J. Clin. Exp. Hematopathol
Vol. 46, No. 1, Mar 2006

Original Article

p27^{Kip1} is Detected on Most Gastric MALT Lymphomas, but not Large Cell Lymphomas

Hiaki Sato[1,2], Yumiko Sato[1], Koichi Ichimura[1], Takashi Oka[1], Eisaku Kondo[1], Takehiro Tanaka[1], Takami Kondo[1], Nobuya Ohara[1], Kiyoshi Takahashi[2] and Tadashi Yoshino[1]

We investigated the relationship of gastric mucosa-associated lymphoid tissue (MALT) lymphoma tumorigenesis to *Helicobacter pylori* infection, the t (11 ; 18) translocation, and alterations in cell cycle regulators. We sought to assess the implications of altered expression of p27^{Kip1}, a cyclin-dependent kinase inhibitor, on high-grade transformation and responsiveness to eradication therapy. We used immunohistochemistry to examine p27^{Kip1}, p53, and Ki-67 expression in 23 MALT lymphomas, five diffuse large B-cell lymphomas (DLBCLs), and four DLBCLs with associated MALT lymphoma. All of the MALT lymphomas were positive for p27^{Kip1} expression and negative for p53 with a low Ki-67 index, regardless of the sensitivity of these cells to eradication. All DLBCLs were negative for p27^{Kip1} and positive for p53, exhibiting a high Ki-67 index. In DLBCLs with MALT lymphoma, p27^{Kip1} expression was absent from both the MALT and large cells components. In all of these lymphomas, the MALT components were negative for p53 and displayed a low Ki-67 index, while the large cell components were positive for p53 with a high Ki-67 index. The expression patterns of the DLBCLs differed significantly from those of the MALT lymphomas. p27^{Kip1} was not detected in either component of DLBCL with MALT lymphoma, suggesting that decreased expression of p27^{Kip1} in the MALT component may be related to high-grade transformation. Thus, p27^{Kip1} expression in morphological MALT lymphomas could be useful tool to predict high-grade transformation to DLBCL.
Key words p27^{Kip1}, Gastric mucosa-associated lymphoid tissue (MALT) lymphoma, stomach, p53, Ki-67

INTRODUCTION

Gastric mucosa-associated lymphoid tissue (MALT) lymphomas are a low grade B cell lymphoma with a unique character ; most of these tumors regress with the eradication of *Helicobacter pylori*, despite being "neoplastic". Gastric lymphomas are composed of MALT lymphomas and diffuse large B-cell lymphomas (DLBCLs). A subset of the latter neoplasms exhibit a MALT lymphoma component, thought to be a high-grade transformation of the initial tumor. This transformation is related to the overexpression of bcl-6 and p53[1]. Sakugawa *et al*. reported the correlation of histological evaluation and PCR-based clonality in their analysis of gastric MALT lymphomas[2].

A number of studies have described the association of gastric MALT lymphoma tumorigenesis with cell cycle and genetic abnormalities[3-5]. In this report, we attempted to clarify the relationship between cell cycle-related molecules, by measuring Ki-67 index and p27^{Kip1} expression, and high-grade transformation. p27^{Kip1} is a cyclin-dependent kinase inhibitor (CDKI) of the KIP family, inhibiting S phase entry into the cell cycle by binding to cyclin E/CDK2 and cyclin D/CDK4. Tumor aggressiveness may be attributable to decreased p27^{Kip1} expression ; decreased levels of p27^{Kip1} were associated with aggressive tumor biology and poor prognosis for a variety of malignancies[6].

p27^{Kip1} is expressed at lower levels in high-grade B cell lymphomas, such as DLBCL, mantle cell lymphoma, and Burkitt's lymphoma, than in low-grade B cell lymphomas, including chronic B cell lymphoma, follicular lymphoma, and marginal zone B cell lymphoma[3,7,8,10]. In contrast, a number of studies have reported that high levels of p27^{Kip1} expression are detectable on DLBCL that exhibit high rates of cell proliferation[4]. Therefore, the relationship between p27^{Kip1} expression and high-grade transformation has not been determined ; in addition, little is known of p27^{Kip1} expression in gastric MALT lymphomas and DLBCLs.

A number of MALT lymphomas are associated with the t (11 ; 18) (q21 ; q21) translocation ; gastric tumors with this abnormality are resistant to eradication therapy. We attemp-

Received : Aug 29, 2005
Revised : Oct 8, 2005
Accepted : Nov 1, 2005
[1]Department of Pathology, Okayama University, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan
[2]Faculty of Health Sciences, Okayama University School of Medicine.
Address Correspondence and reprint request to Tadashi Yoshino, Department of Pathology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama 700-8558, Japan

ted to examine the influence of $p27^{Kip1}$ expression on high-grade transformation and responsiveness to eradicatioin therapy.

MATERIALS AND METHODS

Examined cases

We examined 23 cases of gastric MALT lymphoma from the surgical pathological files of the Department of Pathology, Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama University. Informed consent was obtained from all patients.

Specimens obtained from biopsy were processed routinely by fixation in 10% formaldehyde and embedding in paraffin. These specimens were diagnosed histopathologically according to previously described criteria[1,9] and the WHO classification scheme[11]. These gastric MALT lymphomas did not contain a large cell component. DLBCL with MALT lymphoma is characterized by the sheet-like appearance of medium-size neoplastic cells with a nuclear size greater than twice the size of a normal lymphocyte. *H. pylori* was detected in biopsy specimens lacking the t (11 ; 18) (q21 ; q21) translocation by H&E and Giemsa stainings. All patients were treated with combined antibiotic therapy. Of these 23 cases, complete remission was achieved in four cases ; the remaining 19 cases were resistant to eradication therapy (Table 2). In patients with eradication-resistant tumors, t (11 ; 18) (q21 ; q21) was detected in four cases. These patients ranged in age from 46 to 82 years, with a mean of 63.1 years. The male to female ratio was 12 : 11. The difference in patient age between these two groups was statistically significant (Table 1, $p<0.05$). We reviewed the clinical data and histology of 23 such cases and examined samples from these cases for t (11 ; 18) (q21 ; q21) and the patterns of $p27^{Kip1}$, p53, and MIB-1 expression.

Table 1. Summary of clinical deta.

	n	Sex	Age (y)	Mean (yr)
MALT lymphomas				
all patients	23	F : M=12 : 11	46-82	63.1
t (11 ; 18)				
+	4	F : M=2 : 2	53-80	68.8
-	19	F : M=10 : 9	46-82	61.9
CR*				
+	4	F : M=2 : 2	46-62	55
-	19	F : M=10 : 9	46-82	64.8
DLBCLs with MALT lymphoma	4	F : M=2 : 2	50-70	56.8
DLBCLs	5	F : M=4 : 1	47-79	63.2

Chi-square test. * ; $p<0.05$

We also examined five cases of gastric DLBCL lacking a MALT component and four cases of DLBCL with MALT lymphoma. Lymph nodes exhibiting reactive hyperplasia were used as positive controls of $p27^{Kip1}$ expression.

Detection of t (11 ; 18) (q21 ; q21) was performed by reverse transcriptase-polymerase chain reaction (RT-PCR) as described previously[12].

Immunohistological examination

Immunohistological examination utilized a panel of monoclonal antibodies specific for $p27^{Kip1}$ (SX53G8 ; DAKO ; dilution 1 : 100), wild-type/mutant p53 (clone DO-7 ; DAKO ; dilution 1 : 50), and the Ki-67 nuclear proliferation antigen (clone MIB-1 ; DAKO ; dilution 1 : 25).

Immunohistochemical staining was performed using an automated immunostainer (Ventana Medical Systems, Tucson, AZ), according to the manufacturer's protocol.

$p27^{Kip1}$ was expressed in nuclei. Endothelial cells, stromal cells, and small lymphocytes were internal positive controls for $p27^{Kip1}$ expression. Substitution of non-immune mouse serum for the primary antibody served as a negative control. Reactive lymph node tissue sections were stained as material controls.

$p27^{Kip1}$, p53, and Ki-67 expression patterns were also detected in epithelial cells of the gastric glands. We used epithelial cells of gastric glands as internal positive controls for p53 and Ki-67 staining. p53 expression was regarded as positive when the staining intensity was stronger than that of the epithelial cells of gastric glands.

Statistical analysis

Chi-square test was used to assess the correlations of clinical data.

The correlation between $p27^{Kip1}$ and p53 or Ki-67 expression and $p27^{Kip1}$, p53, and Ki-67 expression and malignancy were analyzed by Fisher's exact test. Results were considered to be statically significant when $p<0.05$. SPSS software for Windows, Release 11.5, was used for statistical analysis.

RESULTS

Immunohistology of $p27^{Kip1}$, p53 and MIB-1 expressions is demonstrated in Figure 1-3. $p27^{Kip1}$ nuclear expression was also detected in non-neoplastic small lymphocytes and plasma cells and the epithelial cells of gastric glands. The intensity

Table 2. p27^{Kip1}, p53 and MIB-1 proteins expression in MALT lymphomas, MALT lymphomas with DLBCL and DLBCLs

No.	Pt. Age (yr) / Sex	Eradication	Diagnosis	t (11 ; 18)	p27^{Kip1}	p53	MIB-1
1	46/ F	complete (CR : 9M)	MALT	-	+(90%)	-	-(<10%)
2	54/ F	complete (CR : 10M)	MALT	-	+(90%)	-	-(<10%)
3	58/ M	complete (CR : 3-5M)	MALT	-	+(90%)	-	-(<10%)
4	62/ M	complete (CR : 5M)	MALT	-	+(90%)	-	-(<10%)
5	53/ M	no change	MALT	+	+(90%)	-	-(<10%)
6	80/ F	no change	MALT	+	+(90%)	-	-(<10%)
7	72/ M	no change	MALT	+	+(90%)	-	-(<10%)
8	70/ F	no change	MALT	+	+(90%)	-	-(<10%)
9	59/ F	no change	MALT	-	+(90%)	-	-(<10%)
10	67/ M	no change	MALT	-	+(90%)	-	+(<20%)
11	49/ M	no change	MALT	-	+(90%)	-	-(<10%)
12	62/ M	no change	MALT	-	+(90%)	-	-(<10%)
13	75/ F	no change	MALT	-	+(90%)	-	-(<10%)
14	74/ F	no change	MALT	-	+(90%)	-	-(<10%)
15	66/ M	no change	MALT	-	+(80%)	-	-(<10%)
16	46/ F	no change	MALT	-	+(90%)	-	-(<10%)
17	82/ M	no change	MALT	-	+(90%)	-	+(<20%)
18	58/ M	no change	MALT	-	+(80%)	-	-(<10%)
19	53/ M	no change	MALT	-	+(80%)	-	-(<10%)
20	72/ F	no change	MALT	-	+(90%)	-	-(<10%)
21	65/ F	no change	MALT	-	+(90%)	-	-(<10%)
22	68/ F	no change	MALT	-	+(90%)	-	-(<10%)
23	61/ F	no change	MALT	-	+(90%)	-	-(<10%)
24	50/ F	N. D.	DLBCL	N. D.	-	+	+(>30%)
			MALT		-	-	-(<10%)
25	50/ M	N. D.	DLBCL	N. D.	-	+	+(>30%)
			MALT		-	-	-(<10%)
26	70/ F	N. D.	DLBCL	N. D.	-	+	+(>30%)
			MALT		-	-	-(<10%)
27	57/ M	N. D.	DLBCL	N. D.	-	+	+(>30%)
			MALT		-	-	-(<10%)
28	79/ F	N. D.	DLBCL	N. D.	-	+	+(50%)
29	63/ M	N. D.	DLBCL	N. D.	-	+	+(50%)
30	60/ F	N. D.	DLBCL	N. D.	-	+	+(50%)
31	67/ F	N. D.	DLBCL	N. D.	-	+	+(50%)
32	47/ F	N. D.	DLBCL	N. D.	-	+	+(50%)

-, positive cells are 10%. ; MALT, mucosa associated lymphoid tissure ; DLBCL, diffuse large B cell lymphoma ; ND, not done

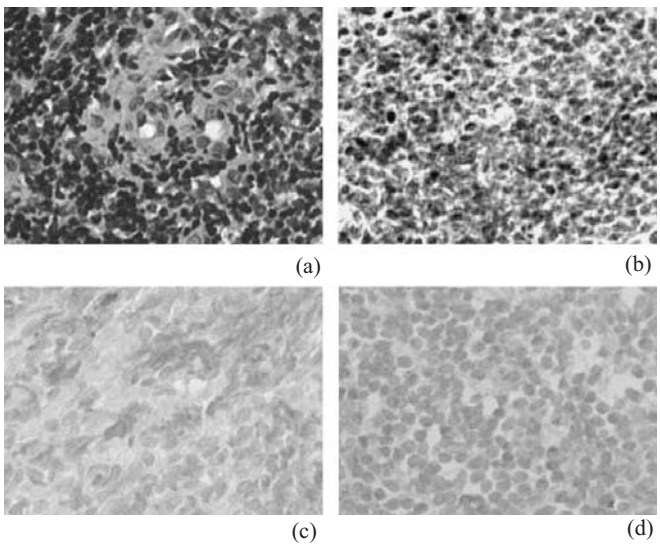


Figure 1.
(a) H&E staining in MALT lymphomas.
Immunostaining in MALT lymphomas. (b) High $p27^{Kip1}$ expression in the nucleus. (c) Low MIB-1 index. (d) Low p53 expression.
Original magnification x200.

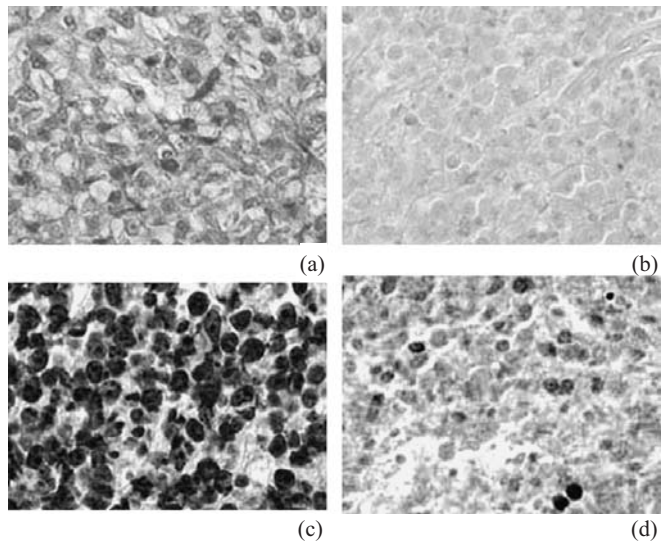
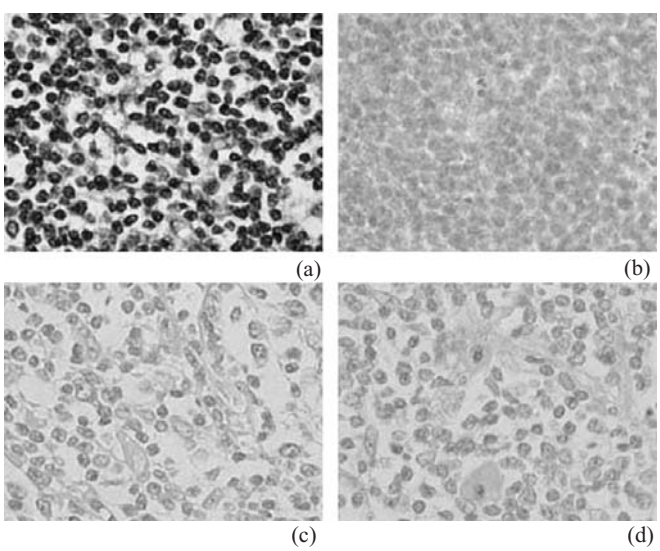


Figure 2.
(a) H&E staining in DLBCLs.
Immunostaining in DLBCLs. (b) Low $p27^{Kip1}$ expression. (c) High MIB-1 index. (d) High p53 expression.
Original magnification x200.



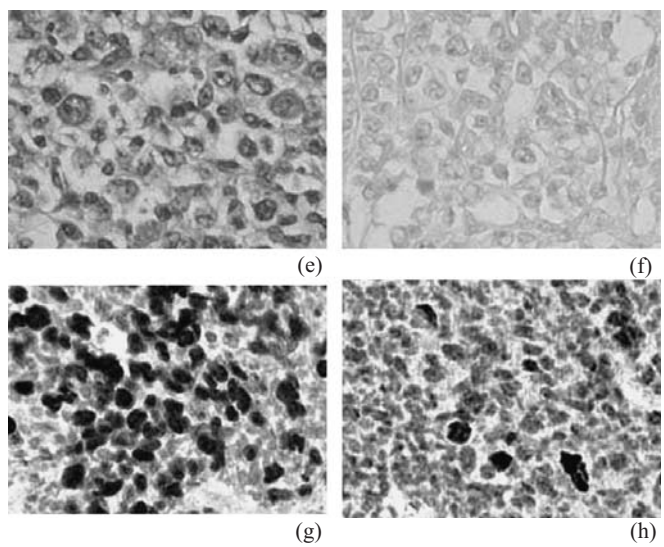


Figure 3.
H&E staining in DLBCLs with MALT lymphomas (a) MALT components (e) DLBCL components.
Immunostaining in DLBCLs with MALT lymphomas. (b) Low $p27^{Kip1}$ expression in MALT components. (f) Low $p27^{Kip1}$ expression in DLBCL components. (c) Low MIB-1 index in MALT components. (g) High MIB-1 index in DLBCL components. (d) Low p53 expression in MALT components. (h) High p53 expression in DLBCL components.
Original magnification x200.

of the $p27^{Kip1}$ staining was weaker in MALT lymphoma cells than in non-neoplastic small lymphocytes. No staining was observed in the cytoplasm. $p27^{Kip1}$ expression was detected in the nuclei of all MALT lymphoma cases lacking a large-cell component, regardless of their sensitivity to eradication therapy.

In lymph nodes with reactive hyperplasia, $p27^{Kip1}$ was detected in a subset of germinal center lymphocytes, the quiescent small lymphocytes of the follicular mantle zone, and interfollicular lymphocytes. The small cleaved germinal center cells exhibited strong coloration, while proliferating large centroblasts were negative for $p27^{Kip1}$.

p53 overexpression was not detected in any of the MALT lymphomas examined. Ki-67 stained fewer than 10% positive in all MALT lymphomas cases, except for two cases that were resistant to eradication. These cases of MALT lymphomas (case No. 10 and 17) displayed a scattering of intermingled large cells, 10 to 20% of which were positive for Ki-67.

t (11 ; 18) (q21 ; q21) and efficacy of eradication therapy did not correlate with p53 or Ki-67 expressions (Table 2).

All DLBCLs lacking a MALT lymphoma component were negative for $p27^{Kip1}$ and positive for p53. The Ki-67 indices in these cases were greater than 50%. In DLBCLs with MALT lymphoma, $p27^{Kip1}$ expression was negative in both the MALT and large cell components. In all cases, the MALT components were negative for p53 and Ki-67 (less than 10%), while the large cell components were positive for both (more than 30%).

DISCUSSION

Gastric MALT lymphomas with the t (11 ; 18) (q21 ; q21) translocation are highly resistant to eradication therapy. A second class of eradication-resistant tumors lacking t (11 ; 18) (q21 ; q21), however, has not yet been well characterized. Thus, we attempted to examine the expression of $p27^{Kip1}$ and p53 and Ki-67 in MALT lymphomas with regard to the sensitivity of these tumors to eradication. Next, we compared this expression pattern with that seen in DLBCLs. As both $p27^{Kip1}$ and t (11 ; 18) (q21 ; q21) may be associated with the regulation of apoptosis, we examined $p27^{Kip1}$ throughout the pathogenesis of gastric MALT lymphomas.

$p27^{Kip1}$ expression was detected in all 23 cases of MALT lymphoma examined, regardless of their sensitivity to eradication (Table 1). Neither the expression levels of p53 nor Ki-67 were related to the efficacy of therapy. These findings suggested that resistance to eradication is not related to these factors.

According to our data, MALT lymphomas that are negative for $p27^{Kip1}$ expression may transform into DLBCL. We do not know why there were no such cases in our series ; these legions would have been in complete remission following appropriate therapy. In addition, no high-grade tumors were found in this sample population.

The expression patterns of $p27^{Kip1}$, p53, and Ki-67 in DLBCLs lacking a MALT lymphoma component were quite different from those seen in MALT lymphomas, correlating with the results of previous reports[3,10]. This study demonstrated that $p27^{Kip1}$ expression in gastric MALT lymphomas and DLBCLs correlated with the grade of the lymphoma, irrespective of the stage[13]. While $p27^{Kip1}$ was not detectable in either the large cell or MALT components of DLBCLs with MALT lymphoma, $p27^{Kip1}$ expression in the MALT component was not linked to p53 and Ki-67 expression. Although it is difficult to determine a clear relationship from these findings due to the small number of cases examined, the decreased expression of $p27^{Kip1}$ by MALT components may be related to high-grade transformation. $p27^{Kip1}$ is detected on mantle cell lymphomas[7,8,10], which usually exhibit low proliferative activity despite a rather aggressive clinical course. This result indicates that $p27^{Kip1}$ expression is not always associated with proliferation activity, as in our MALT lymphoma with a large cell component. Although the molecular mechanism by which $p27^{Kip1}$ functions in tumorigenesis have not yet been clarified, the $p27^{Kip1}$ expression pattern in morphological MALT lymphomas may be a useful predictor of high-grade transformation to DLBCL.

Acknowledgment

We thank Ms. Hiromi Nakamura and Ms. Mutumi Okabe for their excellent technical assistance.

REFERENCES

1 Omonishi K, Yoshino T, Sakuma I, Kobayashi K, Moriyama M, Akagi T : bcl-6 protein is identified in high-grade but not low-grade mucosa-associated lymphoid tissue lymphomas of the stomach. Mod Pathol 11 : 181-185, 1998

2 Sakugawa ST, Yoshino T, Mannami T, Munemasa M, Ichimura K, Okada K, Mizuno M, Tanimoto M, Harada M, Akagi T : Correlation between histological evaluation and PCR-based clonality analysis of gastric MALT lymphomas treated by eradication of *Helicobacter pyloy*. J Clin Exp Hematopathol 42 : 61-67, 2002

3 Bai M, Vlachonikolis J, Agnantis NJ, Tsanou E, Dimou S, Nicolaides C, Stefanaki S, Pavlidis N, Kanavaros P, Kanavarous P : Low expression of p27 protein combined with altered p53 and Rb/p16 expression status is associated with increased expression of cyclin A and cyclin B1 in diffuse large B-cell lymphomas. Mod Pathol 14 : 1105-1113, 2001

4 Sanchez-Beato M, Sanchez-Aguilera A, Piris MA : Cell cycle deregulation in B-cell lymphomas. Blood 101 : 1220-1235, 2003

5 Andrés JM, Maurilio P, Giancarlo P, Massimo F, Roberta R, Stefania D, Luca B, Roberto B, Nadia C, Eugenio V, Giuseppe V : Immunoreactivity for $p27^{Kip1}$ and cyclin E is an independent predictor of survival in primary gastric non-Hodgkin's lymphoma. Int J Cancer 94 : 599-604, 2001

6 Lloyd RV, Erickson LA, Jin L, Kulig, Qian X, Cheville JC, Scheithauer BW : $p27^{Kip1}$: A multifunctional cyclin-dependent kinase inhibitor with prognostic significance in human cancers. Am J Pathol 154 : 313-323, 1999

7 Quintanilla-Martinez L, Davies-Hill T, Fend F, Calzada-Wack J, Sorbara L, Campo E, Jaffe ES, Raffeld M : Sequestration of $p27^{Kip1}$ protein by cyclin D1 in typical and blastic variants of mantle cell lymphoma (MCL) : implications for pathogenesis. Blood 101 : 3181-3187, 2003

8 Kremer M, Dirnhofer S, Nickl A, Hoefler H, Quintanilla-

Martinez L, Fend F : $p27^{Kip1}$ immunostaining for the differential diagnosis of small B-cell neoplasms in trephine bone marrow biopsies. Mod Pathol 14 : 1022- 1029, 2001

9 Isaacson PG, Norton AJ : Extranodal Lymphomas. Churchill Livingstone, 1994.

10 Chiarle R, Budel LM, Skolnik J, Frizzera G, Chilosi M, Corato A, Pizzolo G, Magidson J, Montagnoli A, Pagano M, Maes B, Wolf-Peeters CD, Inghirami G : Increased proteasome degradation of cyclin-dependent kinase inhibitor p27 is associated with a decreased overall survival in mantle cell lymphoma. Blood 95 : 619- 626, 2000

11 Jaffe ES, Harris N, Stein H, Vardiman JW : Tumours of hematopoietic and lymphoid tissues. International Agency For Research on Cancer. 2001

12 Inagaki H, Okabe M, Seto M, Nakamura S, Ueda R, Eimoto T : API2-MALT1 fusion transcripts involved in mucosa-associated lymphoid tissue lymphoma : multiplex RT-PCR detection using formalin-fixed paraffin-embedded specimens. Am J Pathol 158 : 699- 706, 2001

13 Shirin H, Kravtsov V, Shahmurov M, Petchenko P, Boaz M, Moss SF, Avni Y, Avinoach I : $p27^{kip1}$ expression is inversely related to the grade of gastric MALT lymphoma. Int J Gastrointest Cancer 35 : 25- 32, 2005