Gastric Cancers Emerging after *H. pylori* Eradication Arise Exclusively from Non-Acid-Secreting Areas

Katsunori Iijima,1 Yasuhiko Abe,1 Tomoyuki Koike,1 Kaname Uno,1 Hiroyuki Endo,1 Waku Hatta,1 Naoki Asano,1 Kiyotaka Asanuma,1 Akira Imatani1 and Tooru Shimosegawa1

1Division of Gastroenterology, Tohoku University Graduate School of Medicine, Sendai, Japan

Although *Helicobacter pylori* (*H. pylori*) eradication has some inhibitory effects on the subsequent development of gastric cancer, there are sporadic cases of gastric cancer even after successful eradication. The pathogenesis of gastric cancer emerging after *H. pylori* eradication remains to be clarified.

In this study, employing Congo-red chromoendoscopy, which is capable of visualizing the acid-secreting fundic mucosa, we investigated the topographic relationship of the acid secretion pattern to the occurrence site of gastric cancers emerging after eradication. Fourteen consecutive patients who suffered from new gastric cancer after eradication, defined as lesions that were discovered at least 2 years after the eradication, were prospectively enrolled. Whether the neoplasias arose from acid-secreting or non-acid-secreting areas was evaluated with Congo-red chromoendoscopy. Biopsy specimens taken from the two areas were subjected to histologic evaluation and immunohistochemistry for Ki-67 and p53. The mean period from the eradication to the subsequent occurrence of gastric cancer was 74 (44) months. There were two cancer lesions in 5 cases, and thus there was a total 19 lesions from 14 cases. Congo-red chromoendoscopy revealed that all 19 lesions arose exclusively from non-acid-secreting areas. Histological examination revealed sustained hyperproliferation and accumulation of p53 protein was frequently detectable in non-acid-secreting areas. Genetic alteration such as p53 mutation seems to be already present in the residual non-acid-secreting areas after eradication, areas that could be the origin of gastric carcinogenesis after eradication. Identification of such high-risk areas should be a promising approach for estimating the individual cancer risk after eradication.

Keywords: Congo-red chromoendoscopy; gastric cancer; *Helicobacter pylori* eradication; immunohistochemistry; p53


The vast majority of gastric cancer arises from *H. pylori*-infected stomach mucosa (Uemura et al. 2001). Recent studies consistently reported that *H. pylori* eradication had some inhibitory effects on the subsequent development of gastric cancer, at least in certain conditions (Take et al. 2005; Takenaka et al. 2007; Fukase et al. 2008; Ogura et al. 2008). However, it also has been revealed that there are sporadic cases of gastric cancer even after successful eradication (Kamada et al. 2005; Yanaoka et al. 2009; de Vries et al. 2009). Hence, careful follow-up with endoscopic examination for possible gastric cancer is necessary even after successful eradication of *H. pylori* (Kamada et al. 2005; de Vries et al. 2009; Yanaoka et al. 2009). Using endoscopic surveillance after eradication, the sites where new cancer preferentially develops could be determined. Additionally, identifying the underlying mucosal milieu that promotes the development of gastric cancer after successful eradication is important to clarify the pathogenesis of persisting carcinogenesis after the primary carcinogen, *H. pylori* infection, has been removed.

Many investigators, including our group, consistently reported that the suppressed gastric acid secretion in *H. pylori*-positive patients with corpus gastritis with or without atrophy recovered at least partially after eradication of the infection (El-Omar et al. 1997; Tucci et al. 1998; Iijima et al. 2004). In this context, employing Congo-red chromoendoscopy, which is known to be capable of visualizing the acid-secreting fundic area by means of a pH-dependent color reaction (Tatsuta et al. 1973), we found that the area of the acid-secreting mucosa in the fundus was promptly expanded after eradication, especially in the greater curvature (Sekine et al. 2006). However, in the subsequent long-term follow-up (mean periods of 62 months after eradication) study using that technique, we also recognized that
there were varying degrees of residual non-acid-secreting (functionally irreversible) areas in the fundus in most cases, indicating incomplete recovery in terms of the regional acid-secreting capacity (Iijima et al. 2009a). Given that the non-acid-secreting areas were characterized not only by residual inflammation, extensive gastric mucosal atrophy and intestinal metaplasia, but also by sustained, high levels of epithelial proliferation, we hypothesized that such functionally irreversible mucosa could reflect increased malignant potential where new cancer could develop after eradication (Iijima et al. 2009a).

In this study, in order to test our hypothesis concerning the preferential site of cancer after the eradication of \textit{H. pylori}, we enrolled patients who actually suffered from gastric cancer long after the eradication of \textit{H. pylori} infection. Then, we evaluated whether the neoplasias occurring after eradication had arisen from acid-secreting or non-acid-secreting areas identified by the Congo-red chromoendoscopy and then compared the histological characteristics between the respective areas in relation to the residual carcinogenic potential.

**Material and Methods**

**Patients**

From January 2005 to August 2010, 14 all consecutive patients who suffered from new gastric cancer after eradication of \textit{H. pylori} at Tohoku University Hospital were prospectively enrolled in this study. These patients had received eradication therapy from 1996 to 2008 at the hospital, and, after confirming that the treatment was successful, they returned for periodic endoscopic examination. In this trial, gastric cancers after eradication were defined as lesions that were discovered at least 2 years after the eradication. \(^{13}\)C-urea breath test confirmed that all 14 patients had remained free from re-infection at the time of cancer occurrence. Those with a history of gastric surgery, and those taking anti-secretary agents such as \(\text{H}_2\) blocker and proton pump inhibitor, were excluded from the study subjects. The study was approved by Tohoku University School of Medicine Ethics Committee and each subject gave written informed consent.

**Congo-red chromoendoscopy**

Congo-red chromoendoscopy was performed as we recently reported (Sekine et al. 2006; Iijima et al. 2009a). Briefly, after an overnight fast, pentagastrin at a dose of 6 \(\mu\)g/kg (pentagastrin; Sigma, St. Louis, Mo, USA) was administered to the subjects via an intramuscular injection prior to endoscopy. Immediately following the routine endoscopic inspection, a solution of 0.3\% Congo-red and 0.2M sodium bicarbonate was sprayed over the entire surface of the stomach through a spray catheter. Five minutes after spraying, the acid-secreting fundic mucosa turned a blue-black color, whereas the mucosa without acid production remained red. The areas of fundic mucosa with color shifting from red to blue-black were semi-quantitatively evaluated in two separate portions, namely “lesser curvature” and “greater curvature” (Sekine et al. 2006). In the lesser curvature, the areas with color shift were classified into 4 types: “all”, “large”, “small”, and “none”; while, in the greater curvature, they were classified into five types: “all”, “large”, “intermediate”, “small”, and “none”. Then, we determined whether the neoplastic lesion arose from the acid-secreting or non-acid-secreting area discriminated by Congo-red chromoendoscopy. Finally, two gastric biopsy specimens were taken in the fundus from the acid-secreting and non-acid-secreting areas within 2 cm of the boundary between the two areas and were subjected to the following histologic and immunohistochemical evaluation.

**Histology**

Using the Updated Sydney system (Misiewicz et al. 1990), the degrees of inflammation, activity, atrophy, and intestinal metaplasia were scored from 0 to 3 by two independent pathologists in a blinded manner (YA and AI).

**Immunohistochemistry**

Serial sections from paraffin-embedded biopsy specimens were assessed immunohistochemically for Ki-67 and p53 (Yabuki et al. 1997). Cell proliferation in the gastric epithelium was determined immunohistochemically using the monoclonal antibody MIB-1 (Dako, Glostrup, Denmark) against the nuclear proliferation-associated antigen Ki-67. Tumor suppressor p53 was determined immunohistochemically using DO-7 antibody (Novocastra, Newcastle upon Tyne, U.K.). For immunostaining, the tissue sections were incubated with the Ki-67 and p53 antibodies at a dilution of 1:100 at room temperature for 1 hour. The Ki-67 and p53 labeling indices were determined by counting the number of Ki-67-positive and p53-positive cells in at least three well-oriented gastric pits (more than 500 epithelial cells) and expressing the mean percentage of the total number of cells counted in each gastric pit.

**Serum pepsinogen concentrations**

Fasting serum samples obtained from all participants were centrifuged immediately at 4°C and stored at −20°C until measurement. Serum concentrations of PG I were measured using a CLEIA kit (Lumipulse pepsinogen I, Fujirebio Inc, Tokyo, Japan) (Iijima et al. 2009b).

**Statistics**

Clinical parameters of the enrolled subjects were expressed as mean (s.d.). Significant difference in histological scores, and Ki-67 and p53 labeling indices were determined by the Mann-Whitney test. \(P\) values < 0.05 were considered to be statistically significant.

**Results**

The characteristic features of the 14 enrolled patients in this analysis are listed in Table 1. Ten of 14 patients were male and the mean age at the occurrence of gastric cancer after eradication was 71 (9) years. The indications for eradication therapy were endoscopic mucosal resection for early gastric cancer in 10 cases (Fukase et al. 2008), gastric ulcer in 2 cases, duodenal ulcers in 1, and idiopathic thrombocytopenic purpura in 1 case (Kohda et al. 2002), and the mean period from the eradication to the subsequent occurrence of gastric cancer was 74 (44) months. In 10 patients who had received endoscopic mucosal resection previously, since none of the new cancerous lesions emerged from the preceding resection scar, they were unlikely to have been residual cancer due to incomplete endoscopic procedure. The cancerous lesions were duplicated in 5 cases, thus there were 19 lesions from 14 cases
Table 1. Characteristics of enrolled patients who suffered from gastric cancer at least 2 years after *H. pylori* eradication.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>Age</th>
<th>GI Disease prior to eradication</th>
<th>Location of primary gastric cancer</th>
<th>Current smoking</th>
<th>Current drinking</th>
<th>Time after eradication (M)</th>
<th>Number of cancers</th>
<th>Histology of cancers</th>
<th>Diameter of cancer (mm)</th>
<th>Macroscopic type</th>
<th>Location of cancers</th>
<th>Depth of cancers</th>
<th>Serum PG I</th>
<th>Occurrence site</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>66</td>
<td>GC</td>
<td>MB-AW &amp; LB-LC-GC</td>
<td>no</td>
<td>no</td>
<td>28</td>
<td>2</td>
<td>Intestinal</td>
<td>10 &amp; 10</td>
<td>0-IIc &amp; 0-IIc</td>
<td>UB-LC &amp; MB-PW</td>
<td>M &amp; M</td>
<td>11.5</td>
<td>NAS for both</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>64</td>
<td>GC</td>
<td>Ant-AW</td>
<td>no</td>
<td>yes</td>
<td>26</td>
<td>1</td>
<td>Intestinal</td>
<td>16</td>
<td>0-IIc</td>
<td>UB-PW</td>
<td>M</td>
<td>24.8</td>
<td>NAS</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>60</td>
<td>GC</td>
<td>Ant-LC &amp; LB-LC-GC</td>
<td>no</td>
<td>no</td>
<td>62</td>
<td>1</td>
<td>Intestinal</td>
<td>10</td>
<td>0-IIc</td>
<td>LB-AW</td>
<td>M</td>
<td>30.8</td>
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<tr>
<td>4</td>
<td>M</td>
<td>72</td>
<td>GU</td>
<td>-</td>
<td>no</td>
<td>no</td>
<td>105</td>
<td>1</td>
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<td>UB-PW</td>
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<tr>
<td>5</td>
<td>M</td>
<td>74</td>
<td>GC</td>
<td>LB-LC &amp; MB-PW-GC</td>
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<td>no</td>
<td>150</td>
<td>2</td>
<td>Intestinal</td>
<td>13 &amp; 5</td>
<td>0-IIc &amp; 0-IIb</td>
<td>UB-PW &amp; UB-PW</td>
<td>SM &amp; M</td>
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<td>6</td>
<td>M</td>
<td>80</td>
<td>GC</td>
<td>MB-LC</td>
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<td>no</td>
<td>58</td>
<td>2</td>
<td>Intestinal</td>
<td>20 &amp; 10</td>
<td>0-IIc &amp; 0-IIb</td>
<td>Ang-AW &amp; Ang-GC</td>
<td>M &amp; M</td>
<td>26</td>
<td>NAS for both</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>76</td>
<td>GC</td>
<td>Ant-GC</td>
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<td>no</td>
<td>91</td>
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<td>Intestinal</td>
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<td>Ang-AW</td>
<td>M</td>
<td>3.6</td>
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<tr>
<td>8</td>
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<td>MB-PW</td>
<td>no</td>
<td>yes</td>
<td>69</td>
<td>2</td>
<td>Intestinal</td>
<td>20 &amp; 10</td>
<td>0-IIa &amp; 0-IIa</td>
<td>Ang-LC &amp; Ang-LC</td>
<td>M &amp; M</td>
<td>NAS</td>
<td>NAS for both</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>78</td>
<td>GC</td>
<td>UB-LC</td>
<td>no</td>
<td>no</td>
<td>26</td>
<td>1</td>
<td>Intestinal</td>
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<td>0-IIc</td>
<td>MB-LC</td>
<td>M</td>
<td>NAS</td>
<td>NAS</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>76</td>
<td>AG</td>
<td>-</td>
<td>no</td>
<td>no</td>
<td>36</td>
<td>1</td>
<td>Intestinal</td>
<td>12</td>
<td>0-IIa</td>
<td>Ant-AW</td>
<td>M</td>
<td>49.6</td>
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</tr>
<tr>
<td>11</td>
<td>F</td>
<td>81</td>
<td>GC</td>
<td>Ant-LC</td>
<td>no</td>
<td>no</td>
<td>162</td>
<td>2</td>
<td>Intestinal</td>
<td>9 &amp; 6</td>
<td>0-IIa &amp; 0-IIa</td>
<td>Ang-AW &amp; Ang-GC</td>
<td>M &amp; M</td>
<td>95</td>
<td>NAS for both</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>70</td>
<td>GU</td>
<td>-</td>
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<td>no</td>
<td>85</td>
<td>1</td>
<td>Intestinal</td>
<td>8</td>
<td>0-IIc</td>
<td>Ant-AW</td>
<td>M</td>
<td>NAS</td>
<td>NAS</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>74</td>
<td>GC</td>
<td>Ant-AW</td>
<td>no</td>
<td>no</td>
<td>96</td>
<td>1</td>
<td>Intestinal</td>
<td>6</td>
<td>0-IIa</td>
<td>Ant-LC</td>
<td>M</td>
<td>3.8</td>
<td>NAS</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>48</td>
<td>DU</td>
<td>-</td>
<td>no</td>
<td>yes</td>
<td>36</td>
<td>1</td>
<td>Intestinal</td>
<td>5</td>
<td>0-IIc</td>
<td>Ant-GC</td>
<td>SM</td>
<td>73.3</td>
<td>NAS</td>
</tr>
</tbody>
</table>

**Total M/F: 10/4, GC:10, GU:2, DU:1, AG:1**

Yes/no: 0/14, 3/11, (44) 19 lesions, 10/6 (28.9) lesions.

Deta represent mean (s.d.). The macroscopic type of gastric cancer was classified according to the Japanese Classification of Gastric carcinoma. PG, pepsinogen; GC, gastric cancer; GU, gastric ulcer; AG, atrophic gastritis; DU, duodenal ulcer; UB, upper body; MB, middle body; LB, lower body; Ang, angularis; Ant, antrum; GC, greater curvature; LC, lesser curvature; AW, anterior wall; PW, posterior wall; M, mucosal infiltration; SM, submucosal infiltration; NAS, non-acid-secreting area; AS, acid-secreting area.
for the site-directed analysis. The diameters of the cancerous lesions ranged from 5 to 20 mm, averaging 10 mm, and when dividing the neoplastic lesion into the intestinal- and diffuse-type according to the Lauren classification (Lauren 1965), all lesions in this study were defined as the intestinal-type. The histological evaluation of the endoscopically and surgically resected specimens revealed that all 19 lesions were superficial cancers. The serum pepsinogen I concentration was less than 50 ng/ml in 9 of 11 available subjects, indicating the residual extensive fundic atrophy in these patients. Regarding the locations of the 19 cancerous lesions within the stomach, 13 lesions were located on the corpus including the angularis and the remaining 6 on the antrum. No lesions involved the cardia (Table 1). The greater curvature of the corpus escaped from the post-eradication carcinogenesis, while there was no such tendency in the antrum.

Congo-red chromoendoscopy revealed the predominance of acid-secreting areas in the greater curvature over those of the lesser curvature (Table 2). That is, while the whole or the majority of the greater curvature was occupied by acid-secreting areas in 10 of 14 subjects, there was no acid-secreting area observed in the lesser curvature in 8 of 14 subjects. Additionally, there was no acid-secreting area anywhere in the whole stomach, representing complete achlorhydria, in 2 subjects, one of whom was found to be anti-intrinsic factor-positive in the subsequent blood test and judged to be complicated by type-A autoimmune gastritis.

Regarding the distribution of cancer development within the stomach in relation to the regional acid-secreting capacity, all 19 cancerous lesions arose exclusively from non-acid-secreting areas (Table 1). Notably, the finding that 13 lesions were located on the corpus including the angularis implies that those lesions arose from irreversibly dysfunctional mucosa after eradication, considering that the acid secretory capacity is an intrinsic property of these anatomical sites in subjects who have never been \textit{H. pylori} positive. Fig. 1 shows a representative patient of a patient (74 y.o., male) who suffered from double superficial gastric cancer detected in the posterior wall of the upper body 13 years after eradication. The Congo-red chromoendoscopy of this patient revealed that both of the cancerous lesions arose from non-acid-secreting areas. Fig. 2 shows another representative patient (81 y.o., female) of a new, minute, gastric cancer detected 14 years after eradication. She underwent Congo-red chromoendoscopy prior to eradication as described in our previous study (Sekine et al. 2006; Iijima et al. 2009a). Although non-acid-secreting areas had

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Lesser curvature</th>
<th>Greater curvature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>Intermediate</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>Large</td>
</tr>
<tr>
<td>3</td>
<td>Small</td>
<td>All</td>
</tr>
<tr>
<td>4</td>
<td>None</td>
<td>Large</td>
</tr>
<tr>
<td>5</td>
<td>None</td>
<td>Large</td>
</tr>
<tr>
<td>6</td>
<td>None</td>
<td>Intermediate</td>
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<td>None</td>
<td>None</td>
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<tr>
<td>8</td>
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<td>Large</td>
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</tr>
<tr>
<td>11</td>
<td>Large</td>
<td>All</td>
</tr>
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<td>12</td>
<td>Small</td>
<td>All</td>
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<td>13</td>
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<td>None</td>
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<tr>
<td>14</td>
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<td>All</td>
</tr>
</tbody>
</table>

Acid-secreting mucosa was assessed by Congo-red chromoendoscopy, and the area was semiquantitatively classified into 4 groups in the lesser curvature and 5 groups in the greater curvature, respectively.

Fig. 1. Representative endoscopic images of patient 5.

Shown are the representative endoscopic images of routine endoscopy (a) and Congo-red chromoendoscopy (b) of patient 5. a) 0-IIc type gastric cancer was detected in the posterior wall of the upper body (arrowhead). In the vicinity of the lesion, another minute cancer lesion was also identified (arrow). b) Congo-red chromoendoscopy revealed that both lesions arose from non color-shifting (non-acid-secreting) areas.
been predominant in her stomach prior to eradication (Fig. 2A), acid-secreting areas expanded so remarkably by 7 months after eradication that non-acid-secreting areas remained in only a small portion of the lower body (Fig. 2B). Thereafter, she was followed with endoscopic examination once a year, and a minute gastric cancer was eventually detected 14 years after eradication (Fig. 2C). Congo-red chromoendoscopy at that time clearly revealed that the cancerous lesion arose from the residual non-acid-secreting-area (Fig. 2D).

Histologically, there were striking differences in the grade of mucosal atrophy and intestinal metaplasia between the acid-secreting and non-acid-secreting areas ($P < 0.01$ for both parameters). While the non-acid-secreting areas showed marked degrees of residual mucosal atrophy and intestinal metaplasia, the acid-secreting areas were free of such histological changes in the majority of cases (Fig. 3). In addition, a substantial degree of residual inflammation was also found in the non-acid-secreting areas in most cases, whereas only mild inflammation was detected in acid-secreting areas, and the difference in inflammation scores between the two areas was significant ($P < 0.01$). There was no neutrophil infiltration observed in any biopsy specimens (activity score $= 0$).

Immunohistochemistry for Ki-67 as a marker for cell proliferation revealed that there were remarkable (approximately 6 fold) differences in the protein expression between the two areas ($P < 0.01$, Fig. 4C); marked Ki-67 positivity was diffusely observed in the epithelium of non-acid-secreting areas (Fig. 4A), while the protein expression was detected only in the nuclei of the cells of the glandular neck in acid-secreting areas (Fig. 4C). Likewise, p53 immunohistochemistry was also significantly different between the two areas ($P < 0.01$, Fig. 5C). Substantial nuclear expression of p53 was identified in the epithelium of the non-acid-secreting areas (Fig. 5A), while positivity was rarely observed in acid-secreting areas (Fig. 5B).

**Discussion**

Using Congo-red chromoendoscopy, we have already shown that acid-secreting areas were significantly expanded shortly after $H. pylori$ eradication, mainly with the resolu-
tion of inflammation (Sekine et al. 2006). However, the restoration was incomplete in that residual, non-acid-secreting areas remained in the fundus even in the long-term follow-up (Iijima et al. 2009a). The present study extended our previous observation by showing that gastric cancer newly emerged after the eradication exclusively from the residual, non-acid-secreting areas. Therefore, such non-acid-secreting areas identified by Congo-red chromoendoscopy could be regarded as the major carcinogenic background for gastric cancer arising after *H. pylori* eradication.

There have been several reports that showed the incidence and characteristic features of gastric cancer after *H. pylori* eradication (Take et al. 2005; Kamada et al. 2005; Takenaka et al. 2007; Fukase et al. 2008; Ogura et al. 2008; Yanaoka et al. 2009; de Vries et al. 2009). However, many of these included cases whose cancer lesions developed relatively soon after eradication, namely within 2 years after the treatment (Take et al. 2005; Kamada et al. 2005; Takenaka et al. 2007; Fukase et al. 2008; Ogura et al. 2008). In the present study, we defined post-eradication gastric cancers as lesions that were discovered at least 2 years after the eradication to minimize the possibility of mistaking a minute cancer that had already existed at the time of the eradication therapy. Consequently, during the long-term periodic follow-up, with a mean duration of more than 6 years, we detected 14 cases in which the discovered cancer

![Fig. 3. Differences in histological findings between acid-secreting and non-acid-secreting areas.](image)

Biopsy samples from acid-secreting and non-acid-secreting areas of each subject were semi-quantitatively evaluated regarding inflammation, atrophy, and intestinal metaplasia. In two subjects, there was no-acid-secreting area in their stomachs and the data only from the non-acid-secreting areas were plotted. NAS: non-acid-secreting areas; AS: acid-secreting areas; IM: intestinal metaplasia.

*represents statistical difference with $p < 0.01$.

![Fig. 4. Comparison of immunohistochemical analysis on Ki-67 between acid-secreting and non-acid-secreting areas.](image)

a) Immunohistochemical image of Ki-67 from the non-acid-secreting area showed diffuse Ki-67 positivity in the epithelium. b) Immunohistochemical image of Ki-67 from the acid-secreting area of the same subject showed only scattered positivity in the nuclei of the cells of glandular neck. c) A comparison of the labeling index for Ki-67 between the two areas showed large differences between them ($P < 0.01$). Note that the data for acid-secreting areas were not available in 2 subjects, because acid-secreting areas did not appear in these patients. NAS: non-acid-secreting area; AS: acid-secreting area.
lesions were still tiny, with a mean diameter of 10 mm, strongly suggesting that the lesions of our present case series developed de novo after eradication.

Before the recognition of *H. pylori* infection in a human stomach, Tatsuta et al (Tatsuta et al. 1979) found, using Congo-red chromoendoscopy, that gastric cancer developed chiefly from non-acid-secreting areas and, in a particular intestinal type of cancer, invariably from such areas, and that such functionally impaired areas were characterized by mucosal inflammation as well as mucosal atrophy. The present study extends this previous observation by showing that, although the non-acid-secreting areas are considerably reduced by the elimination of *H. pylori* (Sekine et al. 2006; Iijima et al. 2009a), gastric carcinoma of the intestinal type still arises from residual, non-acid-secreting areas after eradication. We also found that such non-acid-secreting areas were mainly characterized by marked mucosal atrophy and intestinal metaplasia. Regarding the distribution of gastric cancer within the stomach, the greater curvature of the fundus and angularis was completely free of cancer, which might reflect our previous observation that acid secretion is more easily restored after eradication in the greater curvature compared to the lesser curvature (Sekine et al. 2006).

In our previous long-term follow-up study of non-cancer patients (Iijima et al. 2009a), we already reported that the non-acid-secreting gastric mucosa remaining after *H. pylori* eradication showed extensive residual inflammation, mucosal atrophy, intestinal metaplasia, and sustained hyperproliferation compared with acid-secreting areas. In the present study of patients who suffered from gastric cancer after eradication, we found similar differences in the histological features between the two functionally distinct areas, but the differences between the two areas were more prominent in the patients of the present study compared with those of the previous study (e.g. for Ki-67 immunostaining, 6 fold difference between the 2 areas in the former study vs. 2.5 fold in the latter (Iijima et al. 2009a)). Such severe histological abnormalities persisting after eradication could predispose some patients to gastric carcinogenesis.

It has been reported that immunohistochemical accumulation of p53 was found in gastric mucosa infected with *H. pylori* infection and that it significantly decreased after eradication (Jones et al. 1997; Nardone et al. 1999; Satoh et al. 2001; Kodama et al. 2007). On the other hand, another study pointed out that significantly higher accumulation of p53 was detected in the gastric mucosa of patients with multifocal atrophic gastritis 1 year after eradication compared with that of patients with non-atrophic superficial gastritis (Guarner et al. 2005). Partly consistent with this study, the present study clearly revealed a site-specific difference in the p53 accumulation in *H. pylori*-eradicated patients, that is, sustained expression of p53 protein was observed in non-acid-secreting areas, while the protein was...
rarely expressed in the neighboring acid-secreting areas, suggesting that accumulation of the protein could persist after eradication in non-acid-secreting areas. Previous studies have indicated that over-expression of p53 protein detected by immunohistochemistry generally represents mutant p53 (Nardone et al. 1999; Murakami et al. 1999; Kodama et al. 2007), suggesting that the immunohistochemical accumulation of p53 is an indicator of the loss of the p53 tumor suppressor function. Therefore, the p53 protein accumulation detected in non-acid-secreting areas might represent important molecular events involved in the persistent gastric carcinogenesis after *H. pylori* eradication.

The induction of fundic gastric cancer post-eradication exclusively from the functionally irreversible, non-acid-secreting areas could suggest that such areas had already progressed to an irreversible stage of histological change leading to genetic alterations of genes that are crucial to gastric carcinogenesis, such as p53. Thus, the progress from atrophy and intestinal metaplasia to cancer might be an autonomous, *H. pylori*-independent process (de Vries et al. 2009). Simultaneously, the slightly but significantly greater residual inflammation in the non-acid-secreting areas could also be responsible for the carcinogenesis in the areas after eradication through the sustained pro-carcinogenic actions of sustained inflammatory cytokines (Gonda et al. 2009).

One advantage of the present study is the enrollment of patients with superficial cancers so that we could pinpoint the origin of the cancer whether they arose from acid-secreting or non-acid-secreting mucosa. This is an important point since cancer lesions often arose in the vicinity of the boundary of the two areas. On the other hand, a potential limitation is the small numbers of the subjects enrolled. Since the occurrence of gastric cancer after *H. pylori* eradication is still relatively scarce, subjects were strictly screened by enrolling only those in whom gastric cancer was discovered at least 2 years after the eradication, which prevented us from enrolling more patients despite the 5-year enrollment period. Another limitation is that all cancer lesions in the present analysis were the intestinal type. Although the present results on the cancer histology are in agreement with a recent review describing that most gastric cancers that developed after eradication were the intestinal-type (Ito et al. 2009), further studies on the diffuse-type of gastric cancer after *H. pylori* eradication are required.

In conclusion, gastric cancer emerged de novo after eradication exclusively from non-acid-secreting areas. Because this study employing Congo-red chromoendoscopy confirms the significance of non-acid-secreting areas as the origin of carcinogenesis after eradication, further trials for the identification of such high risk areas with recently advanced endoscopy, such as high-resolution endoscopy with narrow band imaging (NBI) (Uedo et al. 2006; Bansal et al. 2008), should be a promising approach for estimating the individual cancer risk in subjects after eradication. Interestingly, a recent study has shown that, in patients with fundic atrophy, the re-emergence of the normal fundic fine structure (fundic pit) after eradication could be detected by magnifying endoscopy (Yagi et al. 2005). This might suggest that advanced endoscopy could make it possible to discriminate between non-acid-secreting areas with high carcinogenic potential from the remaining acid-secreting areas.

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**Conflict of Interest**

All authors have no conflict of interest in this study.

**References**


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