STOMACH

Distinct methylated profiles in *Helicobacter pylori* dependent and independent gastric MALT lymphomas

Y Kaneko, S Sakurai, M Hironaka, S Sato, S Oguni, Y Sakuma, K Sato, K Sugano, K Saito

Gut 2003;52:641–646

See end of article for authors' affiliations

Correspondence to:
Dr S Sakurai, Department
of Pathology, Jichi Medical
School, 3311-1 Yakushiji,
Minamikawachi-machi,
Kawachi-gun, Tochigi,
Japan; ssakurai@jichi.ac.jp

Accepted for publication
19 November 2002

Background: Gastric mucosa associated lymphoid tissue (MALT) lymphomas are clinically subdivided into *Helicobacter pylori* dependent and independent, according to *H pylori* infection and the therapeutic course. In previous reports it has been suggested that *H pylori* independent cases develop from *H pylori* dependent cases, and sometimes transform into high grade diffuse large B cell lymphomas (DLBCLs).

Methods: To better understand the pathogenesis of *H pylori* dependent and independent MALT lymphomas, we analysed the methylation profiles of eight independent CpG islands, including p15, p16, p73, hMLH1, death associated protein kinase, MINT1, MINT2, and MINT31 in *H pylori* dependent and independent MALT lymphomas, DLBCLs, and *H pylori* associated chronic gastritis.

Results: We first confirmed that *H pylori* independent cases had a high incidence of t(11;18)(q21;q21) (4/8 cases) and aberrant BCL10 expression (7/8 cases) compared with *H pylori* dependent cases and gastric DLBCLs. In the methylation pattern study, all 13 *H pylori* dependent MALT lymphomas had more than four methylated loci while *H pylori* independent cases had less than two. According to the previous criterion, all *H pylori* dependent MALT lymphomas (13/13, 100%) and five of 10 (50%) DLBCLs were classified as CpG island methylator phenotype positive (CIMP+). In contrast, all *H pylori* independent MALT lymphomas were CIMP–.

Conclusion: The distinct methylation pattern together with lack of chromosomal translocation in *H pylori* dependent MALT lymphomas suggest that *H pylori* dependent and independent MALT lymphomas have a different pathogenesis.

Gastric mucosa associated lymphoid tissue (MALT) lymphoma is a definitive clinicopathological entity characterised by its unique histological and clinical features.¹ *Helicobacter pylori* infection is one of the major causes of the pathogenesis of gastric MALT lymphoma.^{1,2} Previous reports have shown that most cases of gastric MALT lymphomas arise from *H pylori* associated chronic gastritis, and that eradication therapy for *H pylori* is highly effective.^{1,2} In contrast, some cases of gastric MALT lymphomas show resistance to eradication therapy or absence of *H pylori* infection, and the growth of those cases is thought to be independent of *H pylori*.^{1–4} Some of these cases have been suggested to develop from *H pylori* dependent MALT lymphomas.^{5,6} Given the clonal identities of the immunoglobulin heavy chains between low grade MALT lymphomas and coexisting diffuse large B cell lymphomas (DLBCLs) in a considerable number of cases,^{7,8} MALT lymphomas are, in part, likely to transform into DLBCLs.

Recently, the t(11;18)(q21;q21) translocation, which results in expression of a chimeric transcript fusing the apoptosis inhibitor 2 (API2) and MALT lymphoma translocation 1 (MALT1) genes, has been reported to be present in 30–50% of MALT lymphomas.^{9–11} Importantly, the t(11;18)(q21;q21) translocation is predominantly found in *H pylori* independent cases^{3,11,12} and is rare in *H pylori* dependent cases and gastric DLBCLs,^{10–13} suggesting that *H pylori* independent MALT lymphomas have a distinct pathogenesis. This hypothesis is also supported by the study of BCL10, one of the key molecules in the development of MALT lymphomas, showing that aberrant nuclear localisation of the BCL10 protein is highly frequent in translocation positive MALT lymphomas¹⁴ but less frequent in DLBCLs.¹⁵

A recent analysis using microsatellite markers in gastric MALT lymphomas has also produced a similar result.¹⁶ These

findings have compelled us to redefine the pathogenesis of gastric MALT lymphomas.

In this study, we compared DNA methylation patterns among *H pylori* dependent and independent MALT lymphomas, gastric DLBCLs, and *H pylori* associated chronic gastritis to better understand the pathogenesis of gastric MALT lymphomas. Inactivation of tumour suppressor genes by methylation of the CpG islands is one of the important steps in the development of clinically overt tumours.¹⁷ Recent genome wide analyses for DNA methylation in various human tumours has allowed the subclassification of tumours with DNA methylation patterns. The hypermethylated subtype in tumours, also called the CpG island methylator phenotype (CIMP),¹⁸ is a novel marker for tumour progression. Using the CIMP phenotype, we have characterised *H pylori* dependent and independent MALT lymphomas.

MATERIALS AND METHODS

Patients and evaluation of *H pylori* infection

Twenty one cases of primary low grade gastric MALT lymphomas, 10 cases of gastric DLBCLs, and 10 cases of *H pylori* associated chronic gastritis were selected at random from patients treated at Jichi Medical School Hospital. The study was approved by the research ethics committee of Jichi Medical

Abbreviations: MALT, mucosa associated lymphoid tissue; DLBCLs, diffuse large B cell lymphomas; CIMP, CpG island methylator phenotype; API2, apoptosis inhibitor 2; PCR, polymerase chain reaction; RT, reverse transcriptase; GAPDH, glyceraldehydes-3-phosphate dehydrogenase; MSP, methylation specific PCR; ROS, reactive oxygen species; DAP, death associated protein; MINT, methylated in tumours; hMLH, human Mut L homologue.

Table 1 Clinicopathological data for patients with primary low grade gastric mucosa associated lymphoid tissue (MALT) type lymphomas

Patient No	Sex	Age (y)	<i>H. pylori</i> infection (culture/histology/PCR/serology)	Remission after antibiotic therapy	Follow up period (months)
M1	F	60	+/+/+/+	Complete	54
M2	M	57	+/+/+/+	Complete	36
M3	F	41	+/+/+/ND	Complete	54
M4	F	51	+/+/+/+	Complete	12
M5	F	71	+/+/+/ND	Complete	33
M6	M	69	+/+/+/+	Complete	24
M7	F	59	+/+/+/+	Complete	24
M8	F	67	-/+/+/ND	Complete	3
M9	M	46	+/+/+/ND	Complete	10
M10	F	53	+/+/+/ND	Complete	36
M11	F	47	+/+/+/+	Complete	6
M12	M	44	+/+/+/ND	Complete	24
M13	M	53	+/+/+/ND	Complete	30
M14	M	67	+/+/+/ND	No response	9
M15	M	63	+/+/+/ND	No response	19
M16	M	53	-/-/-/-	NT	42
M17	F	51	-/-/-/-	NT	12
M18	M	59	-/-/-/-	NT	12
M19	F	55	-/-/-/-	NT	12
M20	M	60	-/-/-/-	NT	1
M21	M	73	-/-/-/-	NT	1

NT, patients were not treated with antibiotics to eradicate *H. pylori*.

ND, not done.

PCR, polymerase chain reaction.

School. Clinicopathological data for the low grade gastric MALT lymphomas are summarised in table 1. All MALT lymphoma cases were in stage IE according to the Ann Arbor staging system. Tumour regression after eradication therapy was histologically evaluated according to the criteria of Wotherspoon and colleagues.¹ Patients were followed up by repeated gastric endoscopic biopsies for 3–54 months.

H. pylori infection status was evaluated by bacterial culture, histology, polymerase chain reaction (PCR), and/or a serum antibody test (GAP test IgG; Biomerica Inc., Newport Beach, California, USA). The PCR conditions and primer sets used in this study have been described previously.^{19–20} The primer sets used for this analysis are listed in table 2. As shown in table 1, these independent analyses gave almost identical results for *H. pylori* infection. Of 21 MALT lymphomas, cases M1–M15 were accompanied by *H. pylori* infection and were treated by eradication therapy. In the remaining cases (patients M16–M21), tests for *H. pylori* infection were completely negative during the follow up period. After successful eradication therapy, we followed patients by repeated gastric endoscopic biopsies for 3–54 months. In patients M1–M13, lymphomas regressed completely within 3–16 months after eradication and were classified as *H. pylori* dependent, while patients M14–M15 showed no signs of endoscopic or histological changes after eradication. The remaining five cases without *H. pylori* infection were followed for 1–42 months before radiation or chemotherapy. Both *H. pylori* eradication non-responsive and *H. pylori* negative lymphoma cases were grouped together in the *H. pylori* independent group for this study.

H. heilmannii infection, which has been suggested to be another cause of MALT lymphoma in the stomach,^{21–22} was not detected by histology or PCR in any of the examined cases (data not shown).

Immunohistochemistry for BCL10

Formalin fixed paraffin embedded sections were pretreated in a microwave oven to recover antigenicity and incubated with an anti-BCL10 antibody (1:80; Zymed Laboratories, San Francisco, California, USA). Sections were visualised using the ENVISION method (Dako, Kyoto, Japan)

Reverse transcriptase (RT)-PCR and sequencing for t(11;18)(q21;q21)

Total cellular RNA was extracted from formalin fixed paraffin embedded sections using the Pinpoint Slide RNA Isolation System (Zymo Research, California, USA). Then, cDNA was synthesised using a First-strand cDNA synthesis kit (Amersham Pharmacia Biotech, Tokyo, Japan). According to the method of Inagaki *et al*, multiplex nested PCR analysis was performed to detect t(11;18)(q21;q21).²³ PCR for glyceraldehydes-3-phosphate dehydrogenase (GAPDH) and β -actin, which were used as controls to ensure the suitability of the used RNA, was performed on the same cDNA samples. To confirm the breakpoints of the fusion transcripts, direct sequencing was carried out using an ABI PRISM 377 DNA Sequencer (Perkin Elmer, Massachusetts, USA) with the same primers used for PCR amplification.

DNA extraction

Genomic DNA was extracted from formalin fixed paraffin embedded sections, as described previously.²⁴ Briefly, lymphoma cells or non-neoplastic gastric mucosae were scraped from 3 mm thick paraffin sections using a serial haematoxylin-eosin stained section as a guide, and digested in 50 mmol/l Tris HCl buffer (pH8.5) containing 1 mmol/l EDTA, 200 mg/ml proteinase K, and 0.5% Tween20. After eight hours of incubation at 62°C, Chelex-100 (Bio-Rad) was added to a final concentration of 30%. Samples were then boiled for 10 minutes and centrifuged for five minutes at 12 000 rpm. The liquid phase was used for PCR.

Methylation specific PCR (MSP)

Extracted DNA was subjected to chemical modification using a CpGenome DNA modification kit (Intergen, New York, USA) according to the manufacturer's manual. MSP was performed using the primer sets listed in table 2 according to the methods of previous reports.^{25–28} We analysed for the CIMP using a panel of eight independent CpG islands, including tumour suppressor genes and related genes (p15, p16, p73), a cancer associated gene (death associated protein (DAP) kinase), a mismatch repair gene (hMLH (human Mut L homologue) 1), and three methylated clones (MINT (methylated in tumours)

Table 2 List of primer sets used for evaluation of the *Helicobacter* genus infection, MSP, and RT-PCR

Name	Sense primer	Antisense primer
<i>H. pylori</i>		
1st	5'-AGGATGAAGGTTTATAGATT-3'	5'-CTGGAGAGACTAAGCCCTCC-3'
2nd	5'-ATTACTGACGCTGATTGTGC-3'	5'-CTGGAGAGACTAAGCCCTCC-3'
<i>Helicobacter</i> genus		
1st	5'-CGTGGAGGATGAAGGTTTAA-3'	5'-TACACCAAGAATCCACCTA-3'
2nd	5'-CGTGGAGGATGAAGGTTTAA-3'	5'-AATTCACCTACCTCTCCC-3'
MSP		
p15-M	5'-GCGTTCGTATTTTGGGTT-3'	5'-CGTACAATAACCGAACGACCGA-3'
p15-U	5'-TGTGATGTGTTTGTATTTTGTGGT-3'	5'-CCATACAATAACCAACAACCAA-3'
p16-M	5'-TTATTAGAGGTTGGGGCGGATCGC-3'	5'-GACCCCGAACCGCGACCGTAA-3'
p16-U	5'-TTATTAGAGGTTGGGGTGGATTGT-3'	5'-CAACCCCAACCAACCAACATAA-3'
p73-M	5'-GGACGTAGCGAAATCGGGTTC-3'	5'-ACCCCGAACATCGACGCTCCG-3'
p73-U	5'-AGGGGATGTAGTGAATGGGGTTT-3'	5'-ATCACAACCCCAACATCAACATCCA-3'
hMLH1-M	5'-TTAATAGGAAGAGCGGATAGC-3'	5'-CTATAAATTACTAAATCTCTTCG-3'
hMLH1-U	5'-TTAATAGGAAGAGTGGATAGT-3'	5'-TCTATAAATTACTAAATCTCTTCA-3'
MINT1-M	5'-AATTTTTTATATATATTTTGAAGC-3'	5'-AAAAACCTCAACCCCGCG-3'
MINT1-U	5'-AATTTTTTATATATATTTTGAAGTGT-3'	5'-AACAAAAAACCTCAACCCCAACA-3'
MINT2-M	5'-TTGTTAAAGTGTGAGTTCGTC-3'	5'-AATAACGACGATTCCTGACG-3'
MINT2-U	5'-GATTTTGTAAAGTGTGAGTTTGT-3'	5'-CAAAATAATAACAACAATCCATACA-3'
MINT31-M	5'-TGTGGGGAAGTGTTCGCGC-3'	5'-CGAAACGAAACGCGCCGCG-3'
MINT31-U	5'-TAGATGTTGGGGAAGTGTTCGCG-3'	5'-TAAATACCCCAACCAACCAACCA-3'
DAP kinase-M	5'-GGATAGTCGGATCGAGTTAAGTC-3'	5'-CCCTCCCAACGCGCA-3'
DAP kinase-U	5'-GGAGGATAGTGGATTGAGTTAATGT-3'	5'-CAAATCCCTCCCAACCAACCA-3'
RT-PCR		
t(11;18)(q21;q21)	5'-TTCATCCGTCAGTTCAGC-3'	5'-TTGAACAAAAGGATGCCAG-3'
1st	5'-TACTCAATGCAGAGATGA-3'	5'-ACTGTAAAACCAATGTGCTG-3'
	5'-CAAGAGGAAGTGTGATACG-3'	5'-TTCCTATCAAAAGGGCAACC-3'
t(11;18)(q21;q21)	5'-AGCCAGTACCCTCATCTAC-3'	5'-CAGCCAAGACTGCCTTTGAC-3'
2nd-A		5'-GGCATCAGCTTTGGGAAGT-3'
		5'-AAAGGCTGGTCAGTTGTTG-3'
t(11;18)(q21;q21)	5'-GAAATAAGGAAGAGGAGAG-3'	5'-CAGCCAAGACTGCCTTTGAC-3'
2nd-B		5'-GGCATCAGCTTTGGGAAGT-3'
		5'-AAAGGCTGGTCAGTTGTTG-3'
t(11;18)(q21;q21)	5'-ATTGCAGCCACTGTATTAG-3'	5'-CAGCCAAGACTGCCTTTGAC-3'
2nd-C		5'-GGCATCAGCTTTGGGAAGT-3'
		5'-AAAGGCTGGTCAGTTGTTG-3'

RT-PCR, reverse transcriptase-polymerase chain reaction; MSP, methylation specific PCR.

1, MINT2, MINT31) originally recovered from a colorectal carcinoma cell line.²⁹ Toyota *et al* described two types of DNA methylation: methylation in a cancer specific manner (type C methylation) and age dependent methylation (type A methylation).¹⁸ Methylation of p16, hMLH1, MINT1, MINT2, and MINT31 belonged to type C methylation and offered good discrimination for the CIMP.^{18 28 30 31} Methylation of p15, p16, p73, hMLH1, and DAP kinase gene was detected in some cases of lymphomas.³²⁻³⁶

RESULTS

Aberrant nuclear BCL10 expression and t(11;18)(q21;q21)

The results of immunohistochemistry, RT-PCR, and the break-point of each product are summarised in figs 1A and 2. All *H. pylori* independent lymphomas, except for case M14 in which staining was too weak to evaluate BCL10 localisation, showed BCL10 expression predominantly in the nuclei of tumour cells (fig 1A), and 4/8 *H. pylori* independent lymphomas contained t(11;18)(q21;q21) (fig 2). In contrast, only 4/13 (31%) *H. pylori* dependent MALT lymphomas showed nuclear BCL10 expression. None of the lymphocytes in *H. pylori* associated chronic gastritis showed nuclear BCL10 expression. In DLBCLs, nuclear BCL10 expression was seen in 4/10 (40%) cases. None of the *H. pylori* dependent MALT lymphomas or DLBCLs contained t(11;18)(q21;q21) (fig 2).

DNA methylation state of multiple CpG islands

The results of MSP are summarised in fig 2. Unmethylated alleles of each locus were successfully amplified in all samples examined in this study. Typical results of the MSP are shown in fig 1B. On average, 6.1 (4-8) loci were methylated in the

Figure 1 Immunohistochemistry for BCL10 (A) and methylation specific polymerase chain reaction for death associated protein (DAP) kinase CpG island methylation (B). Almost all *Helicobacter pylori* independent mucosa associated lymphoid tissue (MALT) lymphomas show aberrant nuclear BCL10 expression (A, left), but most *H. pylori* dependent MALT lymphomas showed only cytoplasmic BCL10 expression (A, right). (B) Both aberrant methylated (M) and unmethylated (U) alleles of DAP kinase CpG island were amplified in lanes 2, 3, and 5. Only unmethylated allele was amplified in lanes 1 and 4. Lanes 1, 2: *H. pylori* associated chronic gastritis; lane 3: *H. pylori* dependent MALT lymphoma; lane 4: *H. pylori* independent MALT lymphoma; lane 5: gastric diffuse large B cell lymphoma.

Figure 3 Two distinct pathways for the development of *Helicobacter pylori* dependent and independent mucosa associated lymphoid tissue (MALT) lymphomas. *H. pylori* dependent MALT lymphomas with the CpG island methylator phenotype (CIMP) tend to lack BCL10 aberration, and some transform into diffuse large B cell lymphomas (DLBCLs). In contrast, *H. pylori* independent MALT lymphomas with aberrant BCL10 expression lack the CIMP (CIMP-) and other genetic aberrations for transformation into DLBCLs. DLBCLs without the CIMP may develop along other pathways such as de novo lymphomagenesis. DAP, death associated protein. *From Starostik and colleagues,¹⁶ Bertoni and colleagues,⁴⁷ and Du and Isaacson.⁴⁸

H. pylori dependent and independent cases in this study. Thus characterisation of DNA methylation provides us with a novel aspect for understanding the pathogenesis of MALT lymphomas.

Based on previous reports and our own results,^{16, 47, 48} we propose the following model for gastric MALT lymphoma development and progression (fig 3). *H. pylori* dependent lymphomas arise from *H. pylori* associated chronic gastritis due to genetic alterations, such as p15, p16, and DAP kinase methylation, and 3q26.2-27 amplification,¹⁶ but lack t(11;18)(q21;q21). In contrast, *H. pylori* independent MALT lymphomas develop along a pathway determined by nuclear BCL10 expression due to t(11;18)(q21;q21)¹⁴ but lack methylation of the CpG islands. Given that half of the DLBCLs showed the CIMP phenotype in this study, it is likely that some cases of *H. pylori* dependent MALT lymphomas without t(11;18)(q21;q21) transform into DLBCLs at progression, and that the residual half of the DLBCLs, which were CIMP- and did not have t(11;18)(q21;q21) in this study, may develop along other pathways such as de novo lymphomagenesis.

In this model, we grouped together *H. pylori* negative lymphomas and lymphomas from *H. pylori* eradication non-responsive cases into the *H. pylori* independent lymphoma group. In our results, both types of lymphomas were CIMP-. Moreover, t(11;18)(q21;q21) was found in both *H. pylori* negative lymphomas and *H. pylori* eradication non-responsive lymphomas in this study and previous reports.^{3, 11, 12} Thus it seems reasonable to group both types of lymphomas as *H. pylori* independent although the number of cases in this study was too small to draw definitive conclusions.

In summary, based on studies of DNA methylation, we have shown here that *H. pylori* dependent MALT lymphomas have a distinct methylated phenotype. In contrast, *H. pylori* independent cases have lower methylation patterns, and the frequent MALT specific translocation t(11;18)(q21;q21) in association with aberrant BCL10 expression. Taken together with the results from DLBCLs and *H. pylori* associated chronic gastritis, our results seem to further strengthen the current hypothesis that the pathogenesis of gastric MALT lymphomas has two distinct pathways.

ACKNOWLEDGEMENTS

We are grateful to Dr Hiroshi Inagaki at the Department of Pathology, Nagoya City University Medical School, for the generous donation of positive controls for the multiplex RT-PCR method.

Authors' affiliations

Y Kaneko, Departments of Pathology and Gastroenterology, Jichi Medical School, 3311-1 Yakushiji, Minamikawachi-machi, Kawachi-gun, Tochigi, Japan

S Sakurai, M Hironaka, S Sato, S Oguni, Y Sakuma, K Saito, Department of Pathology, Jichi Medical School, 3311-1 Yakushiji, Minamikawachi-machi, Kawachi-gun, Tochigi, Japan

K Sato, K Sugano, Department of Gastroenterology, Jichi Medical School, 3311-1 Yakushiji, Minamikawachi-machi, Kawachi-gun, Tochigi, Japan

REFERENCES

- 1 **Wotherspoon AC**, Dogliani 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* 1993;**342**:575-7.
- 2 **Bayerdorffer E**, Neubauer A, Rudolph B, *et al*. Regression of primary gastric lymphoma of mucosa-associated lymphoid tissue type after cure of *Helicobacter pylori* infection. *Lancet* 1995;**345**:1591-4.
- 3 **Nakamura T**, Nakamura S, Yonezumi M, *et al*. The t(11;18)(q21;q21) translocation in *H. pylori*-negative low-grade gastric MALT lymphoma. *Am J Gastroenterol* 2000;**95**:3314-15.
- 4 **Ruskone-Fourmestraux A**, Lavergne A, Aegerter PH, *et al*. Predictive factors for regression of gastric MALT lymphoma after anti-*Helicobacter pylori* treatment. *Gut* 2001;**48**:297-303.
- 5 **de Jong D**, Boot H, van Heerde P, *et al*. Histological grading in gastric lymphoma: pretreatment criteria and clinical relevance. *Gastroenterology* 1997;**112**:1466-74.
- 6 **Isaacson PG**. Gastric MALT lymphoma. From concept to cure. *Ann Oncol* 1999;**10**:637-45.
- 7 **Montalban C**, Manzanal A, Gastrillo JM, *et al*. Low grade gastric B-cell MALT lymphoma progressing into high grade lymphoma. Clonal identity of the two stages of the tumor, unusual bone involvement and leukemic dissemination. *Histopathology* 1995;**27**:89-91.
- 8 **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* 1997;**30**:425-9.
- 9 **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**:1183-8.
- 10 **Mathijs B**, 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**:1433-9.
- 11 **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* 2001;**357**:39-40.
- 12 **Sugiyama T**, Asaka M, Nakamura T, *et al*. API2-MALT1 chimeric transcript is a predictive marker for the responsiveness of *H. pylori* eradication treatment in low-grade gastric MALT lymphoma. *Gastroenterology* 2001;**120**:1884-5.
- 13 **Alpen B**, Neubauer A, Dierlamm J, *et al*. Translocation t(11;18) absent in early gastric marginal zone B-cell lymphoma of MALT type responding to eradication of *Helicobacter pylori* infection. *Blood* 2000;**95**:4014-4015.

- 14 **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 BCL 10. *Blood* 2001;**98**:1182–7.
- 15 **Ye H**, Dogan A, Karan L, *et al.* BCL 10 expression in normal and neoplastic lymphoid tissue: nuclear localization in MALT lymphoma. *Am J Pathol* 2000;**157**:1147–54.
- 16 **Starostik P**, Patzner J, Greiner A, *et al.* Gastric marginal zone B-cell lymphomas of MALT type develop along 2 distinct pathogenetic pathways. *Blood* 2002;**99**:3–9.
- 17 **Esteller M**, Corn PG, Baylin SB, *et al.* A gene hypermethylation profile of human cancer. *Cancer Res* 2001;**61**:3225–9.
- 18 **Toyota M**, Ahuja N, Ohe-Toyota M, *et al.* CpG island methylator phenotype in colorectal cancer. *Proc Natl Acad Sci U S A* 1999;**96**:8681–6.
- 19 **Ho SA**, Hoyle JA, Lewis FA, *et al.* Direct polymerase chain reaction test for detection of *Helicobacter pylori* in humans and animals. *J Clin Microbiol* 1991;**29**:2543–9.
- 20 **Trebesius K**, Adler K, Vieth M, *et al.* Specific detection and prevalence of *Helicobacter heilmannii*-like organisms in the human gastric mucosa by fluorescent in situ hybridization and partial 16S ribosomal DNA sequencing. *J Clin Microbiol* 2001;**39**:1510–16.
- 21 **Stolte M**, Kroher G, Meining A, *et al.* A comparison of *Helicobacter pylori* and *H. heilmannii* gastritis. A matched control study involving 404 patients. *Scand J Gastroenterol* 1997;**32**:28–33.
- 22 **Morgner A**, Lehn N, Andersen LP, *et al.* *Helicobacter heilmannii*-associated primary gastric low-grade MALT lymphoma: complete remission after curing the infection. *Gastroenterology* 2000;**118**:821–8.
- 23 **Inagaki H**, Okabe M, Seto M, *et al.* API1-MALT1 fusion transcripts involved in mucosa-associated lymphoid tissue lymphoma. *Am J Pathol* 2001;**158**:699–706.
- 24 **Sakurai S**, Sano T, Nakajima T. Clinicopathological and molecular biological studies of gastric adenomas with special reference to p53 abnormality. *Pathol Int* 1995;**45**:51–7.
- 25 **Hermans JG**, Graff JR, Myohanen S, *et al.* Methylation-specific PCR: A novel PCR assay for methylation status of CpG islands. *Proc Natl Acad Sci U S A* 1996;**93**:9821–6.
- 26 **Liu M**, Taketani T, Li R, *et al.* Loss of p73 gene expression in lymphoid leukemia cell lines is associated with hypermethylation. *Leuk Res* 2000;**25**:441–7.
- 27 **Kang GH**, Shim Y-H, Jung H-Y, *et al.* CpG island methylation in premalignant stages of gastric carcinoma. *Cancer Res* 2001;**61**:2847–51.
- 28 **Chan AO**, Issa JP, Morris JS, *et al.* Concordant CpG island methylation in hyperplastic polyposis. *Am J Pathol* 2002;**160**:529–36.
- 29 **Toyota M**, Ho C, Ahuja N, *et al.* Identification of differentially methylated sequences in colorectal cancer by methylated CpG island amplification. *Cancer Res* 1999;**59**:2307–12.
- 30 **Strathdee G**, Appleton K, Illand M, *et al.* Primary ovarian carcinomas display multiple methylator phenotype involving known tumor suppressor genes. *Am J Pathol* 2001;**158**:1121–7.
- 31 **Kanai Y**, Ushijima S, Kondo Y, *et al.* DNA methyltransferase and DNA methylation of CpG islands and peri-centromeric satellite regions in human colorectal and stomach cancers. *Int J Cancer* 2001;**91**:205–12.
- 32 **Martinez DB**, Fernandez PJ, Garcia MJ, *et al.* Hypermethylation of a 5' CpG island of p16 is a frequent event in non-Hodgkin's lymphoma. *Leukemia* 1997;**11**:425–8.
- 33 **Baur AS**, Shaw P, Burri N, *et al.* Frequent methylation silencing of p15(INK4b) (MTS2) and p16(INK4a) (MTS1) in B-cell and T-cell lymphomas. *Blood* 1999;**94**:1173–81.
- 34 **Kawano S**, Miller CW, Gombart AF, *et al.* Loss of p73 gene expression in leukemias/lymphomas due to hypermethylation. *Blood* 1999;**94**:1113–20.
- 35 **Katzenellenbogen RA**, Baylin SB, Herman JG. Hypermethylation of the DAP-kinase CpG island is a common alteration in B-cell malignancies. *Blood* 1999;**93**:4347–53.
- 36 **Ping Siu LL**, Cheung Chan JK, Wong KF, *et al.* Specific patterns of gene methylation in natural killer cell lymphomas. *Am J Pathol* 2002;**160**:59–66.
- 37 **Shen L**, Ahuja N, Shen Y, *et al.* DNA methylation and environmental exposures in human hepatocellular carcinoma. *J Natl Cancer Inst* 2002;**94**:755–61.
- 38 **Issa JP**, Ahuja N, Toyota M, *et al.* Accelerated age-related CpG island methylation in ulcerative colitis. *Cancer Res* 2001;**61**:3573–7.
- 39 **Sato F**, Harpaz N, Shibata D, *et al.* Hypermethylation of the p14^{ARF} gene in ulcerative colitis-associated colorectal carcinogenesis. *Cancer Res* 2002;**62**:1148–51.
- 40 **Hsieh CJ**, Klump B, Holzmann K, *et al.* Hypermethylation of the p16^{INK4a} promoter in colectomy of patients with long-standing and extensive ulcerative colitis. *Cancer Res* 1998;**58**:3942–5.
- 41 **Tanaka T**, Iwasa Y, Kondo S, *et al.* High incidence of allelic loss on chromosome 5 and inactivation of p15INK4B and p16INK4A tumor suppressor genes in oxystress-induced renal cell carcinoma of rats. *Oncogene* 1999;**18**:3793–7.
- 42 **Cerda S**, Weitzman SA. Influence of oxygen radical injury on DNA methylation. *Mutat Res* 1997;**386**:141–52.
- 43 **Bagchi D**, McGinn TR, Ye X, *et al.* *Helicobacter pylori*-induced oxidative stress and DNA damage in a primary culture of human gastric mucosal cells. *Dig Dis Sci* 2002;**47**:1405–12.
- 44 **Shimoyama T**, Fukuda S, Liu Q, *et al.* Production of chemokines and reactive oxygen species by human neutrophils stimulated by *Helicobacter pylori*. *Helicobacter* 2002;**7**:170–4.
- 45 **Matolcsy A**, Nagy M, Kisfaludy N, *et al.* Distinct clonal origin of low-grade MALT- type and high-grade lesions of a multifocal gastric lymphoma. *Histopathology* 1999;**34**:6–8.
- 46 **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* 2002;**440**:209–14.
- 47 **Bertoni F**, Cavalli F, Cotter FE, *et al.* Genetic alterations underlying the pathogenesis of MALT lymphoma. *Hematol J* 2002;**3**:10–13.
- 48 **Du MQ**, Isaacson PG. Gastric MALT lymphoma: from aetiology to treatment. *Lancet Oncol* 2002;**3**:97–104.