Chapter 6

Intestinal Metaplasia Related to Gastric Cancer: An Outcome Analysis of Biomarkers for Early Detection

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1. Introduction

Although the overall incidence of gastric cancer is decreasing worldwide, it is the main cause of cancer death both worldwide and in East Asia including Japan. Gastric cancer is histologically divided into two types, intestinal and diffuse types (Lauren, 1965). Helicobacter pylori (*H. pylori*) infection is considered to be a major risk factor for the development of intestinal-type gastric cancer (Correa, 1990; Sipponen & Hyvärinen, 1993; International Agency for Research on Cancer [IARC], 1994; Graham, 2000; Uemura et al., 2001). It has been postulated that *H. pylori* infection causes chronic gastritis, gastric atrophy, usually with gastric intestinal metaplasia (IM) and dysplasia, and finally gastric cancer. The stepwise course of this inflammatory process, which usually continues over decades, has been defined as a sequence of histological events that confer an increasing risk of malignant transformation, as described in Correa's hypothesis (Correa, 1995). Long-term interactions between *H. pylori* infection and human increase the risk for precancerous lesions such as atrophic gastritis and IM (Peek & Blaser, 2002; Mera et al., 2005). Based on several long-term prospective studies involving large groups of patients, it is believed that eradication of *H. pylori* infection may prevent the development of gastric cancer (Uemura et al., 2001; Mera et al., 2005; You et al., 2000; Take et al., 2005). According to a prospective, randomized, placebo-controlled, population-based study from China, *H. pylori* eradication significantly decreased the development of gastric cancer in participants without precancerous lesions, i.e., gastric atrophy, IM, or gastric dysplasia (Wong et al., 2004). Furthermore, it has been reported from Japan that *H. pylori* treatment reduces the risk of developing new gastric carcinoma in patients who have a history of gastric cancer and are thus at high risk for such develop-

ment (Fukase et al., 2008). On the other hand, it is evident that gastric cancer still occurs to some degree after endoscopic resection and successful eradication of *H. pylori* (Wong et al., 2004; Fukase et al., 2008). Therefore, establishment of predictable markers for the identification of patients at high risk for recurrent gastric cancer development despite eradication of *H. pylori* infection is clinically needed.

Gastric cancer develops through the accumulation of molecular alterations (Tamura, 2006). Current knowledge of the molecular mechanisms underlying gastric carcinogenesis indicates that two major genetic instability pathways are involved in the pathogenesis of gastric cancer: microsatellite instability (MSI), and chromosome instability, including loss of heterozygosity (Lengauer et al., 1998). We have recently reported that genetic instability in IM may be associated with gastric carcinogenesis (Tanaka A et al., 2006; Zaky et al., 2008; Watari et al., 2012). Previous reports have shown that MSI may play an important role in the development of synchronous or metachronous gastric cancer (MGC) and that it may be used clinically as a molecular marker for the prediction of multiple gastric cancers (Miyoshi et al., 2001; Hasuo et al., 2007). DNA methylation changes in cancer cells are characterized by regional CpG island hypermathylation and generalized genomic hypomethylation. Epigenetic inactivation of tumor-related genes by promoter hypermethylation is increasingly recognized to play an important role on tumorigenesis (Laird, 2005; Robertson, 2005). Previous reports on promoter hypermethylation showed that epigenetic alterations are frequently observed in precancerous lesions as well as in gastric cancer (Kang et al., 2001; To et al., 2002; Chan et al., 2006; Leung et al., 2006; Perri et al., 2007; Dong et al., 2009; Park et al., 2009).

Das et al. have developed a novel monoclonal antibody (mAb), Das-1 (formerly known as $7E_{12}H_{12}$, IgM isotype), which specifically reacts with the colonic epithelium (Das et al., 1987). We have reported that IM of a colonic phenotype detected by mAb Das-1, is strongly associated with gastric cancer, thus suggesting that mAb Das-1 positivity in IM could be a sensitive and specific marker related to gastric carcinogenesis (Mirza et al., 2003; Watari et al., 2008). Furthermore, we have recently shown that *H. pylori* eradication does not reduce the histological IM score, but changes the cellular phenotype of IM, as identified by this mAb in a prospective, 4-year follow-up study (Watari et al., 2008).

Epidemiological data on pre-malignant lesions such as *H. pylori*-related IM are relevant for predicting intestinal-type gastric cancer and for evaluating screening and surveillance practices (Mirza et al., 2003; Watari et al., 2008). We followed patients who received and did not receive *H. pylori* treatment after endoscopic resection for early gastric cancer for 1 year, and then examined the changes in MSI and mAb Das-1 in IM in response to *H. pylori* therapy. Here we show our study as to whether these biomarkers are predictive markers of MGC development after *H. pylori* treatment.

2. Patients and methods

We performed a hospital-based, case-control study of 75 patients, including 50 mucosal cancer patients who had undergone endoscopic resection (Group DYS), and 25 age- and sex-

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matched chronic gastritis patients for whom *H. pylori* had been successfully eradicated (control). In this study, the patients with duodenal ulcer only were excluded because most of duodenal ulcers were categorized as 'antral predominat gastritis' (Uemura et al., 2001) or 'non-atrophic gastritis' (Mera et al., 2005), which is considered to be a low risk group of gastric cancer. Additionally, Group DYS patients were divided into 2 groups: 25 successfully *H. pylori*-eradicated (eradicated group) and 25 un-eradicated patients (persistent group). Mucosal gastric cancer was defined as any cancer in which invasion was limited to the mucosa based on Japanese criteria (Japanese Gastric Cancer Association. Japanese Classification of Gastric Carcinom, 2010). In all patients, biopsy specimens were taken to assess *H. pylori* infection, two each from the greater curvature of the antrum and the greater curvature of the corpus. The presence of *H. pylori* status was determined by a positive result for either or both Wartin-Starry staining or *H. pylori* culture. For eradication, patients were treated with lansoprazole (30 mg), amoxicillin (750 mg), and clarithromycin (400 mg), each taken twice daily for 1 week. All patients in the eradicated group underwent endoscopic resection for their mucosal cancer and then received treatment for *H. pylori*. All patients were followed for 1 year. In patients from the eradicated group and control (chronic gastritis), clearance of *H. pylori* was confirmed by negative results by both Wartin-Starry staining and *H. pylori* culture at a follow-up endoscopy.

3. Intestinal metaplasia

All IM cases investigated here were goblet cell metaplasia, namely incomplete-type IM obtained from the antrum (Filipe et al., 1994). Serial sections (4 μm) were made, and consecutive sections were used for histologic examination by H&E staining and for immunohistochemistry.

3.1. DNA extraction

From paraffin-embedded blocks, two 7-μm tissue sections were cut. DNA was extracted from the IM samples. In this DNA extraction procedure, the sample was precisely microdissected under microscopic visualization using a PALM MG III Laser Capture Microdissection System (MEIWAFOSIS, Tokyo, Japan) to avoid DNA contamination of inflammatory or stromal cell nuclei based on the previously described methodology (Tanaka et al., 2006; Zaky et al., 2008; Watari et al., 2012).

3.2. Analysis of MSI

The MSI was analyzed as reported previously (Tanaka et al., 2006; Zaky et al., 2008; Watari et al., 2012). We examined five microsatellite markers (two mono- and three dinucleotide repeats) for MSI based on the revised Bethesda panel (Umar et al., 2004) as follows: 2p (BAT26), 4q (BAT25), 2p (D2S123), 5q (D5S346), and 17p (D17S250). Briefly, polymerase chain reaction (PCR) amplification was carried out in a reaction volume of 10 μL, which contained 100 ng of genomic DNA, 1X PCR buffer (Perkin Elmer Applied Biosystems, Foster

City, CA), 200 μmol/L of dNTP, 600 μmol/L of each primer, and 1.5 units of AmpliTaq Gold polymerase (Perkin Elmer). The MgCl2 concentration was 1.5 mmol/L. The following PCR cycle conditions were used for amplification: 95°C for 10 min, 30 cycles of 95°C for 45 sec, 55°C for 1 min, and 72°C for 30 sec. The PCR products were evaluated for MSI by capillary electrophoresis using an ABI prism 310 Genetic Analyzer (Perkin Elmer) and automatic sizing of the alleles using a Gene Scan (Applied Biosystems). The MSI status was judged according to previous reports (Tanaka et al., 2006; Zaky et al., 2008; Watari et al., 2012). MSI was defined as positive when unequivocal extra peak bands in tumor DNA were observed that differed by multiples of 2 base pairs in dinucleotide markers or 1 base pair in mononucleotide markers from DNA in normal mucosa, and was also characterized by the appearance of additional alleles in the tumor DNA. The former type MSI was judged as the minor pattern (Figure 1A) and the latter type as the major pattern (Figure 1B), as reported previously (Tanaka et al., 2006; Zaky et al., 2008; Watari et al., 2012). IM was defined as having high MSI (MSI-H) when unstable loci were observed in two or more of five microsatellite markers and as having low MSI (MSI-L) when an unstable locus was observed in only one of the five markers studied based on the criteria established in 2002 (Umar et al., 2004). The lesion was considered microsatellite stable (MSS) if no unstable loci were found. The MSI phenotype was categorized into two groups, MSI-H and MSI-L/MSS. In our study, a sample was defined as MSI only when it qualified as MSI-H.

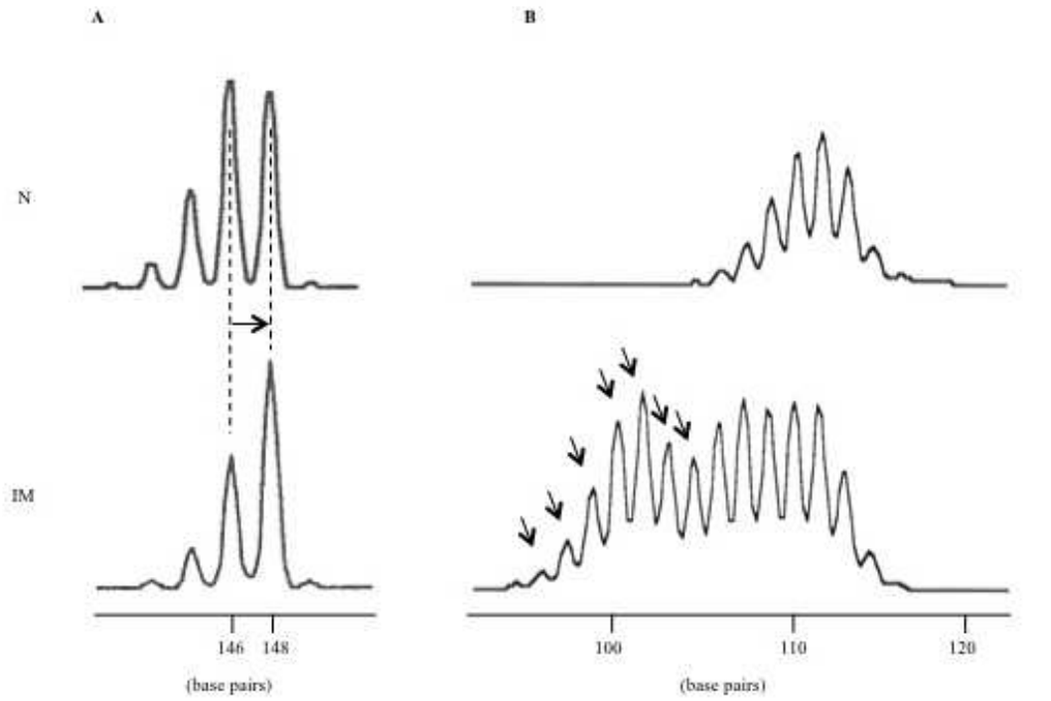

Figure 1. Examples of microsatellite instability (MSI) were detected in intestinal metaplasia (IM) by high-resolution fluorescent microsatellite analysis. DNA was isolated from IM and matching normal mucosa without IM (N). (A) Representative case of a minor pattern of MSI on D17S250. MSI is seen as an unequivocal extra peak shift (arrow) compared with normal mucosa. (B) Representative case of a major pattern of MSI on BAT26. MSI is characterized by the appearance of multiple additional alleles (arrows).

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4. Immunoperoxidase assays with mAb Das-1

Serial sections were stained with mAb Das-1 using sensitive immunoperoxidase assays as described previously (Watari et al., 2012; Das et al. 1987; Mirza et al., 2003; Watari et al., 2008). Reactivity to mAb Das-1 was considered positive if cells were stained a crisp golden brown. A substantial number of cells and more than one gland had to be reactive to this mAb before a specimen was considered positive. If only an occasional goblet cell was stained, the sample was defined as negative (Watari et al., 2012; Das et al. 1987; Mirza et al., 2003; Watari et al., 2008; Das et al., 1994).

4.1. Statistical analysis

The data were assessed by the Mann-Whitney *U*-test, the chi-square test, and Fisher's exact test. Statistical significance was defined by a p value of <0.05. Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) for each biomarker (MSI and mAb Das-1 reactivity) by a case-control study were calculated using StatView Ver. 5.0 for Macintosh (SAS Institute Inc., North Carolina, USA). Risk factors with a p value of < 0.10 in univariate analyses were included in a multiple logistic regression model and analyzed using the backward approach. The 95% CI of the OR was used to assess the statistical significance at the conventional level of 0.05.

4.2. Results

4.2.1. Patient characteristics

The mean ages of patients in the eradicated, persistent, and control groups were 70.8 (range 54-79), 70.9 (range 59-80), and 69.5 (range 62-77), respectively. Male patients made up 76% of the eradicated group, 92% of the persistent group, and 80% of the control group. There were no significant differences in age and gender among the three groups.

5. MSI and mAb Das-1 reactivity in IM

The observed incidences of MSI were 28.0% (14 of 50) in Group DYS and 8.0% (2 of 25) in the control; these results were significantly different (p<0.05). Similarly, mAb Das-1 reactivity of IM was also more frequently observed in Group DYS (66.0%, 33 of 50) than in the control (32.0%, 8 of 25) (p=0.07) (Table 1). We analyzed the strength of the association between gastric cancer and advanced age (70 years or older), male gender, MSI and mAb Das-1 reactivity by calculating univariate and multivariate logistic regression (Table 2). MSI and mAb Das-1 reactivity were associated with gastric cancer in the univariate analysis. In the multivariate logistic regression analysis, MSI and Das-1 reactivity were strong and independent factors (OR=7.09, 95% CI 1.27-39.6, p=0.03 in MSI; OR=4.96, 95% CI 1.64-15.0, p=0.005 in Das-1 reactivity). The sensitivity, specificity, and positive predictive value of MSI and mAb Das-1 reactivity of IM for gastric cancer were 28.0% (14 of 50), 92.0% (23 of 25), 87.5% (14 of

16), and 66.0% (33 of 50), 68.0% (17 of 25), 80.5% (33 of 41), respectively. If at least one of these 2 biomarkers was expressed in IM, those statistical calculations for gastric cancer became 78.0% (39 of 50), 60.0% (15 of 25) and 79.6% (39 of 49), respectively.

	Microsatellite instability		Das-1 reactivity	
	At initial	After 1-year	At initial	After 1-year
Group DYS (n=50)	14 (28.0) *	-	33 (66.0) **	-
Eradicated group (n=25)	7 (28.0) †	2 (8.0) †	16 (64.0)	12 (48.0) §
Persistent group (n=25)	7 (28.0)	6 (24.0)	17 (68.0)	18 (72.0) §
Control (n=25)	2 (8.0) *	1 (4.0)	8 (32.0) **	4 (16.0)

* p<0.05, ** p<0.01, † p=0.07, § p=0.08, Numbers in parentheses are percentages.

Table 1. Changes in microsatellite instability and Das-1 reactivity in Group DYS and control before and after *H. pylori* eradication

Variable	Univariate	Multivariate analysis		
	p	Odds ratio	95% CI	*p*
Microsatellite instability	0.06	7.09	1.27-39.6	0.03
Das-1 reactivity	0.007	4.96	1.64-15.0	0.005

Table 2. Predictors for development of gastric cancer

6. Changes in MSI and mAb Das-1 reactivity after *H. pylori* treatment

The incidence of MSI in IM 1 year after *H. pylori* treatment tended to decrease from 28.0% (7 of 25) to 8.0% (2 of 25) in the eradicated group (p=0.07), while there was no significant change in the persistent group during the follow-up period. The incidence of MSI also declined in the control, but the difference between pre- and post-eradication rates was not significant (Table 1). Similarly, the immunoreactivity of IM against mAb Das-1 decreased from 64.0% (16 of 25) to 48.0% (12 of 25) in the eradicated group, and from 32.0% (8 of 25) to 16.0% (4 of 25) in the control in response to *H. pylori* treatment; the difference in each group between pre- and post-treatment rates was not significant. In contrast, the incidence of the reactivity in the persistent group showed no changes at the 1-year follow-up. One year after treatment, the incidence of mAb Das-1 reactivity tended to be lower in the eradicated group than in the persistent group (p=0.08) (Table 1).

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7. Newly developed gastric cancer after treatment

MGC was defined as a new carcinoma occurring at a previously uninvolved site in the stomach found more than 1 year after endoscopic resection. We encountered 13 MGCs that developed after endoscopic resection from January 1996 to July 2008, which included 3 (3.8%) of 79 patients for whom *H. pylori* had been eradicated and 10 (9.4%) of 106 patients who were un-eradicated. However, no newly developed gastric cancer was identified in 290 chronic gastritis patients in the same period. Three of 13 cases did not reveal IM in the biopsy at the time of MGC detection. The characteristics of the remaining 10 patients that developed gastric cancer after endoscopic treatment are shown in Table 3. Intriguingly, all patients showed positive for either MSI or mAb Das-1 reactivity in IM.

						At the time of MGC detection	
No.	**Group**	**Age**	**Gender**	**Yrs after eradication**	**Yrs after endoscopic resection**	**Microsatellite instability**	**Das-1 reactivity**
1	Eradicated group	77	M	2.5	5.2	+	+
2	Eradicated group	78	M	2.7	3.4	-	+
3	Eradicated group	68	M	1.1	1.8	-	+
4	Persistent group	65	M	NA	1.8	+	-
5	Persistent group	71	F	NA	1.8	+	-
6	Persistent group	76	M	NA	1.1	+	+
7	Persistent group	74	M	NA	1.2	+	+
8	Persistent group	79	M	NA	1.3	-	+
9	Persistent group	71	M	NA	1.0	-	+
10	Persistent group	81	M	NA	7.3	+	-

Table 3. Clinical characteristics of metachronous gastric cancer (MGC) after endoscopic resection

One of the patients (Case 1) in Group DYS presented a flat, elevated lesion at the antrum (Figure 2A). Endoscopic resection was performed on this lesion, and the histological diagnosis was mucosal cancer. Then, the patient received treatment for *H. pylori*. Thirty months after eradication, at the follow-up endoscopy, a new cancer was found at the lesser curvature of the corpus (Figure 2B and 2C). Interestingly, this patient lacked MSI prior to eradication but exhibited MSI after *H. pylori* therapy, while mAb Das-1 reactivity was positive both before and after eradication (Figure 2D and 2E).

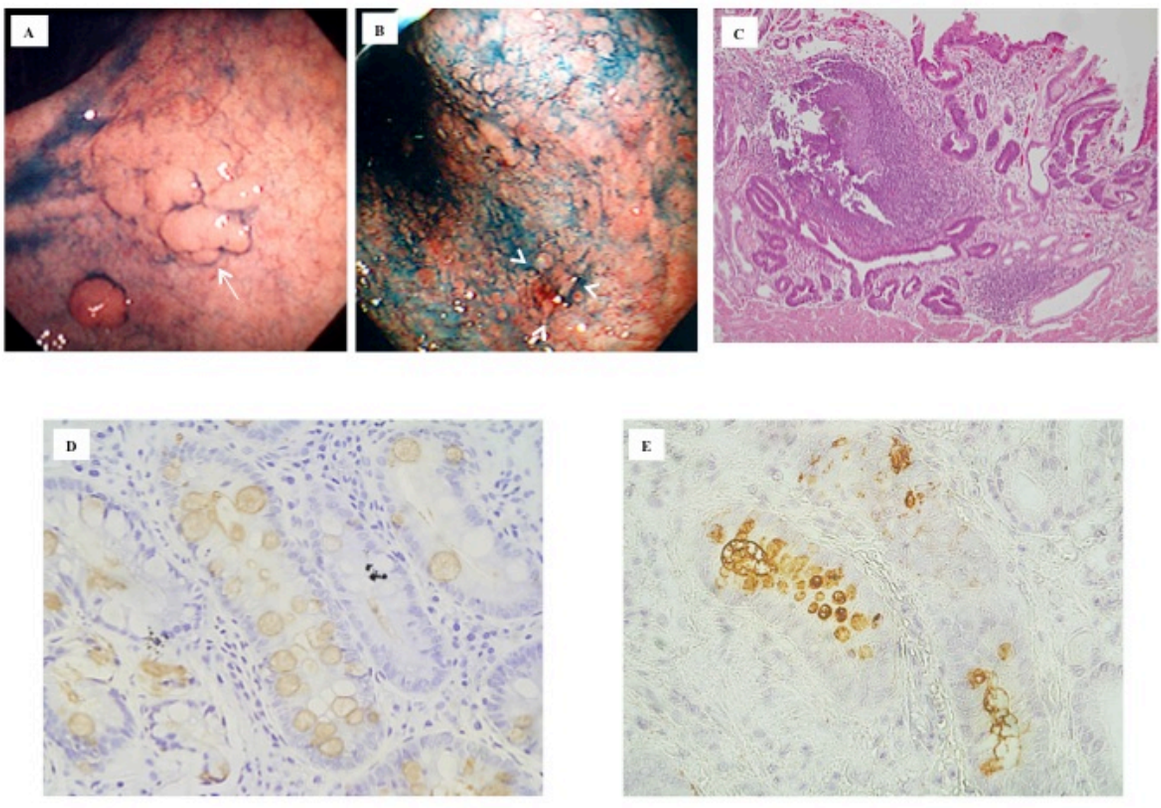

Figure 2

Figure 2. A) Endoscopic findings showed a flat elevated gastric cancer (arrow) at the posterior wall of the antrum. *H. pylori* was eradicated after endoscopic resection to the lesion. (B) At 30 months after eradication, a new lesion was found at the lesser curvature of the lower body of the stomach (arrowhead). (C) The resected sample was diagnosed histologically as mucosal cancer (×100). (D) IM in biopsy obtained from the antrum reacted with Das-1 (×200) prior to *H. pylori* treatment, and (E) the reactivity of Das-1 in IM was also demonstrated at the time a new gastric cancer was found following eradication (×200).

8. Discussion

Recent prospective randomized trial from Japan proved that *H. pylori* eradication significantly prevents the development of MGC after endoscopic mucosal resection to early gastric

cancer (Fukase et al., 2008). In contrast, another Japanese retrospective study showed that the incidence of MGC did not differ between the eradicated group and the persistent groups during a follow-up period of as long as 11.1 years (Maehata et al. 2012). Nonetheless, new gastric cancer does sometimes develop after successful treatment of *H. pylori* infection in both reports (Wong et al., 2004; Maehata et al. 2012). These results indicate that once gastric cancer has developed in the stomach a first time, the background mucosa has increased potential of developing MGC (Wong et al., 2004; Zaky et al., 2008; Maehata et al. 2012). Therefore, efficient strategies to identify individuals who are at a "high risk" for MGC after *H. pylori* treatment are very much needed. We found in this study that MSI or mAb Das-1 reactivity in IM may serve independently as biomarkers to predict the development of gastric cancer regardless of *H. pylori* eradication.

It has previously been reported that MSI possibly plays a role in early events leading to gastric carcinogenesis (Chung et al., 1996; Buonsanti et al., 1997). Until now, we have also reported that genetic instability is frequently observed in the progression of IM in chronic gastritis patients and of IM in patients with gastric cancer and a cancerous area (Tanaka et al., 2006; Zaky et al., 2008). In this case-control study, the frequency of MSI in IM was significantly higher in Group DYS than in the control. These findings indicate that MSI may be a useful marker in identifying "high risk" IM that may develop into gastric cancer. It has since been reported that MSI may be associated with the inflammation from the standpoint of view of the investigation from patients with ulcerative colitis (Brentnall et al., 1996) or chronic hepatitis (Kondo et al., 2000). In the present study, MSI changed, becoming stable in response to the improvement of inflammation following eradication; these findings may indicate that inflammation of the stomach affects the presence of MSI. On the other hand, there is an interesting report by Kashiwagi et al. (Kashiwagi et al., 2000) in which MSI in gastritis, adenoma, and adenocarcinoma were examined retrospectively. According to their results, in all patients (n=6) with gastric adenoma or adenocarcinoma showing MSI, identical MSI patterns had been observed at the stage of gastritis, 1.5 to 7 years before the final diagnosis of adenocarcinoma. Thus, they concluded that MSI in chronic gastritis mucosa may identify patients at risk of developing gastric adenoma and cancer. Taking into account their results and ours, MSI expressed in IM, regardless of successful *H. pylori* eradication may be a predictor for the risk of MGC development.

Consistent with our previous reports (Mirza et al., 2003; Watari et al., 2008), the current study demonstrated that IM reactivity to mAb Das-1 is strongly associated with gastric cancer. However, the reactivity did not show a statistically significant decrease at 1 year after *H. pylori* eradication in the current study although our previous study showed a significant decline in mAb Das-1 positivity following treatment, up to 4 years (Watari et al., 2008). One of the explanations for this discrepancy may be the difference in the length of follow-up. It may be the case that a cellular phenotypic change by *H. pylori* treatment requires substantial time in comparison with MSI. However, mAb Das-1 reactivity declined significantly more in the eradicated group more than in the persistent group as of 1 year after *H. pylori* treatment. Some individuals actually showed persistent immunoreactivity to mAb Das-1 after treatment as shown in the previous report (Watari et al., 2008). Importantly, seven (70%) of 10

newly developed gastric cancers after endoscopic resection were positive for mAb Das-1 reactivity after eradication of *H. pylori*.

Based on our results, then, lesions that are newly or persistently positive for the markers including MSI or mAb Das-1 after *H. pylori* eradication are at a certain stage of progression of IM, and may have passed the "point of no return" (Wong et al., 2004; Wright, 1998). It remains possible, therefore, that these are patients who are at high risk of developing gastric cancer that may warrant more intensive endoscopic surveillance to detect early gastric cancer. Wong et al. reported that eradication of *H. pylori* did not decrease the development of gastric cancer in participants with precancerous lesions such as IM (Wong et al., 2004). In contrast to their report, our results may provide an important clue to the pathogenesis of the observed reduction of gastric cancer following *H. pylori* eradication in some patients with IM from the perspective of MSI or the cellular phenotype related to carcinogenesis.

It is intriguing that all newly developed cancers occurred only from Group DYS, with none from the control, although the number of cases investigated was small. This result indicates that IM in the background mucosa in patients from Group DYS is in an advanced stage compared to that in the control (chronic gastritis), and has more malignant potential. Accordingly, the accumulation of genetic alterations may be continued during the progression of IM. Considering the results in a Japanese population from Take et al., gastric cancer developed in 0.8% (8 of 944) of peptic ulcer patients cured of infection for up to 8.6 yr (mean 3.4 yr) (Take et al., 2005). Moreover, a recent report from a multi-center, open-label, randomized controlled trial from the Japan GastStudy Group suggested that eradication of *H. pylori* reduced by approximately one-third the risk of new gastric cancer in patients with a history of gastric cancer, but could not completely prevent cancer development (Fukase et al., 2008). According to this report, the incidence of new gastric cancer development was 3.5% (9 of 255) during 3 yrs of follow-up after endoscopic resection, significantly different from that reported by Take et al. Taking the findings of these reports and ours into consideration, the background gastric mucosa in patients who have a history of cancer may have more malignant potency in comparison to that in chronic gastritis patients. Thus, the molecular and cellular phenotypic backgrounds of IM can explain the pathogenesis of MGCs, even after resection of tumors due to persistent uncorrected accumulated errors of DNA mismatch repair and colonic phenotype on IM.

Newly developed gastric cancers may be the result of occult gastric cancers that are not detectable at the previous endoscopy, but that have grown enough to be diagnosed at the follow-up. Regarding the definition of MGC after endoscopic resection, therefore, it may be necessary to conduct follow-up for a long time, more than 1 year. In the current study, however, the subset of IM with MSI or mAb Das-1 positivity has the possibility of developing gastric cancer after even this relatively short period of time. Indeed, all patients who developed new cancer showed positive for MSI or mAb Das-1 reactivity in IM (Table 3). The persistence of DNA damage and the colonic phenotype, as detected by mAb Das-1, may identify the "at risk" group of patients with histological IM; thus, these biomarkers in the biopsy specimens of gastric mucosa may predict MGC development following *H. pylori* therapy. In the current study, the number of patients analyzed may be small, particularly for the

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comparison of MSI and cellular phenotypes in the three different groups. Further investigations on a larger series will be required in the future.

First of all, *H. pylori* should be absolutely eradicated for the prevention of MGC development. The efficacy of standard 7-14 day triple therapies is decreasing, mainly due to increasing primary bacterial resistance to antibiotics. Currently, the most effective treatments are either the sequential regimen or the concomitant therapy. The sequential therapy was first introduced in Italy in 2000 (Zullo et al., 2000). This regimen is a 10-day therapy, including a simple dual therapy with a proton pump inhibitor (PPI) plus amoxicillin 1 g (both twice daily) given for the first 5 days, followed by a triple therapy including a PPI, clarithromycin 500 mg, and tinidazole 500 mg (all given twice daily) for the remaining 5 days. Different antibiotic combinations, administered together with a PPI, have been proposed in the last decades. Unfortunately, no available therapy is able to eradicate *H. pylori* in all treated patients. Therefore, new drugs and novel therapeutic approaches are needed. It has been recently reported by a randomized clinical trial that simvastatin as adjuvant to standard therapy improves significantly the *H. pylori* eradication rate (Nseir et al., 2012). The search for novel antibacterial therapies against *H. pylori* is a "work in progress" driven by the goal of preventing gastric cancer, and by worldwide increasing antibiotic resistance (Fiorini et al., 2012).

We believe that MSI and mAb Das-1 reactivity may be reliable biomarkers to identify a subgroup of patients at sufficiently high risk of MGC after endoscopic resection to justify endoscopic surveillance. According to recent reports, *H. pylori* eradication is able to significantly reduce gene methylation thus delaying or reversing *H. pylori*-induced-gastric carcinogenesis (Chan et al., 2006; Leung et al., 2006; Perri et al., 2007). It has been also reported that sonic hedgehog methylation was detected more frequently in the high-risk group for gastric cancer after *H. pylori* treatment (Shiotani et al., 2012). Further studies on genetic and epigenetic alterations, are necessary to clarify the credibility of the markers in different regional populations.

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