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BCL10 expression and localization in Ocular Adnexa MALT lymphomas: a comparative cytogenetic and immunohistochemical study

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Summary. T(1;14) (p22;q32) involving *BCL10* and *IGH* genes is a rare but recurrent chromosomal aberration in MALT-type lymphoma. It is rarely described in ocular adnexa B cell lymphomas, although nuclear BCL10 shuttling seems to be critical for disease progression in this district. We have evaluated the translocations MALT lymphoma-related in a series of 45 ocular adnexa cases, focusing in particular on their relation with BCL10 expression and its cellular topographic distribution. A prognostic tissue microarray (TMA) with ocular adnexa MALT lymphomas was designed. A study of BCL10 expression and its topographic distribution was performed through immunohistochemistry. In addition the assessment of t(14;18) (q32;q21), t(1;14) (p22;q32) and t(11;18) (q21;q21) was determined by Fluorescent In Situ Hybridization (FISH).

Our series revealed t(14;18) (q32;q21) in 6/43 cases (14,3%). t(1;14) (p22;q32), never described in ocular adnexa MALT lymphomas, was observed in 3/31 (9,7%), two of which exhibited the gain of 3' upstream *BCL10* gene signal (4%), whereas no case showed t(11;18) (q21;q21). Moreover, BCL10 expression was observed in 18/45 cases. In particular its nuclear expression was revealed in 12/45 cases, cytoplasmic expression in 5/45 and both cytoplasmic and nuclear expression in 1/45. Statistical analysis demonstrated that while BCL10 cytoplasmic expression is significantly related to the presence of the investigated chromosomal aberrations, in particular with t(14;18) (q32;q21), BCL10 nuclear shuttling does not show any correlation with these translocations. Our data support that BCL10 nuclear distribution is neither related to BCL10 rearrangement nor to other known translocations.

Key words: BCL10, Genetic aberration, MALT lymphoma, Ocular Adnexal B-cell Lymphoma

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Introduction

BCL10 (B-cell lymphocytic lymphoma/leukemia) is a CARD (caspase recruitment domain)-containing protein, playing an important role in the assembly and activation of apoptotic and inflammatory complexes (Willis et al., 1999; Zhang et al., 1999; Park et al., 2007). It was cloned from t(1;14)(p22;q32) breakpoint of extranodal marginal zone lymphoma of Mucosa-Associated Lymphoid Tissue (MALT lymphoma) and is related to disease progression (Willis et al., 1999; Isaacson and Duo, 2004). In fact, BCL10 pathological overexpression leads to constitutive activation of NF-κB, responsible for enhancing cell survival and downstream activation of anti-apoptotic/proliferative signals (Ghosh et al., 1998; Johnson et al., 1999; Karin and Ben-Neriah, 2000; Sanchez-Beato et al., 2003; Liu et al., 2004; Thome, 2004). BCL10 is normally located

in the cytoplasm to relay antigen receptor-mediated signals activating T and B cells NF-κB (Ruland et al., 2001). NF-κB activation through BCL10 is mediated by several factors. Particularly, BCL10 interacts with MALT1 to active IκB kinase via TRAF6 ubiquitine kinase and TAK1 kinase (Sun et al., 2004).

T(1;14)(p22;q32), leading *BCL10* under the control of IgH promoter, has been described in 1-2% of MALT lymphomas (Inagaki, 2007). MALT lymphomas, harboring this chromosomal aberration, seem to be characterized by a strong nuclear BCL10 staining (Karin and Ben-Neriah, 2000; Daibata et al., 2006; Masanori et al., 2006). However, this staining is not always presumptive evidence of t(1;14)(p22;q32) (Ye et al., 2000; Liu et al., 2004; Gallardo et al., 2006). In fact, BCL10 nuclear expression has also been described in gastric MALT lymphomas with t(11;18), which represents the most common chromosomal aberration described in MALT lymphomas, involving *MALT1* and *API2* genes (Liu et al., 2001; Kuo et al., 2004). Besides, it has been reported that nuclear immunostaining of BCL10 occurs in 20% to 50% of MALT lymphomas without t(11;18) and t(1;14) (Liu et al., 2001; Maes et al., 2002; Ye et al., 2003a). Finally these studies suggested that BCL10 oncogenic activity is carried out in the nuclei (Ye et al., 2000, 2003a,b; Liu et al., 2001; Maes et al., 2002; Isaacson and Du, 2004). Nuclear localization of BCL10 was associated with advanced MALT lymphoma (Liu et al., 2001) or even with a worse prognosis (Franco et al., 2006).

Although most studies of BCL10 have focused on BCL10 deregulated expression due to chromosomal translocation, DNA amplification of *BCL10* gene has been described in pancreatic cancer and in Diffuse Large B Cell Lymphomas (DLBCL), suggesting that *BCL10* may play a critical role in different types of tumors (Holzmann et al., 2004; Ye et al., 2006).

MALT lymphomas of Ocular Adnexa account for 14% of all extranodal marginal zone lymphomas, mainly described in intestinal tract (Ferreri et al., 2006; Shaye and Levine, 2006; Solari et al., 2009). Its incidence has progressively risen in recent years, with an annual increase of 6,3% (Moslehi et al., 2006; Stefanovic and Lossos, 2009). As suggested in gastric *H. pylori*-dependent MALT lymphoma pathogenesis, microbial pathogens have been variously proposed as responsible of Ocular Adnexa B-cell MALT Lymphomas (OABMLs) development. A significant association has been recently demonstrated between OABMLs and *Chlamydia psittaci* infection in different groups of patients, both Italian and not (Ferreri et al., 2004; Daibata et al., 2006; Liu et al., 2006; Masanori et al., 2006; Mulder et al., 2006; Rosado et al., 2006; Vargas et al., 2006; Gracia et al., 2007; Ruiz et al., 2007; Carugi et al., 2010; Collina et al., 2012). Although sharing morphological features with MALT lymphomas of other districts, OABML shows some specific biological features. Due to its genetic instability, also in MALT lymphomas of this district, different chromosomal aberrations have been described (Alpen et al., 2000; Liu et al., 2006; Masanori et al., 2006; Daibata et al., 2006; Rosado et al., 2006; Stefanovic et al., 2009), but with different incidence at multiple sites. The most frequent translocation in gastric MALT lymphomas, t(11;18) (q21;q21) (Ye et al., 2003a,b; Streubel et al., 2004; Remstein et al., 2006; Inagaki 2007), has been described as the least frequently occurring in OABMLs (Ye et al., 2003a,b; Franco et al., 2006). t(14;18) (q32;q21) (*IgH/MALT1*) is documented as the most frequently reported translocation in OABMLs (Streubel et al., 2003, 2004; Franco et al., 2006), but in some series its incidence has been found to be relatively low (Sagaert et al., 2006; Wongchaowart et al., 2006; Carugi et al., 2009; van Maldegem et al 2012). Both these translocations are responsible for *MALT1* gene deregulation through its juxtaposition to *API2* gene in t(11;18) (q21;q21) and to *IgH* gene in t(14;18) (q32;q21) (Streubel et al., 2005). Finally, t(1;14)(p22;q34) has been detected in MALT lymphomas of the gastrointestinal tract, lung and parathyroid, but seems to be absent in OABMLs (Streubel et al., 2004; Raderer et al., 2005; Du, 2007; Stefanovic and Lossos, 2009).

In our study we have gathered a series of patients with OABMLs in order to identify the incidence of *BCL10* gene aberration, its relation to nuclear shuttling and chromosomal aberration frequently described in MALT lymphomas.

Materials and methods

Selection of cases and morphology

Forty-five cases of primary OABMLs were collected from the Department of Pathology of the National Cancer Institute "Giovanni Pascale" and "Federico II" University, diagnosed between 1980 and 2004. All the cases were generally constituted by surgical biopsy of ocular adnexa neoplasm. Ocular adnexal lymphoma was considered primary if the ocular adnexa alone were involved, without infiltration of lymphnodes or visceral sites at diagnosis (Bardestein, 2005; Stefanovic and Lossos, 2009)

The cases were included in this study on the basis of the availability of diagnostic paraffin blocks and of clinical information for more than 6 months following diagnosis.

All cases were reviewed by expert pathologists (RF and ADC). Morphology and routine immunohisto-chemical data, comprising CD20, CD3, CD5, Bcl2, BCL6, CD10, CD23, CD43, Cyclin D1 and ki67 (Table 1) were re-evaluated, in order to rule out from the study other low grade lymphomas. Particularly, follicular lymphomas were excluded when germinal centers in the samples showed images of 'follicular colonization' and the interspersed cells were CD10 and BCL6.

Finally, each case of OABML was evaluated for cell population prevalence, monocytoid, centrocyte-type and plasmocytoid, and for large cell presence, either isolated

or in clusters.

Tissue-Microarray design and immunohistochemical study

TMA was built using the Galileo TMA CK 3500 as well as (Franco et al., 2006), selecting two cores of 6 mm of diameter for each case.

Immunostaining was performed in accordance with standard methods as previously described by our group (Franco et al., 2006). Tested antigens are presented in Table 1.

In particular, cytoplasmic BCL10 expression was scored as strong when it was similar to tonsil centroblast positivity, moderate when similar to centrocyte positivity, and weak/absent when similar to tonsil mantle-zone positivity. Nuclear positivity was also recorded. Stained sections were assessed by two different pathologists (RF, GB) with uniform criteria, through simultaneous inspections and results discussion. In addition, light chain restriction was evaluated in the series. Briefly κ/λ normal rate is 4:1. A rate greater than 4:1 or lower than 2:1 was considered clonal respectively for κ and λ (Ho et al., 1994).

The reproducibility of the results thus obtained has been confirmed by comparing the staining of the TMA sections with those from whole sections. Particularly, for routine immunohistochemical staining (CD20, CD3, CD5, Bcl2, BCL6, CD10, CD23, CD43, Cyclin D1 and Ki67) the comparison was performed for all cases. In addition comparisons of TMA/whole sections data were carried out in 15 randomly selected cases, for BCL10 and light chain staining (García et al., 2003).

Fluorescent In Situ Hybridization Study

The interphase FISH was performed on histological sections from paraffin-embedded tissue as described by

Table 1. Details of used antibodies.

Antigen Clone	Source	Dilution	Reactivity	Threshold	Internal control
BCL6 (clone PG-B6p)	DAKO	1:10	Positive/negative	>10% neoplastic cells	GC* B cells
CD10 (clone56C6)	Novocastra	1:10	Positive/negative	Any positive neoplastic cells	GC* B cells
CD20 (clone L-26)	DAKO	1:100	Positive/negative	Any positive neoplastic cells	Reactive lymphocyte
BCL10 (clone 151)	DAKO	1:200	Positive/negative	>10% neoplastic cells	Reactive lymphocyte
CD3 (clone F7.2.38)	DAKO	1:25	Positive/negative	Any positive neoplastic cells	Reactive lymphocyte
CD5 (4C7)	Novocastra	1:50	Positive/negative	>10% positive cells	Reactive lymphocyte
BCL2 (clone 124)	DAKO	1:25	High/Low	>50% neoplastic cells	Small lymphocyte
Ki-67 (clone MIB1)	DAKO	1:100	High/Low	>20% positive cells	Proliferating cells
CD23 (clone MHM-6)	DAKO	Prediluited	Positive/negative	>50% neoplastic cells	Follicular dendritic cells
CD43 (clone DF-T1)	DAKO	Prediluited	Positive/negative	>50% neoplastic cells	Reactive lymphocyte
κ-chain (polyclonal)	DAKO	1:200	Positive/negative	> 4:1 (κ/λ) monoclonal κ	Plasmacells
λ-chain (polyclonal)	DAKO	1:100	Positive/negative	<2:1 (κ/λ) monoclonal λ	Plasmacells
Cyclin D1 (DCS-6)	DAKO	1:100	Positive/negative	Any positive neoplastic cells	Macrofage and endothelial cells

*GC: germinal center.

Table 2. Commercial probes used in FISH analysis.

PROBE name	Probe location	Probe description	Intended Use	Cut- Off	Shipment made by
LSI API2	11q21	Spectrum Orange			
LSI MALT1	18q21	Spectrum Green			
(Dual Color, Dual Fusion Traslocation Probe)			API2/ MALT1 t(11;18)(q21;q21)	>20-30%	Vysis
LSI IGH	14q32	Spectrum Green			
LSI MALT1	18q21	Spectrum Orange			
(Dual Color, Dual Fusion Traslocation Probe)			IGH/MALT1 t(14;18)(q32;q21)	>20%	Vysis
LSI IGH 3' flanking probe ~250kb		Spectrum Orange			
LSI IGHV ~900kb		Spectrum Green			
(Dual color Break-apart probes)	14q32			>20%	Vysis
CEP 18	18p11,1q11,1 α satellite DNA	Spectrum Aqua	Status Cr 18	----	Vysis
LSI BCL10 3' 343kb		Texas Red			
LSI BCL10 5' 630kb		FITC			
(Dual color Break-apart probes)	1p22			>15%	DAKO

Franco et al. (2006).

Slide pre-treatment and protease incubation were performed according to the manufacturers instructions, illustrated in datasheet, of Vyses (paraffin pre-treatment reagent kit II) for t(11;18) (q21;q21), t(14;18) (q32;q21) (*IgH/MALT1*), and of Dako (Histology FISH Kit) for t(1;14)(p22;q32). Commercial probes were employed and are summarized in Table 2. DAPI II (4,6-diamino-2-phenylindole-2-hydrochloride) was used for chromatin counterstaining.

FISH data were collected using an Olympus BX 61 fluorescence microscope equipped with a cooled black-and-white camera controlled by dedicated software (Olympus, Italy). *IGH* Dual Color Break Apart Rearrangement Probes were applied to cells of all t(14;18)(q32;q21) or t(1;14)(p22;q32)-positive lymphomas to confirm the translocation. Additionally, FISH with centromere-specific probes for chromosome 18 (Vysis) was performed in all cases. For validation three FISH positive and six negative cases in TMA

Table 3. Main clinicopathological features of patients.

				IHC		FISH		
Case	Sex/Age	Site	Status	Nuclear Bcl10	Cytoplasmic Bcl10	t(14;18)	t(11;18)	Bcl10
1	M/58	LG	A	ND	ND	+	ND	ND
2	M/68	O	DO	ND	ND	ND	ND	ND
3	M/75	O	DO	-	-	ND	ND	-
4	F27	O	D	-	-	-	-	-
5	F44	O	A	-	-	-	-	-
6	M75	O	D	-	-	-	-	-
7	F/65	O	AWD	+	-	-	-	-
8	M/66	C	DO	+	-	-	-	-
9	F/83	LG	GO	-	ND	-	-	ND
10	F/61	O	A	+	-	-	-	-
11	F/37	O	a	-	-	-	-	ND
12	F/66	LG	A	+	-	+	-	-
13	F/54	LG	A	-	-	-	-	-
14	M/67	C	A	-	+	+	-	-
15	M/49	O	A	-	-	-	-	-
16	M/64	O	AWD	+	-	-	-	ND
17	M/69	O	A	-	-	-	-	-
18	F/67	O	A	-	-	-	-	ND
19	M/79	O	AWD	-	-	-	-	ND
20	F/74	O	AWD	-	-	-	-	-
21	F/76	O	AWD	-	-	-	-	-
22	F/78	O	A	-	-	-	-	-
23	F/55	LG	A	-	-	-	-	-
24	M/62	C	A	+	-	-	-	-
25	M/46	O	A	-	-	-	-	T
26	F/50	O	A	-	-	-	-	-
27	F/78	LG	A	+	-	-	-	-
28	F/30	LG	A	-	-	-	-	ND
29	M/77	O	A	-	+	-	-	ND
30	F/55	E	A	-	+	+	-	-
31	M/70	O	A	+	-	+	-	-
32	F/69	LG	AWD	-	-	-	-	ND
33	M/78	O	A	-	-	-	-	ND
34	M/69	LG	D	+	-	-	-	ND
35	F/84	O	AWD	+	-	-	-	-
36	F/60	O	A	-	-	-	-	-
37	M/65	O	AWD	+	-	-	-	-
38	F/37	O	A	-	-	-	-	ND
39	M/42	O	A	+	-	-	-	-
40	F/46	O	D	-	-	+	-	-
41	M/77	O	D	-	-	-	-	-
42	M/60	C	A	-	+	-	-	T
43	F/70	O	AWD	-	-	-	-	ND
44	F/68	O	AWD	+	+	-	-	T
45	M/76	O	A	-	+	-	-	-

M, male; F, female; ICH, immunohistochemistry; ND, not determined; T, traslocation; A, alive; D, dead; AWD, alive with disease; DO, dead from other causes; O, orbit; C, conjunctiva; LG, lachrymal gland; E, eyelid.

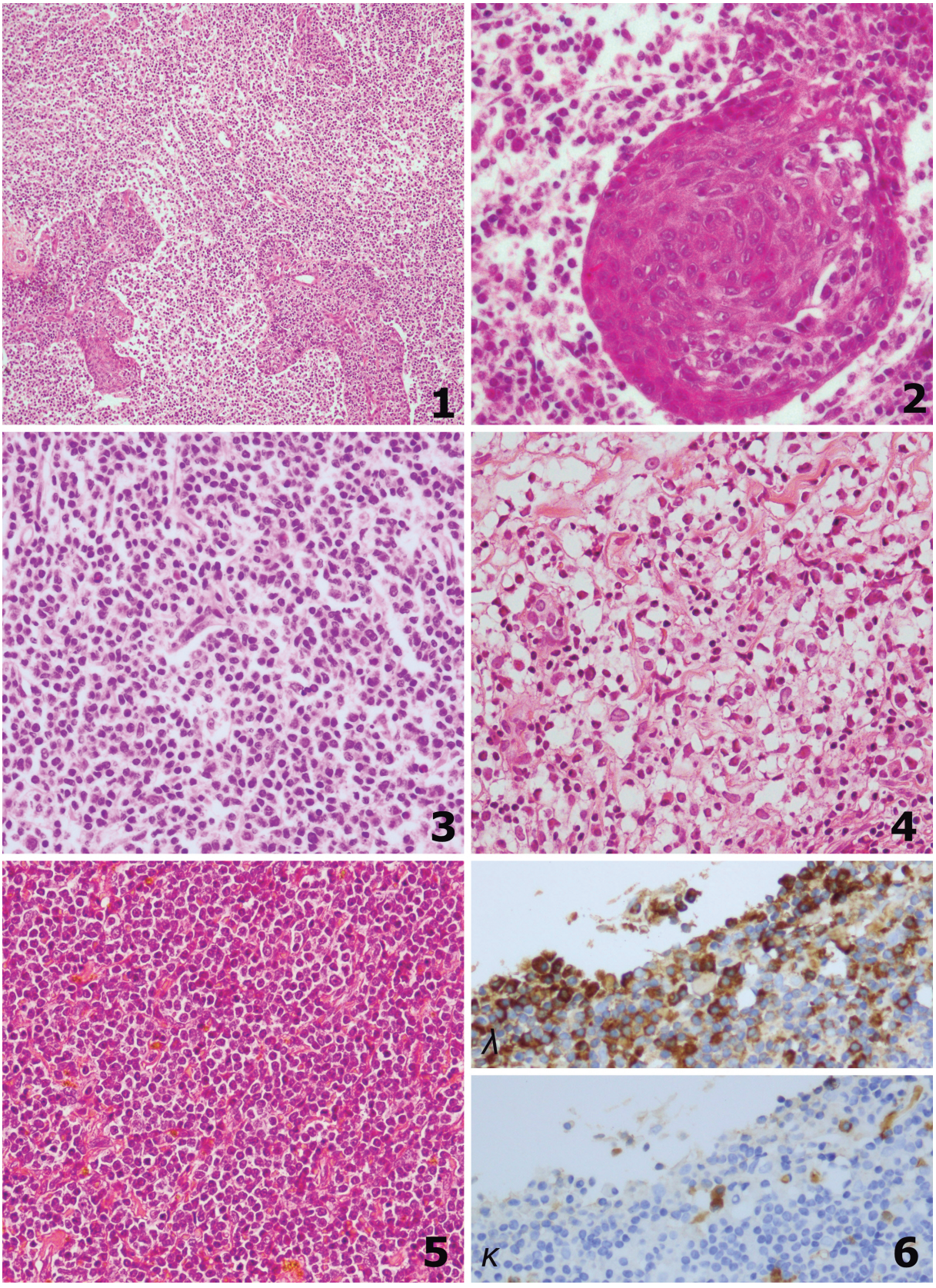

Fig. 1. 1. Infiltration of the lacrimal gland of OABLM. **2.** Lympoepithelial complex in OABLM. **3.** Prevalence of plasmacytoid cells in OABLM. **4.** Prevalence of monocutoid cells in OABLM (H&E). **5.** Prevalence of centrocytic cells in OABLM (H&E). **6.** λ light chain restriction in case 33. 1, x 10; 2-6, x 40

slides were performed on whole sections in order to compare the data.

Statistical analysis

Pearson chi-square test was used to identify any relationships between the frequencies of different markers included in this study. Differences were considered to be significant for values of $p<0.05$. Overall survival (OS) and failure-free survival (FFS) curves were calculated using the Kaplan-Meier method. Statistical significance of associations between individual variables and OS and FFS was determined using the log-rank test. All statistical analyses were performed using SPSS program.

Results

Clinical findings

The MALT lymphoma group included 45 cases (25 females and 20 males). 31 patients were older than 60 years.

The orbital region was involved in 40 cases, with lacrimal gland involvement in 9 cases, the conjunctival mucosa in 4 cases and the eyelid in just 1 case. Bone marrow involvement was observed in 5 cases.

Radiotherapy alone was used as standard in 17 cases associated with chemotherapy in 11 cases. The remaining patients underwent to chemotherapy only. Relapse or persistence of local disease was observed in 14/45 cases. Relapse was recorded at the same stem site in 7 cases and in the contralateral orbital region in 2 cases. Disease persisted after therapy in 2 cases. Dissemination was recorded in 3 cases, to loco-regional lymph nodes in 2 cases, and to the ovary in the third.

The mean OS of the series was 58 months (range from 6 to 215 months). The mean disease-specific OS was 33 months, and the mean FFS was 28 months (range from 0 to 74 months). At the end of the follow-up, 25 patients were alive without disease, 8 were alive with disease, 5 had died from causes unrelated to the disease

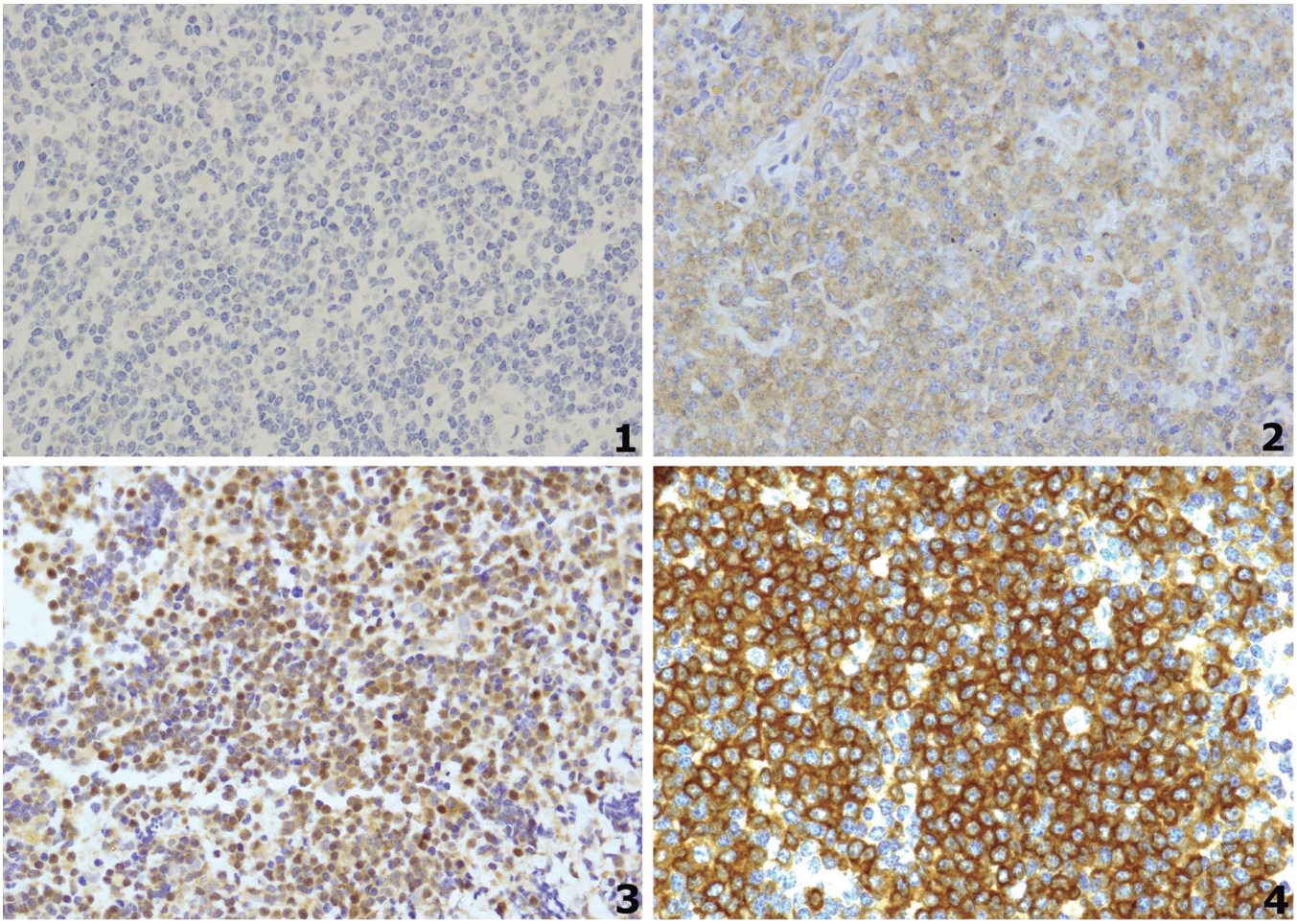

Fig. 2. BCL 10 expression. **1.** Negativity for BCL10. **2.** Low cytoplasmic expression for BCL10. **3.** High nuclear expression for BCL10. **4.** High cytoplasmic expression for BCL10. x 40

and 2 had died from causes attributable to the disease (Table 3).

Morphology and immunohistochemistry

In all cases a generally diffuse or vaguely nodular lymphoid neoplasm was observed. Residual follicles in 14/45 cases, with secondary infiltration of germinal centers by malignant lymphocytes ("follicular colonization"), were clearly evident through follicular dendritic cell CD23 positivity. Clear lymphoepitelial complex was observed in 6 out of 9 cases with involvement of the lachrymal gland. The neoplasia was mostly formed by marginal-zone cells with interspersed lymphoplasmacytoid cells. In 2 cases the neoplastic population was mainly formed by lymphoplasmacytoid cells. Rare single large cells were observed in 27 cases. No large cell clusters were observed. Neoplastic cells showed diffuse positivity for CD20 and CD79a and negativity for germinal-center markers, bcl6 and CD10. CD43 expression was observed in 33/45 cases. Light-chain restriction was demonstrated in 36 cases. In particular, 26/36 showed kappa-chain restriction and 10/36 lambda-chain restriction.

Comparison data of TMA / whole sections demonstrated a perfect correspondence

BCL10 expression and chromosomal aberrations

Among all cases evaluated, 19 showed BCL10

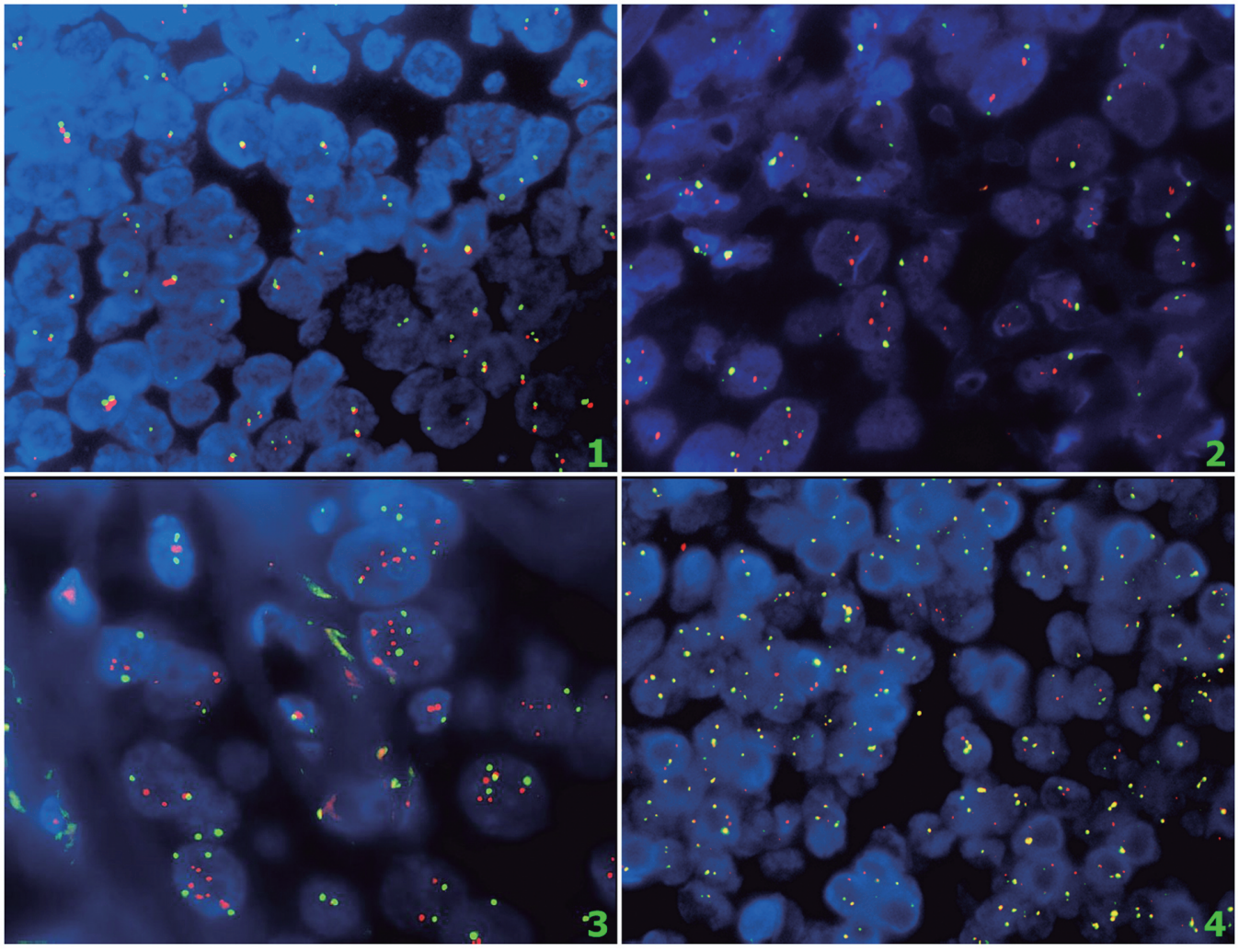

Fig. 3. FISH analysis. **1.** Normal BCL10 gene copies. The FISH DNA probe is a mixture of a Texas Red-labeled DNA probe (BCL10-UPSTREAM) covering centromeric to the *BCL10* breakpoint cluster region, and a fluorescein-labeled DNA probe (*BCL10-Dowstream)* covering telomeric to the BCL10 breakpoint cluster region. Signs of fusion are clearly evident (yellow). **2.** t (1;14) in neoplastic cells. Following the translocation, separate green and red signals are seen (split signals). **3.** BCL10 translocation and gains of 3' upstream BCL10 gene signal in tumor cells (increase of the red signal). **4.** (14,18). Using probes directed at IGH (green) and MALT1 (red), the translocation of MALT1 is demonstrated through fusing signals (yellow). x 60

expression. In particular, 12/43 (27,9%) patients exhibited an aberrant nuclear localization, 3/42 (7,1%) a strong cytoplasmic localization, 2/42 (7,1%) a moderate expression and 1/42 (2,4%) a nuclear and a moderate cytoplasmic localization.

No cases analyzed through FISH showed t (11;18). In 6/43 cases (14%) t (14;18) was detected and in 2/45 cases t (14;18) was not detectable. 2/5 t(14;18) positive cases showed BCL10 nuclear expression, while 2/5 showed cytoplasmic expression. In just one case of the six translocated the immunohistochemical investigation was not evaluable.

T(1;14) was observed in 3/31 (9,67%) and two of these cases also showed gains of 3' upstream BCL10 gene signal. Of these two cases, one showed cytoplasmic BCL10 overexpression, while the other one exhibited both cytoplasmic and nuclear positive immunoreactivity. In 14/45 cases t (1;14) was not detectable.

A break-apart signal of the IgH gene locus was confirmed in all cases with t(14;18) and in the cases with t(1;14). No other case showed IgH locus split signal (Fig. 3).

Comparison data of TMA/whole sections demonstrated a perfect correspondence.

Statistical analysis

The cases with BCL10 nuclear expression showed no significant correlation with analyzed chromosomal aberrations. Cytoplasmic expression was significantly associated with the presence of chromosomal aberrations (p=0.010), in particular with t(14;18) (q32;q21) (p=0.04).

Statistical analysis demonstrates that in univariate analysis shorter FFS significantly relates to nuclear BCL10 expression (p=0.05) (Fig. 4). Other statistical correlations between BCL10 expression and clinico-pathological parameters were not significant.

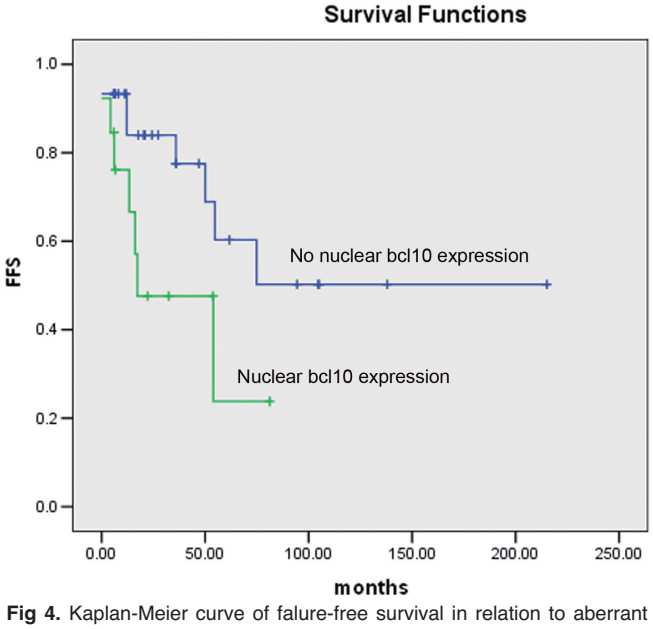

Fig 4. Kaplan-Meier curve of falure-free survival in relation to aberrant bcl10 nuclear expression (p=0.05).

Discussion

MALT lymphomas usually occur in the context of chronic inflammation, where a B-cell clonal proliferation could expand. At this stage, independently from lymphoproliferative inducing factors, genetic alterations and microenvironment might sustain clonal growth (Streubel et al., 2004; Remstein et al., 2006; Tanimoto et al., 2006; Schiby et al., 2007; Verma et al., 2008). T(1;14)(p22;q32), a rare translocation described in MALT lymphomas, leads BCL10 gene (1p22) to the immunoglobulin heavy chain locus (14q32) resulting in an overexpression of BCL10 protein, causing deregulation of the apoptotic mechanism in neoplastic cells, due to the lack of domain for the recruitment of activating apoptosis caspase. BCL10 is normally expressed in the cytoplasm. It is essential for both development and function of normal T and B cells, where it relays antigen receptor-mediated signaling, which culminates in NF-κB activation (Ruland et al., 2001; Xue et al., 2003; Schulze-Luehrmann and Ghosh, 2006). In MALT lymphomas arising in gastrointestinal sites, it is claimed that MALT1, with or without BCL10 cooperation, activates the phosphorylation cascade, leading to IκB-α phosphorylation. IκB-α is a physiological ligand of transcriptional factor NF-κB in the cytoplasm, whose phosphorylation is responsible for the NF-κB release and its migration into the nucleus, leading to up-regulation of the anti-apoptosis related proteins (Ghosh et al., 1998; Johnson et al., 1999; Karin and Ben-Neriah, 2000; Sanchez-Beato et al., 2003; Liu et al., 2004; Thome, 2004).

In the cytoplasm, overexpressed BCL10 also interacts with caspase-8 in order to form a complex that leads to pro-apoptotic caspase-8 activity suppression (Chen et al., 2012).

BCL10 expression varies among cases with different chromosomal translocations. Many studies describe the t(14;18) as the most common aberration in some series of OABMLs (Streubel et al., 2003, 2004; Franco et al., 2006), but in other series it has been found to be less frequent than other chromosomal aberrations (Sagaert et al., 2006) or even absent (Carugi et al., 2009; Wongchaowart et al., 2006). A geographic variability in the incidence of recurring cytogenetic abnormalities in OABML seems to be present in the comparison of European (Streubel et al., 2004) and American (Ruiz et al., 2007) series. Variables influencing these features should be further investigated. In previous studies t(14;18) (q32;q21) positive-MALT lymphomas significantly show strong and homogeneous BCL10

cytoplasmic expression in tumor cells (Streubel et al., 2003; Ye et al., 2005; Sagaert et al., 2006). Our results reveal a significant correlation between BCL10 cytoplasmic expression and the presence of chromosomal aberrations, in particular the t(14;18) (q32;q21), which partially confirms what has been reported in the literature. Therefore, many other unknown biological mechanisms could be responsible for BCL10 cytoplasmic overexpression. As far as BCL10 nuclear expression is concerned, it has been previously underlined that in MALT lymphomas, BCL10 is aberrantly located in the nuclei of neoplastic cells that carry t(1;14) (p22;q32) and t(11;18) (q21;q21) (Capello and Gaidano, 2000; Ye et al., 2000, 2003b; Liu et al., 2001; Kuo et al., 2004). Among our samples, only 3 cases harbor the t(1;14) and one of these showed BCL10 nuclear positivity. In other studies, nuclear positivity was not related to known chromosomal aberrations (Liu et al., 2004; Gallardo et al., 2006). It can be hypothesized that the BCL10 nuclear shuttling is independent from the translocations but it could also involve other biological mechanisms. Thus, Tumor Necrosis Factor (TNF)-α through Akt1 activation induces a molecular complex composed by phosphorilated BCL10 and BCL3, which contains a nuclear localization signal (Yeh et al., 2006; Kuo et al., 2008). In addition, a recent study revealed that, even in gastric DLBCL (MALT), B-cell activating factor of the tumor necrosis factor (TNF) family (BAFF) is associated with nuclear translocation of BCL10 (Kuo et al., 2008). BAFF causes the activation of NF-κB and AKT. Therefore, NF-κB up-regulates BCL10 and AKT generates the formation of BCL10/BCL3 complex, which shuttles to the nucleus (Kuo et al., 2008). BCL10 nuclear shuttling could also increase the activation of NF-κB -regulated genes, contributing to the *H. pylori*-independent neoplastic growth of gastric DLBCL, ex MALT lymphoma (Kuo et al., 2008). Moreover, it has been recently suggested that BCL10 could interact with transcription factor II (TFII), as a potential transcriptional activator (Liu et al., 2004).

To date our data demonstrate 2 cases with gains of the 3' upstream *BCL10* gene signal, one of which with aberrant nuclear BCL10 expression. It is interesting to remark that the level of *BCL10* gene gains was significantly heterogeneous. *BCL10* copy gain has never been described in literature so far, but gene amplification has been observed in pancreatic cancer (Ye et al., 2006) and in one case of DLBCL (Shaye and Levine, 2006).

In conclusion, we report that in OABML chromosomal aberration regarding the *BCL10* gene has been documented in our series, in contrast to what has been published in previous data. In addition a gain of 3' upstream *BCL10* gene signal has been observed. Moreover analyzed chromosomal aberrations seem to be linked to BCL10 cytoplasmic expression. Some cases with BCL10 cytoplasmic expression and BCL10 nuclear localization are not explained by known genomic mutations. Other biological mechanisms responsible for such events, playing a critical role in NF-κB activation, need further investigation.

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