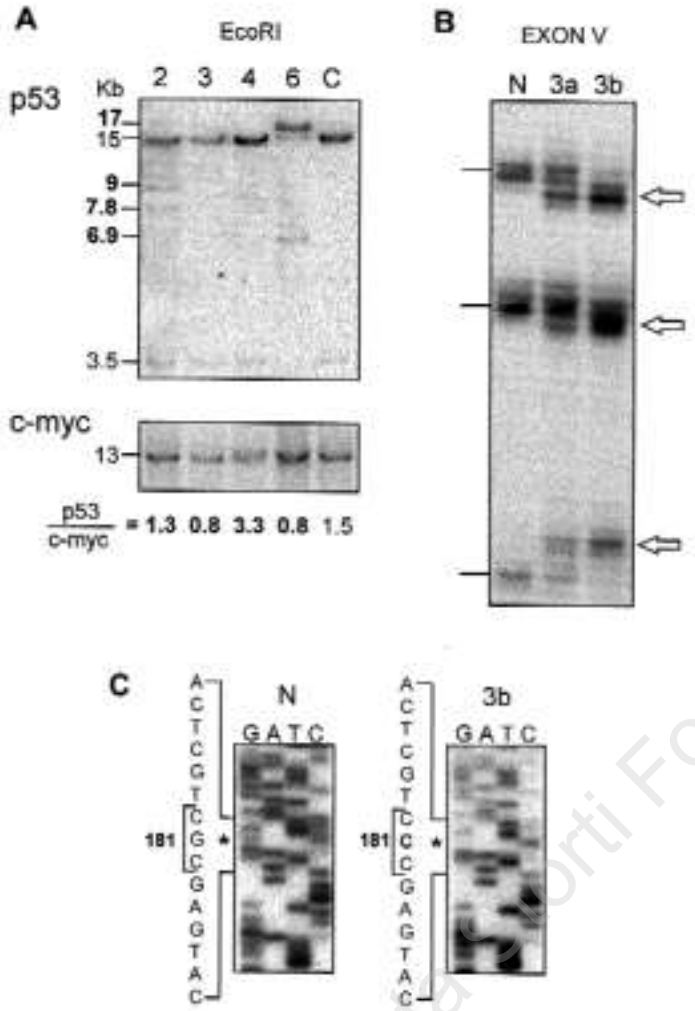

Figure 2. Molecular analysis of the p53 gene.
A. Southern blot analysis of p53 DNA is shown in cases #2, 3, 4, 6 and in one control case (C); sizes of abnormal bands are indicated in bold; the relative amount of p53 DNA was assessed by comparing optical densities of autoradiographic signals of p53 and c-myc EcoRI bands.
B. Detection of p53 gene mutation by PCR-SSCP analysis is shown in two samples from patient #3 obtained at a distance of five years (3a and 3b); arrows indicate abnormal bands.
C. Exon V nucleotide sequence is reported in case #3b and a control case (N). The change (*) is at codon 181, from Pro to Arg.

cases of aCLL and 5 MCL (Table 2). BCL-1/CCND1 transcripts were not detected in the four control cases (see, for example, case C of Figure 1C), whereas they were overexpressed in all the neoplastic cases analyzed (Table 2). Two different BCL-1/CCND1 transcript isoforms of 4.5 and 1.5 Kb were detected: the intensity of the small transcript was frequently higher than that of 4.5 Kb. In case #1, insignificant levels of the 4.5 kb isoform and an additional anomalous transcript of 3.8 Kb were present (cases #1, 3 and 6, shown in Figure 1C). The lower levels of overexpression were observed in 3 aCLL (cases #4, 5 and 7), 2 of which had a rearrangement in the mTC1 (Table 2).

p53 analysis

Gross alterations of p53 gene were detected in four patients. As shown in Figure 2A, case #2 had the expected p53 DNA bands (15 and 3.5 Kb in size) and two additional bands of 7.8 and 9 Kb , indicating a wide rearrangement of one allele. Quantitative alterations in cases #3 (hemizygosity) and 4 (duplication) were found. In case #6, the 15 Kb EcoRI band was markedly reduced in intensity, the 3.5 Kb band and additional EcoRI bands of 17 and 6.9 Kb were also detected. When autoradiographic signals of p53 bands were compared to those of c-myc bands, the relative amount of p53 DNA was approximately normal (1.3) in case #2, half (0.8) in cases #3 and 6, and double (3.3) in case #4, being 1.5 in the control sample.

Fourteen cases were examined for p53 gene mutations in exons 5 through 9 by PCR-SSCP analysis and direct sequencing. As an example, PCR-SSCP electrophoresis and sequence of exon 5 in case #3 are shown in Figures 1B and C. In this case the analysis was performed on two samples (#3a and 3b) obtained after five years, indicating the progression from heterozygosity (#3a) to hemizygosity (#3b) (Figure 2B). The mutation in case #3b at codon 181 (CGC, arginine to CCC, proline) was identified by sequence analysis (Figure 2C). Of the examined cases, 6 showed evidence of p53 gene mutations: case #1 in exon 7, cases #3, 13, 14, 15 and 16 in exon 5; in case #13, a second conformation alteration was found in exon 6.

p53 expression was analyzed at RNA level by Northern blot in 13 cases (8 aCLL and 5 MCL), and at protein level by flow cytometry in 9 cases (7 aCLL

and 2 MCL), yielding concordant results (see Table 2). Different levels of p53 RNA normal band (2.6 Kb in size) were found (data not shown). p53 RNA levels, normalized by GAPDH RNA hybridization,[46] were expressed as + and -, by comparison with non-pathologic control samples, as indicated in Table 2.

The percentage of p53 positive cells varied between 2 to 5% in control samples (normal range). It was below the normal range (<1%) in cases #6 and 14, due to abnormality of the promoter region (case #6) and a stop mutation (case #14). In seven cases (#1, 3, 5, 7, 8, 9 and 15) higher percentages than normal were found, correlating with the presence of missense mutations in three cases (#1, 3, 15).

Discussion

A molecular genetic characterization of atypical B-CLL has not been reported so far. In the present study we investigated a panel of B-lymphoproliferative disorders characterized from morphologic and immunologic standpoints as aCLL and classical MCL (see results), known to have a break within the 11q13 band, as detected by interphase FISH. The distinction of atypical CLL carrying the (11;14) translocation and MCL in leukemic phase is not unequivocal, as recently discussed.[47,48]

As for the involvement of BCL-1 breakpoint regions, no clear cut difference existed between aCLL and MCL. No rearrangements of the BCL-1/CCND1 gene were found in this study. An approximate 50% incidence of lesions at the translocation cluster DNA segments was observed in both aCLL and MCL cases. It is interesting to note that rearrangements in the minor translocation cluster (mTC1) were found in this series only in aCLL and this may represent a difference between these entities which requires confirmation in large studies.

We compared our data with those so far reported in the literature concerning the involvement of the BCL-1 locus in MCL and CLL;[14,23,49-58] data are summarized in Table 3. As expected, BCL-1 locus rearrangements were closely associated with MCL (47.49%) and they occurred much less frequently in cytogenetically-unselected CLLs (7.33%). In agreement with our results, Rimoch *et al.*,[14] found two BCL-1 rearrangements in 4 cases of CLL with t(11;14).

In cases without BCL-1 rearrangements identified in this study, other regions besides those investigated may be involved: rearrangements widely scattered on chromosome 11q13 have in fact already been described.[59]

An interesting finding of our study (see Table 2) was that a moderate-to-weak overexpression of the BCL-1/CCND1 gene was observed, particularly in the cases with involvement of the mTC1, whereas strong overexpression of this gene was documented in MCL with MTC involvement, as previously reported in the literature.[54,60] Therefore, the aCLL cases with t(11;14) studied in this series, showed weaker overexpression of the BCL-1/CCND1 gene than MCL cases with the same translocation.

A high incidence of p53 gene alterations was found in this series (9 of 15 cases analyzed), almost equivalent in aCLL and MCL with t(11;14). Thus, molecular data provide evidence that p53 mutations are frequently combined with translocation t(11;14) in aCLL and leukemic MCL.

We previously reported that cases of typical B-CLL,[42] atypical B-CLL with t(11;14),[25] B-cell prolymphocytic leukemia (B-PLL)[46] and MCL,[61] with mutations of p53 gene, were characterized by rapid progression of the disease and resistance to therapy. In MCL, several reports showed that p53 mutations were more frequent in patients with short survival,[62–64]

Table 3. Review of published MCL and CLL cases with BCL-1 rearrangements in MTC and mTC1 recombination sites.

MCL				CLL				
MTC		mTC1		MTC		mTC1		
%	(R/T)	%	(R/T)	%	(R/T)	%	(R/T)	References
52.6	(10/19[a])	-	-	0.0	(0/36)	-	-	Medeiros, 1990[49]
42.8	(3/7[b])	-	-	28.6	(2[e]/7)	-	-	Rosenberg, 1991[50]
33.3	(9/27[b])	22.2	(6/27)	5.3	(1/19)	0.0	(0/19)	Williams, 1993[51]
48.5	(16/33[c])	0.0	(0/33)	50.0	(2/4[f])	-	-	Rimokh, 1993[14]
-	-	-	-	5.9	(5/84)	-	-	Newman, 1993[52]
30.0	(6/20[d])	20.0	(4/20)	0.0	(0/11)	0.0	(0/11)	de Boer, 1993[53]
45.4	(5/11)	0.0	(0/11)	0.0	(0/6)	16.7	(1[g]/6)	Bosch, 1994[54]
38.5	(5/13)	-	-	-	-	-	-	Hayashi, 1994[55]
25.0	(1/4)	25.0	(1/4)	-	-	-	-	Jadayel, 1994[23]
42.8	(6/14)	7.1	(1/14)	-	-	-	-	Zoldan, 1996[56]
54.5	(6/11)	-	-	-	-	-	-	Garcia-Sanz, 1998[57]
33.0	(39/118)	0.0	(0/118)	-	-	-	-	Chibbar, 1998[58]
40.58 ± 2.87 SE[h]		10.61 ± 4.31 SE[h]		12.83 ± 7.28 SE[h]		5.57 ± 5.57 SE[h]		
(from 25 to 54.5)		(from 0 to 25)		(from 0 to 50)		(from 0 to 16.7)		
47.49 ± 2.67 SE[i] (94/227)				7.33 ± 4.93 SE[i] (2/36)				

MCL: mantle cell lymphoma; CLL: chronic lymphocytic leukemia; MTC: major translocation cluster; mTC1: minor translocation cluster 1; %: percentage values; (R/T): (rearranged/total); a: IDL intermediate differentiation lymphoma ; b: CCL centrocytic lymphoma; c: ILL intermediate lymphocytic lymphoma; d: 11 CCL, 9 IDL; e: 2 CLL with t(11;14); f: with t(11;14); g: the only one with t(11;14); h: mean percentage values; i: values calculated on studies in which both recombination sites were analyzed.

and in the variant cytological types (i.e. anaplastic or blastic) which are clinically aggressive and have a frequent leukemic evolution.[56] It is interesting to note that all our MCL had leukemic involvement and, of the aggressive aCLL forms, 4 out of 5 had p53 alterations. The role played by p53 in the progression was confirmed in 2 cases, who developed a detectable p53 mutation at disease progression[25] and hemizygosity for the mutant allele (SSCP of case #3 in Figure 2). Thus, our observations provide further support to the concept that p53 alterations are strictly associated with aggressiveness.

As regards BCL-2, BCL-6 and c-myc genes, the absence of rearrangements in any of the samples tested indicated that these loci may not be involved in the pathogenesis of lymphoid neoplasias with the t(11;14). While the absence of rearrangements of BCL-2 and c-myc is not surprising, the germline configuration of the BCL-6 gene is of some interest: involvement of BCL-6 has been found in a variety of aggressive as well as indolent lymphoid tumors, including diffuse large cell lymphoma, follicle center cell lymphoma, marginal zone B-cell lymphoma[5] and rare cases of chronic lymphocytic leukemia (CLL),[65] but only occasionally in MCL.[57] The BCL-6 protein is involved in germinal center formation, where the antigen-driven progression of the lymphocytes occurs;[66] it is noteworthy that, unlike MCL, all these tumors including some CLLs, have been shown to derive from cells harboring IgV gene somatic mutations, indicative of a post-GC cell origin.[67,68] Thus, it may be suggested that our aCLL with t(11;14), could share the feature of not rearranged BCL-6 with the pre-GC B-cell MCL.

In conclusion, we characterized cases of aCLL and MCL with the translocation t(11;14)(q13;q32) by molecular genetic methods, showing that: a) the two B-cell chronic lymphoproliferative disorders share similar molecular rearrangements within the BCL-1 locus, b) a relatively high frequency of mTC1 involvement was observed in aCLL; c) overexpression of the BCL-1/CCND1 gene was less pronounced in aCLL than in MCL, especially in those cases having a BCL-1 break within the mTC1.

It is likely that MCL and aCLL with the t(11;14) represent the extremes of a spectrum of disorders of follicle mantle lineage presenting heterogeneous clinical and morphologic features, identifiable by a combination of morphologic, immunologic and molecular cytogenetic techniques. It is reasonable to assume that the transformation of a $CD5^+$ B-lymphocyte of the follicle mantle may give rise to a spectrum of clinicopathologic manifestations, ranging from the classical lymphomatous form of MCL to a primarily leukemic form having the cytological features of CLL/PL. Some authors proposed referring to these leukemias as *mantle cell leukemia*[27,69] and we believe this may be a good option allowing for the distinction of a disorder having a distinct clinical presentation and possibly requiring a specific therapeutic approach.

Contributions and Acknowledgments

CDA and DG performed and analyzed the molecular studies and prepared the manuscript; AC was involved in the conception of the study and reviewed the manuscript for important intellectual content; SM performed and analyzed immunological studies; RB performed cytogenetic and FISH studies; MGR and AB performed and interpreted the cytogenetic analyses; GLC was responsible for the critical revision of the intellectual content; LdS was responsible for the organization and design of the study and for interpretation of the data. All the authors reviewed the manuscript for important intellectual content and approved the final version. The authors thank Dr. Massimo Negrini and Dr. Gianluca Gaidano for their respective gifts of the BCL-1 and BCL-6 probes. This work was supported by M.U.R.S.T. funds Cofin 40% and 60%; CDA was supported by a grant from F.I.R.C.

Disclosures

Conflict of interest: none.

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Potential implications for clinical practice

- No implications for clinical practice; this is a biological study.

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