Journal of Pathology
J Pathol 2005; **205**: 293–301
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/path.1715

Original Paper

MALT lymphoma with t(14;18)(q32;q21)/*IGH-MALT1* is characterized by strong cytoplasmic MALT1 and BCL10 expression

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Received: 3 August 2004
Revised: 18 October 2004
Accepted: 29 October 2004

Abstract

Mucosa-associated lymphoid tissue (MALT) lymphoma is specifically associated with t(11;18)(q21;q21), t(1;14)(p22;q32) and t(14;18)(q32;q21). t(11;18)(q21;q21) fuses the N-terminus of the *API2* gene to the C-terminus of the *MALT1* gene and generates a functional API2-MALT1 product. t(1;14)(p22;q32) and t(14;18)(q32;q21) bring the *BCL10* and *MALT1* genes respectively to the *IGH* locus and deregulate their expression. The oncogenic activity of the three chromosomal translocations is linked by the physiological role of BCL10 and MALT1 in antigen receptor-mediated *NFκB* activation. In this study, MALT1 and BCL10 expression was examined in normal lymphoid tissues and 423 cases of MALT lymphoma from eight sites, and their expression was correlated with the above translocations, which were detected by molecular and molecular cytogenetic methods. In normal B-cell follicles, both MALT1 and BCL10 were expressed predominantly in the cytoplasm, high in centroblasts, moderate in centrocytes and weak/negative in mantle zone B-cells. In MALT lymphoma, MALT1 and BCL10 expression varied among cases with different chromosomal translocations. In 9/9 MALT lymphomas with t(14;18)(q32;q21), tumour cells showed strong homogeneous cytoplasmic expression of both MALT1 and BCL10. In 12/12 cases with evidence of t(1;14)(p22;q32) or variants, tumour cells expressed MALT1 weakly in the cytoplasm but BCL10 strongly in the nuclei. In all 67 MALT lymphomas with t(11;18)(q21;q21), tumour cells expressed weak cytoplasmic MALT1 and moderate nuclear BCL10. In MALT lymphomas without the above translocations, both MALT1 and BCL10, in general, were expressed weakly in the cytoplasm. Real-time quantitative RT-PCR showed a good correlation between *MALT1* and *BCL10* mRNA expression and underlining genetic changes, with t(14;18)(q32;q21)- and t(1;14)(p22;q32)-positive cases displaying the highest *MALT1* and *BCL10* mRNA expression respectively. These results show that MALT1 expression pattern is identical to that of BCL10 in normal lymphoid tissues but varies in MALT lymphomas, with high cytoplasmic expression of both MALT1 and BCL10 characterizing those with t(14;18)(q32;q21).

Keywords: MALT lymphoma; MALT1; Bcl10; translocation; molecular cytogenetics

Introduction

MALT lymphoma is specifically associated with t(11;18)(q21;q21), t(1;14)(p22;q32), and t(14;18)(q32;q21) [1]. t(11;18)(q21;q21) fuses the N-terminus of the *API2* gene to the C-terminus of the *MALT1* gene and generates a functional API2-MALT1 product [2–4]. t(1;14)(p22;q32) and t(14;18)(q32;q21)

bring the *BCL10* and *MALT1* genes respectively to the *IGH* locus and deregulate their expression [5–8]. The oncogenic activity of these translocations is linked by the physiological role of BCL10 and MALT1 in antigen receptor-mediated NFκB activation [9–12]. In normal B-cells, in response to antigen receptor signalling, BCL10 oligomerizes and interacts with MALT1 and mediates its oligomerization, which leads to NFκB activation. In t(1;14)(p22;q32)-positive MALT lymphoma, BCL10 is believed to form oligomers via its N-terminal CARD, while in those with t(14;18)(q32;q21)/*IGH-MALT1*, MALT1 oligomerization is thought to depend on BCL10 [13,14]. In MALT lymphoma with t(11;18)(q21;q21), the API2–MALT1 fusion product is believed to self-oligomerize via the N-terminal BIR domain of the API2 molecule [13,14]. Thus, these independent translocations appear to mediate their oncogenic activities through a common pathway.

In general, the above three translocations are differentially involved in MALT lymphomas of various sites. t(11;18)(q21;q21) occurs most frequently in MALT lymphomas from the lung (40%) and stomach (30%), moderately in those from the ocular adnexae (20%), but rarely in those from the salivary gland, thyroid, and skin [15–21]. In gastric MALT lymphoma, the translocation is significantly associated with those not responding to *Heliobacter pylori* eradication [20,22,23]. However, the translocation is rarely seen in transformed MALT lymphoma [24,25].

t(1;14)(p22;q32) occurs in 5% of MALT lymphomas and is described primarily in those from the stomach and lung. Although BCL10 is expressed predominantly in the cytoplasm of normal germinal centre B-cells, the protein is strongly expressed in the nuclei of lymphoma cells with t(1;14)(p22;q32) [26]. Moderate nuclear BCL10 expression is also seen in t(1;14)(p22;q32)-negative MALT lymphomas, including nearly all those with t(11;18)(q21;q21) and up to 20% cases without t(11;18)(q21;q21) [20,21,27]. The remaining t(11;18)(q21;q21)-negative cases express BCL10 in the cytoplasm.

t(14;18)(q32;q21)/*IGH-MALT1* appears to occur more frequently in non-gastrointestinal MALT lymphomas [7,28,29]. However, its true incidence in MALT lymphoma of various sites remains unclear. In a previous study of a single case of t(14;18)(q32;q21)/*IGH-MALT1*-positive MALT lymphoma, we showed that both MALT1 and BCL10 were highly expressed in the cytoplasm of the tumour cells [8]. It is unknown whether this expression pattern characterizes MALT lymphoma with t(14;18)(q32;q21)/*IGH-MALT1*. In the present study, we examined MALT1 expression in both normal and malignant lymphoid tissues and correlated its expression with that of BCL10 and the presence of the three translocations in MALT lymphomas.

Material and methods

Materials

These included 30 normal lymphoid tissues, 490 B-cell lymphomas consisting of 423 MALT lymphomas (Table 1), 22 follicular lymphomas, 18 mantle cell lymphomas, and 27 diffuse large B-cell lymphomas (DLBCL). Of MALT lymphomas, six cases were known to be t(14;18)(q32;q21)/*IGH-MALT1* positive [7,28]. In addition, 86 normal non-lymphoid tissues of 21 types were studied. Local ethical guidelines were followed for the use of archival paraffin-embedded and frozen tissues for research and such use was approved by the local ethics committees of the authors' institutions where required.

Immunohistochemistry

MALT1 was immunostained with a mouse monoclonal antibody to its N-terminus (Genentech, USA) [8]. Where indicated, MALT1 immunohistochemistry was also performed with a mouse monoclonal antibody to its C-terminus, which was generated by immunization of mice with a C-terminal MALT1 recombinant protein (amino acids 701–808), followed by a hybridoma technique in our laboratory. N-terminal MALT1 antibody recognizes full-length MALT1 but not the API2-MALT1 fusion product, while C-terminal MALT1 antibody reacts with both MALT1 and API2-MALT1 fusion product (Figure 1). BCL10 was stained with mouse monoclonal antibody clone 151 [26].

Reverse transcription–polymerase chain reaction (RT-PCR)

t(11;18)(q21;q21) was detected by RT-PCR of the *API2-MALT1* fusion transcripts [23].

Interphase fluorescence *in situ* hybridization (FISH)

Translocations involving MALT1 were detected by interphase FISH with LSI IGH/MALT1 dual-colour, dual-fusion translocation probes and MALT1 break-apart dual-colour probes (Vysis/Abbott Laboratories Ltd, UK) [8,21].

Real-time RT-PCR

MALT1 and *BCL10* mRNA expression was quantified by real-time RT-PCR using 18S rRNA as an internal control. Total RNA was isolated from tumour cells microdissected from paraffin-embedded tissue sections [23,31]. cDNA was synthesized using random hexamer primers for *MALT1*/18S rRNA and gene-specific primer for *BCL10*/18S rRNA. Real-time PCR was performed using an iCycler iQ system (BIO-RAD, UK) with SYBR Green I. One of each primer pair was designed to span an exon–exon junction to prevent amplification of any contaminated DNA (Table 2)

Table 1. MALT 1 expression pattern in MALT lymphomas with different chromosomal translocations

Site of MALT lymphoma	Number of cases	Translocation status*	Number of cases	Intensity of MALT1 expression		
				Strong	Moderate	Weak/negative
Stomach	185	t(11;18) +ve	40	—	2	38
		t(1;14) +ve	8	—	—	8
		t(14;18) +ve	—	—	—	—
		Translocation −ve	137	—	7	130
Lung	47	t(11;18) +ve	18	—	—	18
		t(1;14) +ve	4	—	—	4
		t(14;18) +ve	3	3	—	—
		Translocation −ve	22	—	—	22
Ocular adnexae	73	t(11;18) +ve	7	—	1	6
		t(1;14) +ve	—	—	—	—
		t(14;18) +ve	5	5	—	—
		Translocation −ve	61	—	7	54
Salivary gland	59	t(11;18) +ve	1	—	—	1
		t(1;14) +ve	—	—	—	—
		t(14;18) +ve	—	—	—	—
		Translocation −ve	58	—	8	50
Thyroid	12	t(11;18) +ve	—	—	—	—
		t(1;14) +ve	—	—	—	—
		t(14;18) +ve	—	—	—	—
		Translocation −ve	12	—	2	10
Skin	37	t(11;18) +ve	—	—	—	—
		t(1;14) +ve	—	—	—	—
		t(14;18) +ve	—	—	—	—
		Translocation −ve	37	—	9	28
Liver	6	t(11;18) +ve	—	—	—	—
		t(1;14) +ve	—	—	—	—
		t(14;18) +ve	1	1	—	—
		Translocation −ve	5	—	—	5
Intestine	4	t(11;18) +ve	1	—	—	1
		t(1;14) +ve	—	—	—	—
		t(14;18) +ve	—	—	—	—
		Translocation −ve	3	—	—	3
Total	423			9(2.1%)	36	378

* t(14;18)(q32;q21)/*IGH-MALT1* and *BCL10* break/t(1;14)(p22;q21) were primarily detected by MALT1 and BCL10 immunohistochemistry followed by interphase FISH with appropriate probes. t(11;18)(q21;q21)/*API2-MALT1*-positive cases were detected by RT-PCR of the *API2-MALT1* fusion transcript, with the exception of one pulmonary case that was initially identified by interphase FISH with MALT1 break apart probes.

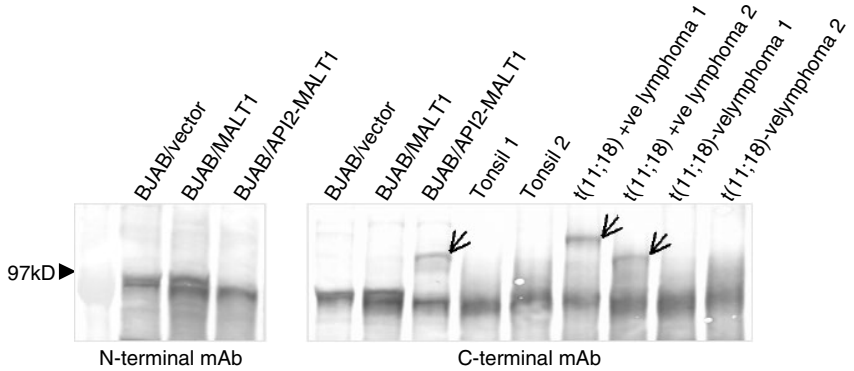

Figure 1. Western blot analysis of human B-cell lymphoma cells (BJAB) transfected with MALT1 or API2-MALT1 expression constructs [30], and MALT lymphoma with and without t(11;18)(q21;q21). The mouse monoclonal antibody (mAb) to the N-terminus of the MALT1 recognizes full-length MALT1, but not the API2-MALT1 fusion product, while the mouse monoclonal antibody to the C-terminus of MALT1 reacts with full-length MALT1 as well as the API2-MALT1 fusion product (indicated by arrows)

[32]. The primers for the *MALT1* gene target its N-terminus and therefore will only amplify wild-type *MALT1* but not *API2-MALT1* transcripts.

The conditions for real-time PCR were optimized prior to data collection. The specificity of the RT-PCR products for each primer set was confirmed by melt-curve analysis. The standard curves were generated by twofold serial dilutions of 100 ng/μl *MALT1* cDNA and 1 ng/μl 18S rRNA cDNA prepared from fresh frozen tonsils, and 100 ng/μl *BCL10* cDNA and

1 ng/μl 18S rRNA cDNA prepared from t(1;14)(p22;q32)-positive frozen tumour tissues. The average coefficient value (R^2) for each standard curve was above 0.99 and the relative efficiency of amplification of *MALT1* and *BCL10* was close to that of 18S rRNA since the absolute value of the slope of log-input amount of cDNA versus ΔC_T was below 0.1.

Once the experimental conditions had been optimized, real-time PCR was performed in a 25 μl reaction mixture containing 12.5 μl SYBR Green Super-Mix (BIO-RAD), 200 nM of each sense and anti-sense primer, and 100 ng (*MALT1* and *BCL10*) or 1 ng (18S rRNA) cDNA. All samples were amplified in triplicate using the following parameters: denaturation at 95 °C for 3 min and annealing and extension at 60 °C for 1 min. Real-time PCR of 18S rRNA was run in parallel for each sample. Melt-curve analysis was performed immediately after the amplification protocol for each case and only samples that showed specific amplification were included in the data analysis. The C_T numbers were obtained from each sample and ΔC_T value was calculated by subtracting the C_T value of 18S rRNA from the C_T value of *MALT1* or *BCL10*.

Statistical analysis

Mann–Whitney *U*-test was used to compare *MALT1* and *BCL10* mRNA expression between MALT lymphomas with different translocations. Pearson's correlation test was used for analysis of the relationship between *MALT1* and *BCL10* mRNA expression.

Results

MALT1 expression in normal lymphoid tissues

Immunohistochemistry with both N-terminal and C-terminal MALT1 antibodies showed that the protein expression pattern was identical to that of BCL10 in both B-cell follicles and thymus [26]. In B-cell follicles of tonsil, lymph node and spleen, both MALT1 and BCL10 are differentially expressed in various germinal centre B-cells, strong in centroblasts, moderate in centrocytes, and weak/negative in mantle zone B-cells (Figure 2). In thymus, MALT1 was weakly positive, while BCL10 was moderately expressed in medullary T-cells. Irrespective of different cell types, both MALT1 and BCL10 are predominantly expressed in the cytoplasm.

MALT1 protein expression appeared to be restricted to lymphoid tissues. It was not found by immunohistochemistry in 21 types of normal tissue, including tongue, oesophagus, duodenum, rectum, liver, gall-bladder, pancreas, bronchus, heart, lung, thyroid, breast, adrenal gland, kidney, bladder, uterus, cervix, ovary, placenta, testis, and skin.

Table 2. Primers used for real-time quantitative PCR of *MALT1* and *BCL10* mRNA

Genes	Primer sequence	Amplicon size (bp)
MALT1*	sense 5′ ctc cgc ctc agt tgc cta ga	104
	anti-sense 5′ caa cct ttt tca ccc att aac ttc a	
BCL10	sense 5′ gaa gtg aag aag gac gcc tta g	80
	anti-sense 5′ aga tga tca aaa tgt ctc tca gc	
18SrRNA	sense 5′ tga ctc aac acg gga aac c	114
	anti-sense 5′ tcg ctc cac caa cta aga ac	

* The primers for the *MALT1* gene were designed to target its N-terminus (nucleotides 363–466 according to its cDNA sequence AF130356), and therefore will only amplify the wild-type *MALT1* but not the *API2-MALT1* transcripts.

MALT1 expression in malignant lymphoma

MALT1 expression in malignant lymphomas was investigated with the N-terminal MALT1 antibody. As reactive lymphoid follicles are commonly seen in MALT lymphoma, MALT1 and BCL10 expression in B-cells of reactive germinal centres provides an excellent internal control. The level of MALT1 and BCL10 expression in lymphoma cells was therefore recorded with reference to that in centroblasts (strong), centrocytes (moderate), and mantle zone B-cells (weak) of reactive lymphoid follicles.

In a previous study of a single case of t(14;18)(q32;q21)/*IGH-MALT1*-positive MALT lymphoma, we showed that both MALT1 and BCL10 were highly expressed in the cytoplasm of the tumour cells [8]. To examine whether strong cytoplasmic expression of both MALT1 and BCL10 characterizes MALT lymphoma with t(14;18)(q32;q21)/*IGH-MALT1*, we studied the expression of these proteins in 423 cases of MALT lymphoma. Among these cases, 364 had data for BCL10 expression, t(1;14)(p22;q32), and t(11;18)(q21;q21) from previous studies [21]. For the remaining cases, these data were collected during the present study. t(11;18)(q21;q21) was detected by RT-PCR of the *API2-MALT1* fusion transcript, while t(1;14)(p22;q32) or variants were screened by BCL10 immunohistochemistry followed by interphase FISH [21].

In total, 12 cases showed strong BCL10 nuclear staining, and 10 of them showed evidence of t(1;14)(p22;q32) by conventional cytogenetics or interphase FISH. None of these cases showed BCL10 gene amplification. Tumour cells in each of the above cases showed weak/negative MALT1 cytoplasmic staining (Figure 2, Table 1). Sixty-seven cases were t(11;18)(q21;q21) positive and tumour cells in these cases showed moderate or weak BCL10 nuclear staining (Figure 2, Table 1). Of the remaining 344 cases lacking t(1;14)(p22;q32) and t(11;18)(q21;q21), nine cases (ocular adnexae five, lung three, and liver one) showed strong homogeneous cytoplasmic expression of both MALT1 and BCL10 in virtually all tumour cells. The intensity of MALT1 staining was similar to that seen in centroblasts of the reactive germinal centre (Figure 2). Of the remaining cases, 33 showed

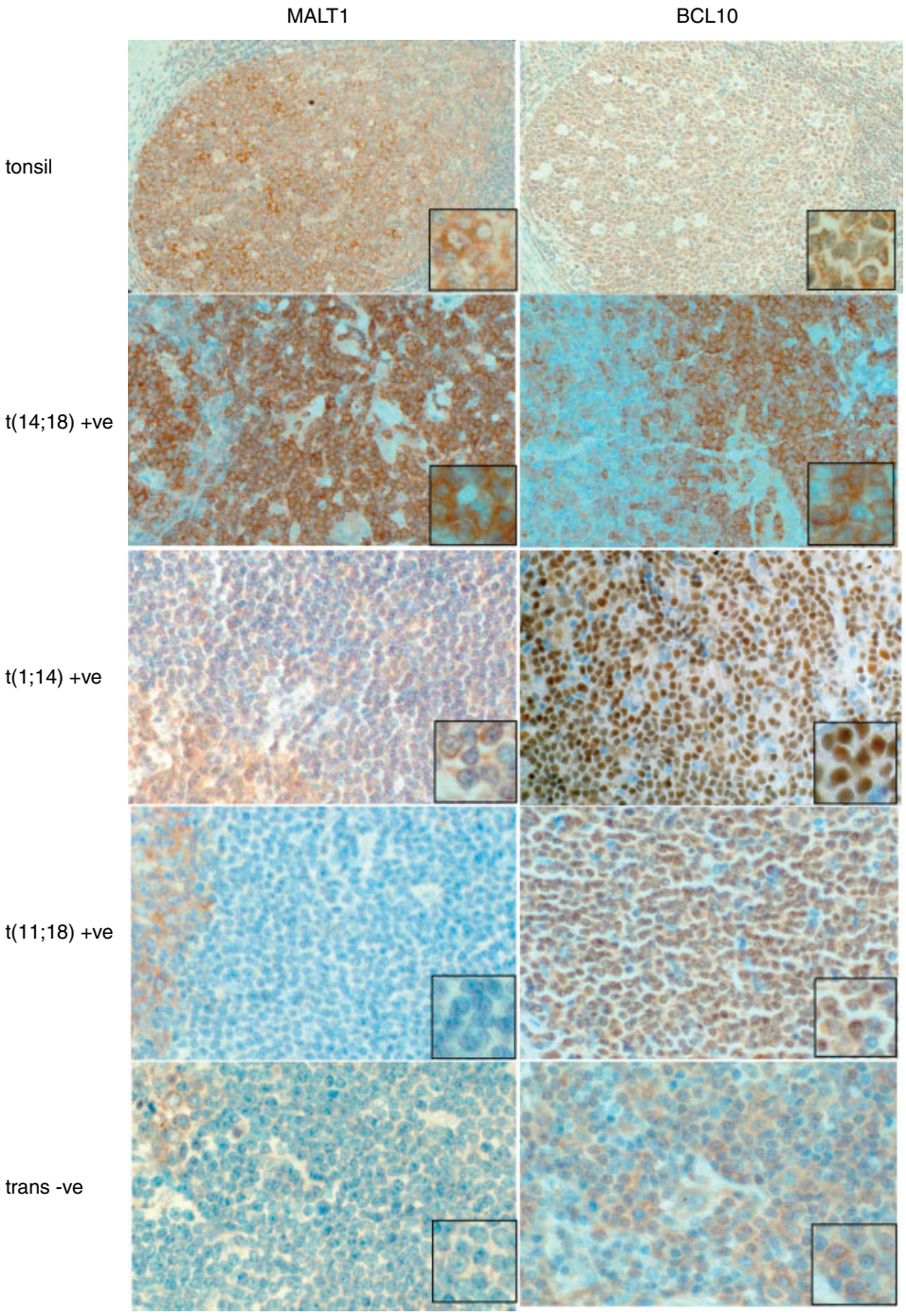

Figure 2. MALT1 and BCL10 expression in reactive tonsil and MALT lymphomas with and without chromosomal translocations. Both MALT1 and BCL10 are similarly expressed in the cytoplasm of various B-cells in reactive tonsil, high in centroblasts, moderate in centrocytes and weak/negative in the mantle zone B-cells. In MALT lymphoma with t(14;18)(q32;q21)/*IGH-MALT1*, all tumour cells show strong MALT1 and BCL10 cytoplasmic expression. In MALT lymphoma with *BCL10* break/t(1;14)(p22;q32), tumour cells express weak MALT1 but strong nuclear BCL10. In MALT lymphoma with t(11;18)(q21;q21)/*API2-MALT1*, tumour cells generally show lack of MALT1 expression but moderate nuclear BCL10. In MALT lymphoma without the above chromosomal translocations, tumour cells show weak MALT1 and moderate BCL10 expression in the cytoplasm. FC, follicle centre

moderate and 302 displayed weak/negative MALT1 cytoplasmic staining (Table 1).

Of the nine cases that showed strong homogeneous cytoplasmic expression of both MALT1 and BCL10, six were t(14;18)(q32;q21) positive, as shown in previous studies [7,28]. For the remaining three cases, we performed interphase FISH using *IGH/MALT1* dual-colour, dual-fusion translocation probes. In each case, signal patterns indicating the presence of the translocation were detected (Figure 3).

To investigate further that t(14;18)(q32;q21)/*IGH-MALT1* was truly negative in MALT lymphoma lacking high cytoplasmic expression of MALT1 and BCL10, we performed interphase FISH using *MALT1* break-apart dual-colour probes. The reliability of the *MALT1* break-apart assay for the detection of

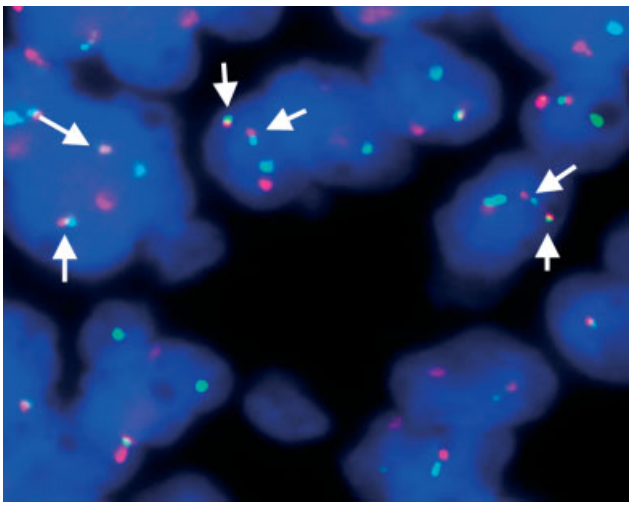

Figure 3. Detection of t(14;18)/*IGH-MALT1* by interphase FISH with dual-colour, dual-fusion translocation probes. An ocular MALT lymphoma with strong cytoplasmic expression of both MALT1 and BCL10 shows co-localization of green and red signals in interphase nuclei, suggestive of t(14;18)/*IGH-MALT1*

chromosomal translocations involving *MALT1* was first validated in 34 t(11;18)(q21;q21)-positive, 5 t(14;18)(q32;q21)/*IGH-MALT1*-positive MALT lymphomas and five negative controls. The *MALT1* break-apart assay showed translocations involving *MALT1* in 39/39 positive cases but not in any of the negative controls.

The *MALT1* break-apart assay was performed in 174 cases of MALT lymphoma (salivary gland 48, ocular adnexae 45, lung 23, stomach 31, thyroid 12, skin 12, liver two and small intestine one) lacking evidence of t(11;18)(q21;q21) and t(1;14)(p22;q32) or variants. With the exception of one case, none of the remaining cases displayed evidence of a breakpoint affecting the *MALT1* gene. The only case that showed a breakpoint at the *MALT1* locus was from lung and subsequently confirmed to be t(11;18)(q21;q21) by interphase FISH with *API2/MALT1* dual-colour, dual-fusion translocation probes. This case was not detected by RT-PCR on RNA samples prepared from paraffin-embedded tissues. It is known that the RT-PCR protocol used misses 7% of rare breakpoints on the *API2* gene [21,23]. Nonetheless, 28 cases showed three copies of the *MALT1* gene in more than 80% of tumour cells, but only three of them displayed moderate MALT1 expression, with the remaining cases exhibiting weak MALT1 expression. There was no evidence of *MALT1* gene amplification in all the cases examined. Figure 4 summarizes the frequencies of t(14;18)(q32;q21)/*IGH-MALT1*, t(11;18)(q21;q21), and t(1;14)(p22;q32) in MALT lymphomas from various sites.

Of the 22 follicular lymphomas examined, 17 showed moderate to strong cytoplasmic MALT1 staining, and the remaining five displayed weak staining. In mantle cell lymphoma, MALT1 expression was weak in 14 and moderate in four cases. Among the 27 DLBCLs studied, 20 showed moderate to strong MALT1 expression and the remaining seven displayed weak staining.

Correlation of MALT1 and BCL10 protein expression with their mRNA expression

In keeping with MALT1 protein expression, *MALT1* mRNA expression was the highest in MALT lymphomas with t(14;18)(q32;q21)/*IGH-MALT1*, significantly higher than those with t(11;18)(q21;q21) or without any of the chromosomal translocations studied (Figure 5). Interestingly, *MALT1* mRNA expression was also significantly higher in MALT lymphomas without any of the chromosomal translocations than in those with t(11;18)(q21;q21) (Figure 5).

Similarly, *BCL10* mRNA expression was the highest in MALT lymphomas with t(1;14)(p22;q32), significantly higher than in those with t(14;18)(q32;q21)/

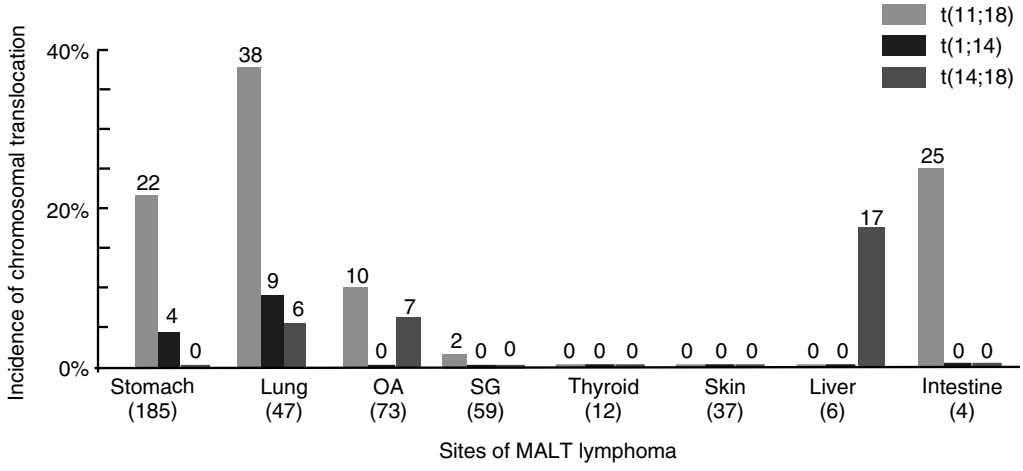

Figure 4. Frequency of t(14;18)(q32;q21)/*IGH-MALT1, BCL10* break/t(1;14)(p22;q32) and t(11;18)(q21;q21)/*API2-MALT1* in MALT lymphomas from various sites. OA, Ocular adnexae; SG, salivary gland. Numbers in parentheses indicate the number of cases studied. t(14;18)(q32;q21)/*IGH-MALT1* and *BCL10* break/t(1;14)(p22;q32) were detected by MALT1 and BCL10 immunohistochemistry followed by interphase FISH. t(11;18)(q21;q21)/*API2-MALT1* was primarily detected by RT-PCR of the *API2-MALT1* fusion transcript, with the exception of one pulmonary case that was identified by interphase FISH with MALT1 break apart probes

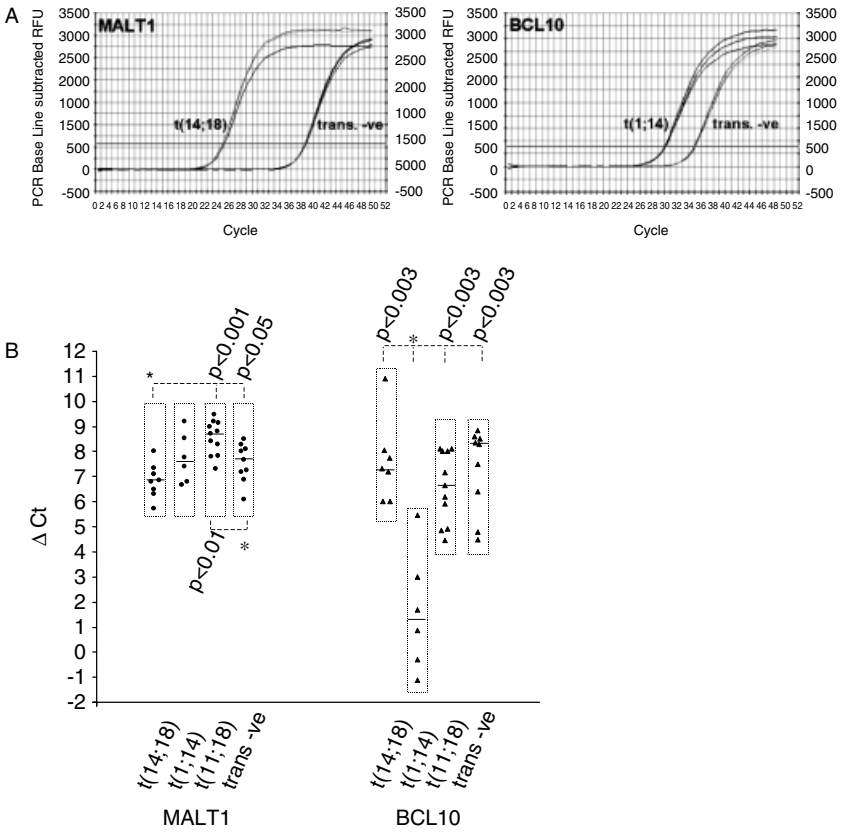

Figure 5. *MALT1* and *BCL10* mRNA expression in MALT lymphoma with different chromosomal translocations. (A) Examples of real-time quantitative RT-PCR with SYBR Green I using an iCycler iQ system. This was carried out in triplicate using RNA samples extracted from tumour cells microdissected from paraffin-embedded tissue sections. For simplicity, reference control 18S rRNA is not shown in the figure. *y*-axis, relative fluorescent units; *x*-axis, number of PCR cycles; trans. −ve, translocation-negative case. (B) Comparison of *MALT1* and *BCL10* mRNA expression in MALT lymphomas with different chromosomal translocation status. Asterisk indicates statistically significant difference. The medians are indicated by horizontal bars in the rectangular boxes

IGH-MALT1 or t(11;18)(q21;q21) or without any of these translocations (Figure 5). There was no correlation between *MALT1* and *BCL10* mRNA expression in individual groups with different chromosomal translocation status or in all groups combined together.

Discussion

Mounting evidence indicates that BCL10 and MALT1 specifically transduce antigen receptor signalling to activate NFκB and play a critical role in the biology of B- and T-cells [33]. This is best demonstrated in BCL10-and MALT1-deficient mice, which are characterized by impaired B-cell development and function, showing a reduced number of marginal zone B-cells and poor humoral responses to both T-cell-dependent and -independent stimulation [9–12]. It is believed that, in normal B- and T-cells, upon antigen receptor stimulation, Carma1 is activated to recruit BCL10 via CARD–CARD interaction and induces BCL10 oligomerization. BCL10 then binds the Ig-like domain of MALT1 and induces MALT1 oligomerization, subsequently leading to NFκB activation [13,34]. The finding of identical expression pattern of MALT1 and BCL10 in B-cell follicles is in line with their roles in B-cell activation and maturation.

One of the remarkable findings of the present study is the characteristic expression pattern of MALT1 and BCL10 in MALT lymphoma with different translocations. In those with t(14;18)(q32;q21)/*IGH-MALT1*, the tumour cells are characterized by strong cytoplasmic expression of both MALT1 and BCL10, while MALT lymphoma cells with t(1;14)(p22;q32) or t(11;18)(q21;q21) show strong or moderate BCL10 nuclear expression respectively, but generally weak MALT1 cytoplasmic expression. Such differential MALT1 and BCL10 expression patterns in MALT lymphoma with various translocations may reflect not only the consequence of these translocations but also the molecular mechanisms involved.

In MALT lymphoma with t(14;18)(q32;q21)/*IGH-MALT1*, strong cytoplasmic MALT1 expression is expected given the strong transcriptional activity of the *IGH* enhancer. The strong cytoplasmic BCL10 expression is, to some extent, a surprising finding, but this could well be explained by the molecular mechanism of MALT1-mediated NFκB activation. MALT1 lacks structural domains that are capable of mediating its self-oligomerization and over-expression of MALT1 alone in fibroblasts does not activate NFκB [13,14,34]. However, MALT1 is synergistic with BCL10 in NFκB activation and it is believed

that the oligomerization and activation of MALT1 depend on BCL10 [14]. Thus, it is likely that, in lymphoma cells with t(14;18)(q32;q21)/*IGH-MALT1*, MALT1 interacts with BCL10 and stabilizes it in the cytoplasm, consequently leading to its accumulation. In line with this notion, no alteration in *BCL10* mRNA expression was seen in MALT lymphoma with t(14;18)(q32;q21)/*IGH-MALT1* as compared with other MALT lymphomas lacking a *BCL10*-associated translocation.

Similarly, in view of the direct interaction between BCL10 and MALT1 in their mediated NFκB activation, one may expect to see MALT1 protein accumulation in lymphoma cells with t(1;14)(p22;q32). Intriguingly, this is not the case: MALT1 is only weakly expressed in the cytoplasm of t(1;14)(p22;q32)-positive cells, in contrast to strong BCL10 expression in the nuclei. Such differential expression of the two proteins in terms of both level and subcellular localization suggests that MALT1 may not be required for BCL10 function. This is supported by knockout mice studies. While BCL10 is essential for antigen receptor mediated NFκB activation in both B- and T-cells, deficiency of MALT1 expression does not critically affect BCL10-mediated NFκB activation in B-cells as it does in T-cells [9,12]. It is believed that there is an alternative pathway in BCL10-mediated NFκB activation in B-cells, which is MALT1 independent. In addition, BCL10 plays an extra role during neurodevelopment, indicating that BCL10 has additional biological activity compared with MALT1 [9].

Similar to t(1;14)(p22;q32)-positive MALT lymphoma, tumour cells with t(11;18)(q21;q21) generally show weak/negative MALT1 cytoplasmic expression but moderate BCL10 nuclear expression. Given that oligomerization of the API2-MALT1 fusion product is most likely mediated by the N-terminal BIR domains of the API2 molecule, API2-MALT1-mediated NFκB activation is unlikely to require BCL10 or MALT1. In addition, only one allele of the intact *MALT1* gene remains in MALT lymphoma with t(11;18)(q21;q21) and the level of *MALT1* mRNA is much lower in these tumours than in those without this translocation. Hence, weak MALT1 staining in MALT lymphoma with t(11;18)(q21;q21) is expected. However, moderate BCL10 nuclear staining is a surprising finding. The mechanism underlying BCL10 nuclear expression is unclear. Nonetheless, this is unlikely to be related to the level of *BCL10* mRNA expression since there was no significant difference in its transcript expression between cases expressing nuclear BCL10 (excluding those with BCL10-involved chromosomal translocation) or those expressing cytoplasmic BCL10.

As shown previously, strong nuclear BCL10 staining is highly indicative of the presence of t(1;14)(p22;q32) or variants. In the present study, we further showed that high levels of cytoplasmic expression of both MALT1 and BCL10 characterize MALT lymphoma with t(14;18)(q32;q21)/*IGH-MALT1*. This characteristic MALT1 and BCL10 expression pattern was seen in 9/9 MALT lymphomas with t(14;18)(q32;q21)/*IGH-MALT1*. The absence of t(14;18)(q32;q21)/*IGH-MALT1* in MALT lymphoma lacking strong MALT1 and BCL10 cytoplasmic expression was further confirmed by interphase FISH analyses of 174 t(11;18)(q21;q21)- and t(1;14)(p22;q32)-negative cases. Based on MALT1 and BCL10 immunohistochemistry, followed by interphase FISH analysis, we have demonstrated t(14;18)(q32;q21)/*IGH-MALT1* in MALT lymphoma of the lung (6%), ocular adnexae (7%), and liver (17%) but not in those of the stomach, salivary gland, thyroid, and skin. These findings are in line with previous reports [7,28,29,35]: the translocation is mutually exclusive from t(11;18)(q21;q21) and *BCL10* break/t(1;14)(p22;q32), and occurs more frequently in extra-gastrointestinal sites.

In view of the characteristic BCL10 and MALT1 expression patterns in MALT lymphoma with different translocations, BCL10 and MALT1 immunohistochemistry may be used for screening for these translocations. Since both BCL10 and MALT1 expression patterns in MALT lymphomas with t(1;14)(p22;q32) or t(14;18)(q32;q21)/*IGH-MALT1* are characteristic and the incidence of both translocations is relatively infrequent in MALT lymphoma, it would be rational to screen for these translocations first by BCL10 and MALT1 immunohistochemistry, followed by confirmation with interphase FISH. For MALT lymphoma with t(11;18)(q21;q21), BCL10 and MALT1 immunohistochemistry does not provide a strong indication for the presence of the translocation as up to 20% of t(11;18)(q21;q21)-negative cases also show moderate BCL10 nuclear expression. Detection of this translocation is best carried out by RT-PCR or interphase FISH.

In summary, we have shown that MALT1 expression pattern is identical to that of BCL10 in normal lymphoid tissues but varies in MALT lymphomas, with high levels of cytoplasmic expression of both MALT1 and BCL10 characterizing those with t(14;18)(q32;q21)/*IGH-MALT1*.

Acknowledgements

The study was supported by research grants from the Leukaemia Research Fund, UK, the Leukemia and Lymphoma Society, USA, and the Deutsche Krebshilfe.

References

1. Isaacson PG, Du MQ. MALT lymphoma: from morphology to molecules. *Nat Rev Cancer* 2004; **4**: 644–653.
2. 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 mucosa-associated lymphoid tissue lymphomas. *Blood* 1999; **93**: 3601–3609.
3. Akagi T, Motegi M, Tamura A, *et al*. A novel gene, *MALT1* at 18q21, is involved in t(11;18) (q21;q21) found in low-grade B-cell lymphoma of mucosa-associated lymphoid tissue. *Oncogene* 1999; **18**: 5785–5794.
4. Morgan JA, Yin Y, Borowsky AD, *et al*. Breakpoints of the t(11;18)(q21;q21) in mucosa-associated lymphoid tissue (MALT)

lymphoma lie within or near the previously undescribed gene *MALT1* in chromosome 18. *Cancer Res* 1999; **59**: 6205–6213.
5. 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.
6. Zhang Q, Siebert R, Yan M, *et al*. Inactivating mutations and overexpression of BCL10, a caspase recruitment domain-containing gene, in MALT lymphoma with t(1;14)(p22;q32). *Nat Genet* 1999; **22**: 63–68.
7. Streubel B, Lamprecht A, Dierlamm J, *et al*. T(14;18)(q32;q21) involving *IGH* and *MALT1* is a frequent chromosomal aberration in MALT lymphoma. *Blood* 2003; **101**: 2335–2339.
8. Sanchez-Izquierdo D, Buchonnet G, Siebert R, *et al*. *MALT1* is deregulated by both chromosomal translocation and amplification in B-cell non-Hodgkin lymphoma. *Blood* 2003; **101**: 4539–4546.
9. Ruland J, Duncan GS, Elia A, *et al*. Bcl10 is a positive regulator of antigen receptor-induced activation of NF-kappaB and neural tube closure. *Cell* 2001; **104**: 33–42.
10. Xue L, Morris SW, Orihuela C, *et al*. Defective development and function of Bcl10-deficient follicular, marginal zone and B1 B cells. *Nat Immunol* 2003; **4**: 857–865.
11. Ruefli-Brasse AA, French DM, Dixit VM. Regulation of NF-kappaB-dependent lymphocyte activation and development by paracaspase. *Science* 2003; **302**: 1581–1584.
12. Ruland J, Duncan GS, Wakeham A, Mak TW. Differential requirement for MALT1 in T and B cell antigen receptor signaling. *Immunity* 2003; **19**: 749–758.
13. Lucas PC, Yonezumi M, Inohara N, *et al*. Bcl10 and MALT1, independent targets of chromosomal translocation in MALT lymphoma, cooperate in a novel NF-kappa B signaling pathway. *J Biol Chem* 2001; **276**: 19 012–19 019.
14. McAllister-Lucas LM, Inohara N, Lucas PC, *et al*. Bimp1, a MAGUK family member linking protein kinase C activation to Bcl10-mediated NF-kappaB induction. *J Biol Chem* 2001; **276**: 30 589–30 597.
15. Remstein ED, James CD, Kurtin PJ. Incidence and subtype specificity of API2-MALT1 fusion translocations in extranodal, nodal, and splenic marginal zone lymphomas. *Am J Pathol* 2000; **156**: 1183–1188.
16. Baens M, Maes B, Steyls A, *et al*. The product of the t(11;18), an API2-MLT fusion, marks nearly half of gastric MALT type lymphomas without large cell proliferation. *Am J Pathol* 2000; **156**: 1433–1439.
17. Motegi M, Yonezumi M, Suzuki H, *et al*. *API2-MALT1* chimeric transcripts involved in mucosa-associated lymphoid tissue type lymphoma predict heterogeneous products. *Am J Pathol* 2000; **156**: 807–812.
18. Nakamura T, Nakamura S, Yonezumi M, *et al*. *Helicobacter pylori* and the t(11;18)(q21;q21) translocation in gastric low-grade B-cell lymphoma of mucosa-associated lymphoid tissue type. *Jpn J Cancer Res* 2000; **91**: 301–309.
19. Kalla J, Stilgenbauer S, Schaffner C, *et al*. Heterogeneity of the API2-MALT1 gene rearrangement in MALT-type lymphoma. *Leukemia* 2000; **14**: 1967–1974.
20. Liu H, Ye H, Dogan A, *et al*. T(11;18)(q21;q21) is associated with advanced mucosa-associated lymphoid tissue lymphoma that expresses nuclear BCL10. *Blood* 2001; **98**: 1182–1187.
21. Ye H, Liu H, Attygalle A, *et al*. Variable frequencies of t(11;18)(q21;q21) in MALT lymphomas of different sites: significant association with CagA strains of *H pylori* in gastric MALT lymphoma. *Blood* 2003; **102**: 1012–1018.
22. Liu H, Ruskone Fourmestraux A, Lavergne-Slove A, *et al*. Resistance of t(11;18) positive gastric mucosa-associated lymphoid tissue lymphoma to *Helicobacter pylori* eradication therapy. *Lancet* 2000; **357**: 39–40.
23. Liu H, Ye H, Ruskone-Fourmestraux A, *et al*. T(11;18) is a marker for all stage gastric MALT lymphomas that will not respond to *H. pylori* eradication. *Gastroenterology* 2002; **122**: 1286–1294.
24. Chuang SS, Lee C, Hamoudi RA, *et al*. High frequency of t(11;18) in gastric mucosa-associated lymphoid tissue lymphomas in Taiwan, including one patient with high-grade transformation. *Br J Haematol* 2003; **120**: 97–100.
25. Remstein ED, Kurtin PJ, James CD, *et al*. Mucosa-associated lymphoid tissue lymphomas with t(11;18)(q21;q21) and mucosa-associated lymphoid tissue lymphomas with aneuploidy develop along different pathogenetic pathways. *Am J Pathol* 2002; **161**: 63–71.
26. Ye H, Dogan A, Karran L, *et al*. BCL10 expression in normal and neoplastic lymphoid tissue: nuclear localization in MALT lymphoma. *Am J Pathol* 2000; **157**: 1147–1154.
27. Maes B, Demunter A, Peeters B, Wolf-Peeters C. BCL10 mutation does not represent an important pathogenic mechanism in gastric MALT-type lymphoma, and the presence of the *API2-MLT* fusion is associated with aberrant nuclear BCL10 expression. *Blood* 2002; **99**: 1398–1404.
28. Remstein ED, Kurtin PJ, Einerson RR, Paternoster SF, Dewald GW. Primary pulmonary MALT lymphomas show frequent and heterogeneous cytogenetic abnormalities, including aneuploidy and translocations involving *API2* and *MALT1* and *IGH* and *MALT1* . *Leukemia* 2004; **18**: 156–160.
29. Murga Penas EM, Hinz K, Roser K, *et al*. Translocations t(11;18)(q21;q21) and t(14;18)(q32;q21) are the main chromosomal abnormalities involving MLT/MALT1 in MALT lymphomas. *Leukemia* 2003; **17**: 2225–2229.
30. Ho L, Davis RE, Conne B, *et al*. MALT1 and -MALT1 confer proliferative advantage and resistance against FAS-induced cell death in B cells. *Blood* (in press).
31. Pan LX, Diss TC, Peng HZ, Isaacson PG. Clonality analysis of defined B-cell populations in archival tissue sections using microdissection and the polymerase chain reaction. *Histopathology* 1994; **24**: 323–327.
32. Liu H, Huang X, Zhang Y, *et al*. Archival fixed histologic and cytologic specimens including stained and unstained materials are amenable to RT-PCR. *Diagn Mol Pathol* 2002; **11**: 222–227.
33. Thome M. CARMA1, BCL-10 and MALT1 in lymphocyte development and activation. *Nat Rev Immunol* 2004; **4**: 348–359.
34. Uren GA, O'Rourke K, Aravind L, *et al*. Identification of paracaspases and metacaspases: two ancient families of caspase-like proteins, one of which plays a key role in MALT lymphoma. *Mol Cell* 2000; **6**: 961–967.
35. Streubel B, Huber D, Wohrer S, Chott A, Raderer M. Frequency of chromosomal aberrations involving MALT1 in mucosa-associated lymphoid tissue lymphoma in patients with Sjogren's syndrome. *Clin Cancer Res* 2004; **10**: 476–480.