VOLUME 22 · NUMBER 17 · SEPTEMBER 1 2004

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Nuclear Expression of BCL10 or Nuclear Factor Kappa B Predicts *Helicobacter pylori*–Independent Status of Early-Stage, High-Grade Gastric Mucosa-Associated Lymphoid Tissue Lymphomas

Sung-Hsin Kuo, Li-Tzong Chen, Kun-Huei Yeh, Ming-Shiang Wu, Hui-Chen Hsu, Pei-Yen Yeh, Tsui-Lien Mao, Chi-Long Chen, Shin-Lian Doong, Jaw-Town Lin, and Ann-Lii Cheng

From the Departments of Oncology, Internal Medicine, and Pathology, National Taiwan University Hospital and National Taiwan University College of Medicine; Cancer Research Center, Graduate Institute of Clinical Medicine, and Graduate Institute of Microbiology, National Taiwan University College of Medicine; Division of Cancer Research, National Health Research Institutes; Department of Pathology, Taipei Medical University, Taipei; and Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan.

Submitted October 10, 2003; accepted June 1, 2004.

Supported by research grants NSC91-3112-B-002-009 from the National Science Council, NHRI-91A1-CANT-1 from the National Health Research Institutes, and NTUH 93-N012 from National Taiwan University Hospital, Taiwan.

S.-H.K. and L.-T.C. contributed equally to this work.

Authors' disclosures of potential conflicts of interest are found at the end of this article.

Address reprint requests to Ann-Lii Cheng, MD, PhD, Department of Internal Medicine and Department of Oncology, National Taiwan University Hospital, No. 7, Chung-Shan S Rd, Taipei, Taiwan; e-mail: andrew@ha.mc.ntu.edu.tw.

0732-183X/04/2217-3491/$20.00

DOI: 10.1200/JCO.2004.10.087

ABSTRACT

Purpose

A high percentage of early-stage, high-grade gastric mucosa-associated lymphoid tissue (MALT) lymphomas remain *Helicobacter pylori* dependent. t(11;18)(q21;q21), a genetic aberration highly predictive of *H pylori*–independent status in low-grade gastric MALT lymphoma, is rarely detected in its high-grade counterpart. This study examined whether nuclear expression of BCL10 or nuclear factor kappa B (NF-κB) is useful in predicting *H pylori*–independent status in patients with stage IE high-grade gastric MALT lymphomas.

Patients and Methods

Twenty-two patients who had participated in a prospective study of *H pylori* eradication for stage IE high-grade gastric MALT lymphomas were studied. The expression of BCL10 and NF-κB in pretreatment paraffin-embedded lymphoma tissues was evaluated by immunohistochemistry and confocal immunofluorescence microscopy. The presence of t(11;18)(q21;q21) was identified by a multiplex reverse transcriptase polymerase chain reaction of the API2-MALT1 chimeric transcript.

Results

Aberrant nuclear expression of BCL10 was detected in seven (87.5%) of eight *H pylori*–independent and in none of 14 *H pylori*–dependent high-grade gastric MALT lymphomas ($P < .001$). All seven patients with nuclear BCL10 expression had nuclear expression of NF-κB, compared with only two of 15 patients without nuclear BCL10 expression ($P = .002$). As a single variable, the frequency of nuclear expression of NF-κB was also significantly higher in *H pylori*–independent tumors than in *H pylori*–dependent tumors (seven of eight [87.5%] *v* two of 15 [12.3%]; $P = .002$). The API2-MALT1 fusion transcript was detected in only one (12.5%) of eight *H pylori*–independent lymphomas.

Conclusion

Nuclear expression of BCL10 or NF-κB is highly predictive of *H pylori*–independent status in high-grade gastric MALT lymphoma, and coexpression of these two markers in the nuclei is frequent.

J Clin Oncol 22:3491-3497. © 2004 by American Society of Clinical Oncology

INTRODUCTION

Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) of the stomach has recently been recognized as a distinct entity of non-Hodgkin's lymphoma.[1] *Helicobacter pylori* infection of the gastric mucosa plays an important role in the development and progression of gastric MALT lymphoma,[2-5] and eradication of *H pylori* results in durable tumor regression in approximately 70% of patients with localized low-grade gastric MALT lymphomas.[5-7] However, high-grade gastric MALT lymphomas, in contrast to their low-grade counterpart, are believed to consist of highly transformed cells, the growth of which is independent of *H pylori*.[8-11]

Recently, our group and other investigators have demonstrated that a substantial portion of early-stage, high-grade gastric MALT lymphomas remain *H pylori* dependent and

can potentially be cured by *H pylori* eradication.[12-14] Although the tumor response to *H pylori* eradication is almost as good as in its low-grade counterpart, early-stage, high-grade gastric MALT lymphomas may rapidly progress if they are unresponsive to *H pylori* eradication therapy. Therefore, identification of cellular or molecular markers that can help predict the *H pylori*–independent status of newly diagnosed high-grade gastric MALT lymphomas is mandatory. It is noteworthy that markers relevant to *H pylori*–independent status may be different between high-grade and low-grade gastric MALT lymphomas. For example, t(11;18)(q21;q21), one of the most important predictors of *H pylori* independence in low-grade gastric MALT lymphomas, is rarely found in high-grade gastric lymphomas.[15-17] Other markers that help predict the *H pylori*–independent status of high-grade gastric MALT lymphomas must be sought.

t(1;14)(p22;q32) is another genetic aberration implicated in the development of MALT lymphoma. t(1;14)(p22;q32) juxtaposes *BCL10* of chromosome 1 to an immunoglobulin gene locus of chromosome 14, and results in strong expression of a truncated BCL10 protein in the nuclei and cytoplasm, in contrast to the weak cytoplasmic expression of BCL10 in normal germinal center B cells.[18,19] It is noteworthy that t(1;14)(p22;q32) was detected in less than 5% of low-grade gastric MALT lymphomas, whereas moderate nuclear expression of BCL10 was found in 30% to 40% of these tumors.[19] In contrast, BCL10 nuclear expression was found to be more closely associated with the genetic aberration t(11;18)(q21;q21) and advanced tumor stages, two of the conditions predictive of *H pylori*–independent status in low-grade gastric MALT lymphoma.[20] The physiologic function of BCL10 in B lymphocytes remains unclear. The mechanism and biologic significance of BCL10 nuclear expression in lymphoma cells without BCL10 gene mutation are largely unknown. In T lymphocytes, BCL10 normally resides in the cytoplasm and specifically relays antigen-receptor–mediated signals to activate nuclear factor kappa B (NF-κB).[21] We hypothesized that upregulation of BCL10 may trigger a constitutive NF-κB activation signal, and therefore contribute to antigen-independent growth and the progression of the gastric MALT lymphoma. Under this condition, expression patterns of NF-κB and BCL10 may be useful markers for predicting *H pylori* independence of high-grade gastric MALT lymphoma.

In this study, we compared the expression patterns of BCL10 and NF-κB in 14 *H pylori*–dependent and eight *H pylori*–independent high-grade gastric MALT lymphomas. We found that these two markers are highly useful in predicting *H pylori* status of high-grade gastric MALT lymphoma.

PATIENTS AND METHODS

Patients, Treatment, and Evaluation of the Tumors

Twenty-two patients who had participated in a prospective study of *H pylori* eradication for stage IE high-grade gastric MALT lymphomas at our institutions from June 1995 through June 2002 were included in the study. The clinicopathologic features of the initial 16 patients have been reported previously.[14] The diagnosis of high-grade gastric MALT lymphoma was made according to the histologic criteria described by Chan et al[22] based on the presence of confluent clusters or sheets of large cells resembling centroblasts or lymphoblasts within predominantly low-grade centrocyte-like cell infiltrate, or the predominance of high-grade lymphoma with only a small residue, low-grade foci, and/or the presence of lymphoepithelial lesions. The histopathologic characteristics of all tumor specimens were independently reviewed by two experienced hematopathologists. Staging was classified according to Musshoff's modification of the Ann Arbor staging system.[23] All patients consented to a brief trial of an *H pylori* eradication therapy.

At the beginning of the study, the eradication regimen consisted of amoxicillin 500 mg and metronidazole 250 mg qid with either bismuth subcitrate 120 mg qid or omeprazole 20 mg bid for 4 weeks, which was changed to amoxicillin 500 mg qid, clarithromycin 500 mg bid, plus omeprazole 20 mg bid for 2 weeks after March 1996. Patients were scheduled to undergo first follow-up upper gastrointestinal endoscopic examination 4 to 6 weeks after completion of antimicrobial therapy, and follow-up was then repeated every 6 to 12 weeks until histologic evidence of remission was found. At each follow-up examination, four to six biopsy specimens were taken from the antrum and body of the stomach for the evaluation of *H pylori* infection, and a minimum of six biopsy specimens were taken from each of the tumors and suspicious areas for histologic evaluation. Diagnosis of *H pylori* infection was based on histologic examination, biopsy urease test, and bacterial culture. Tumor regression after eradication therapy was histologically evaluated according to the criteria of Wotherspoon et al.[5] Tumors that resolved to Wotherspoon grade 2 or less after *H pylori* eradication were considered *H pylori* dependent; other tumors were considered *H pylori* independent.

Immunohistochemistry and Confocal Laser Scanning Microscopy

Formalin-fixed, paraffin-embedded sections cut at a thickness of 4 μm were deparaffinized and rehydrated through xylenes and a graded alcohol series. After antigen retrieval by heat treatment in 0.1 M citrate buffer at pH 6.0, endogenous peroxidase activity was blocked by 3% H_2O_2. Briefly, slides were incubated for 30 minutes in 2.5% normal donkey serum or goat serum. The slides were then incubated overnight at 4°C either with polyclonal goat antihuman BCL10 (1:10; sc-9560; Santa Cruz Biotechnology, Santa Cruz, CA) or polyclonal rabbit antihuman RelA (p65; 1:100; sc-7151; Santa Cruz Biotechnology) and incubated with secondary antibodies (BCL10, donkey antigoat immunoglobulin; RelA, goat antirabbit immunoglobulin; Santa Cruz Biotechnology) according to the manufacturer's instructions. Finally, antibody binding was detected with the avidin-biotin-peroxidase method. Reaction products were developed using 3′, 5′-diaminobenzidine (Dako, Glostrup, Denmark) as a substrate for peroxidase. Sections were counterstained with Mayer's hematoxylin. All of the washes were performed in phosphate-buffered saline (pH 7.4). Staining was considered as positive for BCL10 or NF-κB when the protein was detected in more than 10% of tumor cells with nuclear staining. A semiquantitative method was used to determine the level of expression of RelA. Reactive spleen and lymph node tissue sections were used as controls.

For double-immunolabeling studies, fluorescein isothiocyanate–labeled donkey antigoat immunoglobulin G (IgG) or

rhodamine-labeled goat antirabbit IgG was incubated as a secondary antibody for 60 minutes at room temperature in the dark. The sections were further evaluated under a confocal laser scanning microscope (model TC-SP; Leica, Heidelberg, Germany) equipped with argon and argon-krypton laser sources.

Multiplex Reverse Transcriptase Polymerase Chain Reaction for the API2-MALT1 Fusion Transcript

Given that t(11;18)(q21;q21), which results in chimeric API2-MALT1 fusion protein, is not only the most important predictor of *H pylori*–independent status but also is closely associated with BCL10 nuclear expression in low-grade gastric MALT lymphoma, we examined our patients for the presence of this genetic aberration. Eight patients with low-grade gastric MALT lymphoma were also studied for comparison. Total cellular RNA was extracted from formalin-fixed, paraffin-embedded tissues using an Ambion RNA isolation kit (AMS Biotechnology, Oxon, United Kingdom). Briefly, two to three pieces of 10-μm paraffin sections were deparaffinized in xylene. The tissue was digested with proteinase K (Roche Diagnostics, Mannheim, Germany) for 1 hour at 45°C and solubilized in a guanidinium-based buffer. RNA extracted from the paraffin-embedded tissues was analyzed for API2-MALT1 fusion using multiplex reverse transcriptase polymerase chain reaction (RT-PCR), as described previously.[17] RNA was subjected to first-round multiplex one-tube RT-PCR, then to second-round nested multiplex PCRs (three parallel: second PCR-A, second PCR-B, and second PCR-C). The final PCR products were run on 3% agarose gel and stained with ethidium bromide. The band size ranged from 80 to 179 bp. PCR of low-grade gastric MALT lymphoma samples known to possess API2-MALT1 fusion was used as a positive control. Beta-actin (190 bp) was amplified in parallel as an internal control. Where indicated, PCR products of the API2-MALT1 transcript were either directly sequenced or cloned into a vector (TOPO TA Cloning Kit; Invitrogen, Paisely, United Kingdom) and sequenced with vector primers using dye-labeled terminators (BigDye Terminators; Applied Biosystems, Foster City, CA) and analyzed on a DNA sequencer (model 310; Applied Biosystems).

Statistical Analysis

Fisher's exact test and the χ^2 test were used to analyze the correlation between the *H pylori*–independent status of MALT lymphomas with BCL10 and NF-κB expression patterns.

RESULTS

Patients and Tumor Response

There were 14 patients with *H pylori*–dependent and eight patients with *H pylori*–independent tumors. The clinicopathologic features of these patients are summarized in Table 1. The median duration between *H pylori* eradication and complete histologic remission was 5.6 months (range, 1.5 to 17.7 months). At a median follow-up of 57.5 months (range, 8.6 to 81.8 months), all 14 patients who had achieved complete histologic remission after eradication of *H pylori* were alive and free of lymphoma. One patient

Table 1. Clinicopathologic Features of the Patients and Tumor Expression of BCL-10, NF-κB, and API2-MALT1

Patient No.	Sex	Age (years)	Depth of Tumor Invasion*	Tumor Response to *H pylori* Eradication	Current Status	Immunohistochemistry		*API2-MALT1* Transcript
						BCL10	NF-κB	
1	F	48	Muscularis propria	CR		c	c	–
2	M	21	Muscularis propria	PD	Chemotherapy	c	c	–
3	F	63		SD	Chemotherapy	n/c	n/c	+†
4	F	68		CR		c	c	–
5	F	52	Submucosa	PD	Chemotherapy	n/c	n/c	–
6	F	42	Serosa	CR		c	c	–
7	F	66	Serosa	CR		c	c	–
8	F	71	Muscularis propria	CR		c	n/c	–
9	F	54		CR		c	c	–
10	M	83	Muscularis propria	PD	Chemotherapy	n/c	n/c	–
11	F	52	Submucosa	CR		c	c	–
12	F	73	Muscularis propria	CR		c	n/c	–
13	M	46	Submucosa	CR		c	c	–
14	F	38		CR		c	c	–
15	M	73	Serosa	PD	Chemotherapy	n/c	n/c	–
16	F	45	Muscularis propria	PD	Chemotherapy	n/c	n/c	–
17	F	56	Submucosa	CR		c	c	–
18	F	59	Serosa	PD	C/T + gastrectomy	n/c	n/c	–
19	F	65		CR		c	c	–
20	M	35	Submucosa	CR		c	c	–
21	F	73		CR		c	c	–
22	M	45	Serosa	SD	Chemotherapy	n/c	n/c	–

Abbreviations: NF-κB, nuclear factor kappa B; MALT, mucosa-assocated lymphoid tissue; c, cytoplasmic; n/c, both nuclear and cytoplasmic; CR, complete remission; SD, stable disease; PD, progressive disease; EUS, endoscopic ultrasonography.
*_API2_ breakpoint (A1446); _MALT1_ breakpoint (M1150).
†Evaluated by EUS (15 patients) and histologic examination of surgical specimens (one patient).

(patient 22) had residual high-grade lymphoma cells, whereas low-grade lymphoma cells were completely resolved. In contrast, another patient (patient 3) had residual low-grade components with complete remission of the high-grade components. Six patients whose tumors grossly increased in size or had microscopic findings of increased large-cell fraction and one patient whose tumor remained grossly stable at the first follow-up endoscopic examination were immediately referred for systemic chemotherapy.

Correlation of Nuclear Expression of BCL10 and NF-κB With Tumor Response to H pylori *Eradication*

All patients had variable degrees of cytoplasmic staining of BCL10 (Fig 1). However, aberrant nuclear BCL10 expression was detected in seven (87.5%) of eight *H pylori*–independent patients and in none of the 14 *H pylori*–dependent patients ($P < 0.001$; Fig 1 and Table 1). The correlation between aberrant nuclear expression of BCL10 and disease extent was studied further. Nuclear BCL10 expression was detected in three (37.5%) of eight tumors confined to mucosa or submucosa, and in four (50%) of eight tumors that invaded to the muscular layer or serosa ($P = .5$; Table 1). All seven patients with nuclear BCL10 expression had coexpression of nuclear NF-κB, whereas only two of 15 patients without nuclear BCL10 expression had nuclear NF-κB expression ($P = .002$; Table 1 and Fig 1). The nuclear colocalization of NF-κB and BCL10 was also confirmed by confocal immunofluorescence microscopy (Fig 2). The frequency of nuclear expression of NF-κB was also significantly higher in *H pylori*–independent tumors than in *H pylori*–dependent tumors (seven of eight [87.5%] *v* two of 14 [12.3%]; $P = .002$). The nuclear expression of either BCL10 or NF-κB had a sensitivity of 87.5% in predicting the *H pylori* independence of high-grade gastric MALT lymphomas, whereas the specificity of nuclear expression of BCL10 and NF-κB for predicting *H pylori* independence was 100% and 88.2%, respectively.

API2-MALT1 Fusion Transcript of t(11;18)(q21;q21) Is Rarely Found in High-Grade H pylori*–Independent Gastric MALT Lymphoma Expressing BCL10*

The API2-MALT1 fusion transcript was detected in one (12.5%) of eight patients with *H pylori*–independent and in none of 14 *H pylori*–dependent high-grade gastric MALT lymphomas. In comparison, five (62%) of eight patients with *H pylori*–independent low-grade gastric MALT lymphoma were positive for the API2-MALT1 fusion transcript (Fig 3). The sequencing analysis of the RT-PCR products confirmed the presence of API2-MALT1 fusion transcript in all positive patients. The characteristics of all API2-MALT1 fusion variants were in keeping with those reported previously.[17]

DISCUSSION

In this study, we demonstrated that the nuclear expression of BCL10 and the nuclear expression of NF-κB are two highly useful markers to predict *H pylori*–independent status of high-grade gastric MALT lymphomas. Given that high-grade gastric MALT lymphoma may progress rapidly if unresponsive to *H pylori* eradication therapy, this information is invaluable for selection of first-line treatment. We found that coexpression of BCL10 and NF-κB in the nuclei was a common phenomenon in *H pylori*–independent high-grade gastric MALT lymphomas. In addition, in contrast to its low-grade counterpart, nuclear expression of BCL10 is rarely associated with t(11;18)(q21;q21) in high-grade gastric MALT lymphoma.[15-17]

BCL10 protein is expressed in the cytoplasm of normal lymphoid tissues, whereas the protein partially localizes to the nucleus in a fraction of MALT lymphoma with or without t(1;14)(p22;q32).[19] It has been shown that t(1;14)(p22;q32) or other BCL10 gene mutation is absent in the majority of MALT lymphoma with nuclear expression of BCL10.[24-26] Moreover, two recent studies clearly demonstrated that genomic BCL10 mutations are not responsible for the nuclear localization of BCL10 protein in gastric MALT lymphoma cells.[27,28] However, several researchers have shown that nuclear BCL10 expression is highly correlated with the presence of API2-MALT1 fusion in low-grade gastric MALT lymphomas.[20] In addition, nuclear expression of BCL10 in low-grade gastric MALT lymphoma is closely associated with advanced-stage diseases, particularly those invading beyond the serosa.[20] Given that t(11;18)(q21;q21) is rarely found in high-grade gastric MALT lymphoma, the mechanisms and biologic significance of the aberrant nuclear expression of BCL10 in these tumors are intriguing. In our study, we found that BCL10 nuclear translocation seems to be a major independent event that predicts *H pylori* independence of high-grade gastric MALT lymphoma. We confirmed that BCL10 nuclear translocation was independent of t(11;18)(q21;q21) in the majority of patients with high-grade gastric MALT lymphoma. Nuclear expression of BCL10 may also be detected in some low-grade gastric MALT lymphomas without t(11;18)(q21;q21).[29-31] These findings suggest that the direct interaction between BCL10 and API2-MALT1 fusion protein may not occur in most MALT lymphomas. Additional investigation of the molecular interaction and biologic consequences of nuclear translocation of BCL10 in gastric MALT lymphoma is needed.

BCL10 is an intracellular protein that positively regulates lymphocyte proliferation by linking antigen receptor stimulation to constitutively activate NF-κB signaling.[21,32] Because NF-κB is known to mediate cell survival and anti-apoptotic signals, it has been speculated that upregulation of NF-κB may contribute to the malignant transformation of *H pylori*–independent growth of MALT lymphomas. In

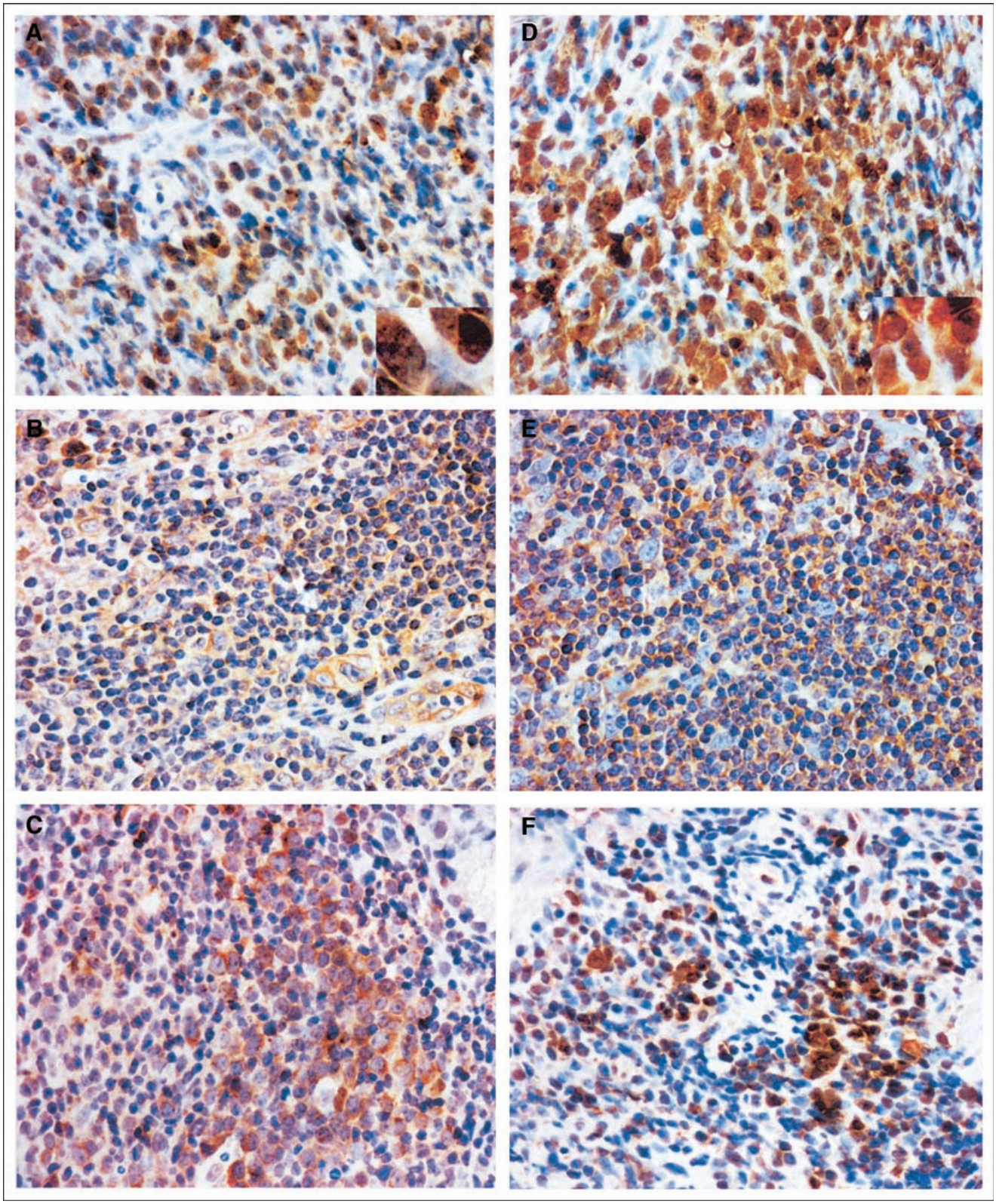

Fig 1. BCL10 and nuclear factor kappa B (NF-κB) protein expression in high-grade gastric mucosa-associated lymphoid tissue lymphoma. (A, B, C) BCL10; (D, E, F) NF-κB; (A, D) nuclear BCL10 and NF-κB expression in *Helicobacter pylori*–independent patients; (B, E) cytoplasmic BCL10 and NF-κB expression in *H pylori*–dependent patients; (C, F) cytoplasmic BCL10 expression and nuclear NF-κB expression in two *H pylori*–dependent patients. Original magnification ×400.

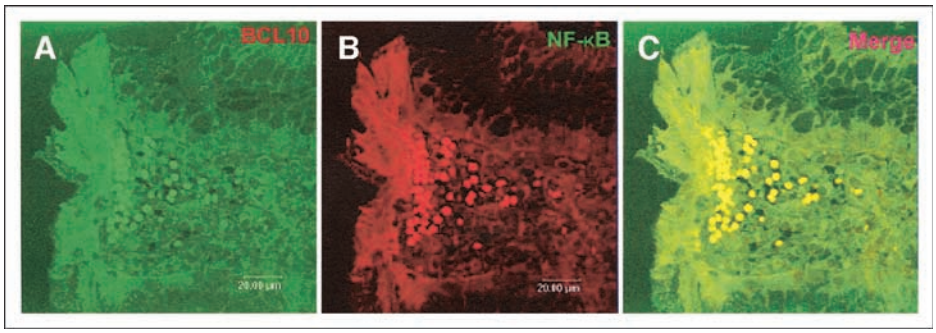

Fig 2. Colocalization of BCL10 and nuclear factor kappa B (NF-κB). Nuclear expression of BCL10 (A), NF-κB (B), and co-expression of BCL10 and NF-κB (C) in the tumor cells of *Helicobacter pylori*–independent patients analyzed by confocal microscopy. Original magnification ×400.

normal lymphoid cells, NF-κB was weakly expressed in the cytoplasm.[28] In contrast, our results showed that NF-κB was strongly expressed in the cytoplasm and/or nucleus in a substantial portion of high-grade gastric MALT lymphoma cells. Furthermore, the nuclear expression of NF-κB was closely associated with the aberrant nuclear expression of BCL10, comparable to the findings of a recent study by Ohshima et al.[28] Interestingly, the nuclear expression of NF-κB was also closely associated with the *H pylori*–independent status of high-grade gastric MALT lymphomas.

In this series, API2-MALT1 was detected in only one patient with *H pylori*–independent high-grade gastric MALT lymphoma. This patient had residual low-grade components with complete remission of the high-grade counterpart. Although high-grade MALT lymphoma is generally believed to be transformed from its low-grade counterpart,[33] recent reports suggest that the high-grade components may evolve independently from coexisting low-grade MALT lymphoma.[34,35] We suspect that this patient may have had coexisting *H pylori*–dependent API2-MALT1–negative high-grade MALT lymphomas and *H pylori*–independent API2-MALT1–positive low-grade MALT lymphomas originating from two different clones. Although we failed to examine API2-MALT1 separately in these two different components of lymphomas, we were able to demonstrate that low-grade and high-grade lymphoma of this patient displayed different pattern of rearranged *IgH* genes, indicating different clonal origin of the low-grade and high-grade lymphomas in this patient. Our results suggest that high-grade gastric MALT lymphoma may not necessarily evolve by transformation of a low-grade MALT lymphoma.

In conclusion, a substantial portion of early-stage, high-grade gastric MALT lymphomas remains *H pylori* dependent and can potentially be cured by *H pylori* eradication. Detection of nuclear expression of either BCL10 or NF-κB can identify *H pylori*–independent patients and help select patients for either cytotoxic or *H pylori* eradication therapy.

Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

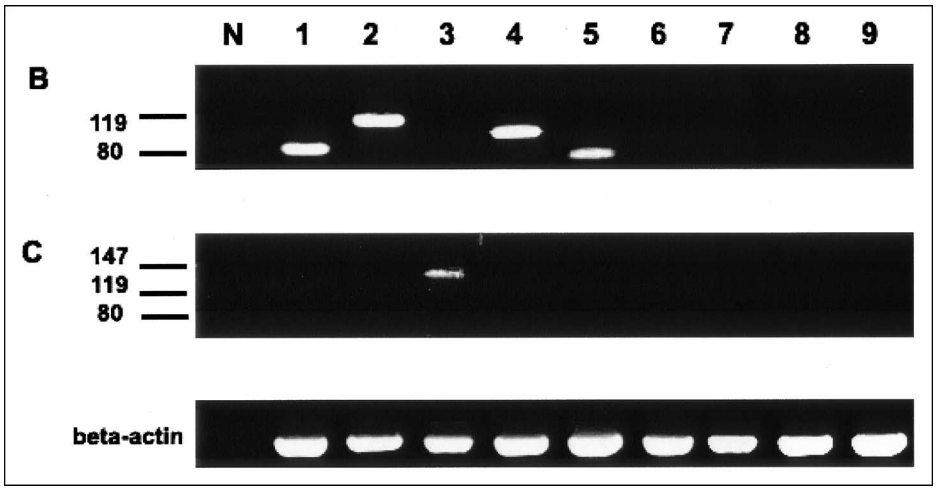

Fig 3. Detection of *API2-MALT1* fusion transcript by multiplex reverse transcriptase polymerase chain reaction (RT-PCR). (B) Second PCR-B. (C) Second PCR-C; lane N, negative control (normal lymph node); lane 1, *Helicobacter pylori*–independent high-grade gastric MALT lymphoma (positive); lanes 2 to 5, *H pylori*–independent low-grade gastric MALT lymphoma (positive); lanes 6 to 9, *H pylori*–independent high-grade gastric MALT lymphoma (negative). Beta-actin mRNA is amplified in all cases.

REFERENCES

1. Harris NL, Jaffe ES, Diebold J, et al: World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: Report of the Clinical Advisory Committee meeting—Airlie House, Virginia, November, 1997. J Clin Oncol 17:3835-3849, 1999

2. Wotherspoon AC, Ortiz-Hidalgo C, Falzon MR, et al: *Helicobacter pylori*–associated gastritis and primary B-cell gastric lymphoma. Lancet 338:1175-1176, 1991

3. Parsonnet J, Hansen S, Rodriguez I, et al: *Helicobacter pylori* infection and gastric lymphoma. N Engl J Med 330:1267-1271, 1994

4. Hussell T, Isaacson PG, Crabtree JE, et al: The response of cells from low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue to *Helicobacter pylori*. Lancet 342: 571-574, 1993

5. Wotherspoon AC, Doglioni C, Diss TC, et al: Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of *Helicobacter pylori*. Lancet 342:575-577, 1993

6. Du MQ, Isaacson PG: Gastric MALT lymphoma: From aetiology to treatment. Lancet Oncol 3:97-104, 2002

7. De Jone D, Boot H: Gastric lymphoma: The revolution of the past decade. Scand J Gastroenterol Suppl 236:27-36, 2002

8. Bayerdorffer E, Neubauer A, Rudolph B, et al: Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after cure of *Helicobacter pylori* infection. Lancet 345:1591-1594, 1995

9. Neubauer A, Thiede C, Morgner A, et al: Cure of *Helicobacter pylori* infection and duration of remission of low-grade gastric mucosa associated lymphoid tissue lymphoma. J Natl Cancer Inst 89:1350-1355, 1997

10. Zucca E, Roggero E, Pileri S: B-cell lymphoma of MALT type: A review with special emphasis on diagnostic and management problems of low-grade gastric tumors. Br J Haematol 100:3-14, 1998

11. Isaacson PG: Gastric MALT lymphoma: From concept to cure. Ann Oncol 10:637-645, 1999

12. Morgner A, Miehlke S, Fishbach W, et al: Complete remission of primary high-grade B cell gastric lymphoma after cure of *Helicobacter pylori* infection. J Clin Oncol 19:2041-2048, 2001

13. Nakamura S, Matumoto T, Suekane H, et al: Predictive value of endoscopic ultrasonography for regression of gastric low grade and high-grade MALT lymphomas after eradication of *Helicobacter pylori*. Gut 48:454-460, 2001

14. Chen LT, Lin JW, Shyu RY, et al: Prospective study of *Helicobacter pylori* eradication therapy in stage I(E) high-grade mucosa-associated lymphoid tissue lymphoma of the stomach. J Clin Oncol 19:4245-4251, 2001

15. Rosenwald A, Ott G, Stilgenbauer S, et al: Exclusive deletion of the t(11;18)(q21;q21) in extranodal marginal zone B cell lymphomas (MZBL) of MALT type in contrast to other MZBL and extranodal large B cell lymphomas. Am J Pathol 155:1817-1821, 1999

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 156:1433-1439, 2000

17. Inagaki H, Okabe M, Seto M, et al: 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

18. 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 96:35-45, 1999

19. Ye H, Dogan A, Karran L, et al: BCL10 expression in normal and neoplastic lymphoid tissue: Nuclear localization in MALT lymphoma. Am J Pathol 157:1147-1154, 2000

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 98:1182-1187, 2001

21. Wang D, You Y, Case SM, et al: A requirement for CARMA1 in TCR-induced NF-kappa B activation. Nat Immunol 3:830-835, 2002

22. Chan JKC, Ng CS, Isaacson PG: Relationship between high grade lymphoma and low-grade B-cell lymphoma of mucosa-associated lymphoid tissue (MALToma) of the stomach. Am J Pathol 136:1153-1164, 1990

23. Musshoff K: Klinische Stadieneinteilung der nicht-Lymphome. Strahlenther Onkol 153: 218-221, 1977

24. Du MQ, Peng H, Liu H, et al: BCL10 gene mutation in lymphoma. Blood 95:3885-3990, 2000

25. Fakruddin JM, Chananti RS, Murthy VV: Lack of BCL10 gene mutations in germ cell tumors and B cell lymphomas. Cell 96:683-684; discussion 686-688, 1999

26. Luminari S, Intini D, Baldini L, et al: BCL10 gene mutations rarely occur in lymphoid malignancies. Leukemia 14:905-908, 2000

27. Maes B, Demunter A, Peeters B, et al: BCL10 gene mutation does not represent an important pathogenic mechanism in gastric MALT-type lymphoma, and the presence of the API2-MALT1 fusion is associated with aberrant nuclear BCL10 expression. Blood 99:1398-1403, 2002

28. Ohshima K, Muta H, Kawasaki C, et al: Bcl10 expression rearrangement and mutation in MALT lymphoma: Correlation with expression of nuclear factor-kappa B. Int J Oncol 19:283-289, 2001

29. Okabe M, Inagaki H, Ohshima, et al: API2-MALT1 fusion defines a distinctive clinicopathologic subtypes of pulmonary extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue. Am J Pathol 162:1113-1122, 2003

30. Ye H, Liu H, Raderer M, et al: High incidence of t(11;18)(q21;q21) in *Helicobacter pylori*-negative gastric MALT lymphoma. Blood 101: 2547-2550, 2003

31. 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 102:1012-1018, 2003

32. Gaide O, Favier B, Legler DF, et al: CARMA1 is a critical lipid raft-associated regulator of TCR-induced NF-kappa B activation. Nat Immunol 3:836-843, 2002

33. Peng H, Du M, Diss TC, et al: Genetic evidence for a clonal link between low and high-grade components in gastric MALT B-cell lymphoma. Histopathology 30:425-429, 1997

34. Matolcsy A, Nagy M, Kisfaludy N, et al: Distinct clonal origin of low-grade MALT-type and high-grade lesions of a multifocal lymphoma. Histopathology 34:6-8, 1999

35. Cabras AD, Weirich G, Fend F, et al: Oligoclonality of a "composite" gastric diffuse large B-cell lymphoma with areas of marginal zone B-cell lymphoma of the mucosa-associated lymphoid tissue type. Virchows Arch 440:209-214, 2002