

CASE REPORT

Helicobacter pylori-negative Differentiated Adenocarcinoma of the Stomach

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Abstract

A 58-year-old Japanese man was diagnosed with differentiated adenocarcinoma of the stomach. Histological findings of the resected specimen revealed well- to moderately-differentiated tubular adenocarcinoma (tub1, tub2), 13 mm in diameter, which invaded into the submucosa (SM1, 300 μm) and lymphovascular lumen (ly1). Serum antibody against Helicobacter pylori (Hp) and the $^{13}$C-urea breath test were negative, and there were no atrophic changes in the tumor-adjacent mucosa. The immunohistochemical analysis showed that gastric mucin (MUC5AC) was strongly positive and intestinal mucin (MUC2) was weakly and partially positive. According to these results, the final diagnosis of Hp-negative well-differentiated early gastric cancer was made.

Key words: early gastric cancer, Helicobacter pylori, differentiated adenocarcinoma, ESD, mucin phenotype


Introduction

Helicobacter pylori (Hp) infection plays an important role in the development of gastric cancer. It was previously reported that no gastric cancer developed in Hp-negative patients in a 9-year prospective study (1). Other factors including genetic factors, Epstein-Barr virus (EBV) infection, a high salt intake, duodenal reflex, and metabolic syndrome, are known to contribute to gastric carcinogenesis. Autoimmune gastritis is known to be a high-risk condition of gastric cancer, however, it is very rare among the Japanese population.

The incidence of Hp-negative gastric cancer (HPNGC) is reported to be 0.42-18.8% (2-6). This great variation is likely derived from differing definitions of HPNGC employed among the previous studies. Despite several useful markers for detecting the presence of Hp infection, the false-negative rate cannot be ignored. Moreover, there is a possibility of natural clearance and incidental eradication of Hp. Therefore, confirming the existence of an atrophic change in the non-cancerous mucosa, in addition to various markers, is important for the accurate diagnosis of HPNGC. Matsuo et al. (3) defined HPNGC as: 1) a serum anti-Hp antibody titer ≤10 U/mL; 2) the absence of Hp and gastritis by a histological examination; 3) the absence of an atrophic change in the gastric mucosa by an endoscopic examination; and 4) a negative urea breath test or rapid urease test. Additionally, Ono et al. (2) defined HPNGC as an adenocarcinoma arising from the gastric mucosa without histological atrophy, endoscopic atrophy, or serological atrophy. According to these strict and valid criteria, these two groups reported that the prevalence of HPNGC was 0.66% and 0.42% of all gastric cancer, respectively. Therefore, HPNGC is considered to be a rare subtype of gastric cancer in the Japanese population, however, its clinical and pathological features re-
A 58-year-old Japanese man was found to have a depressed lesion in the posterior wall of the gastric middle body while undergoing a routine health checkup endoscopic examination. A biopsy specimen revealed well-differentiated tubular adenocarcinoma (tub1). The patient was referred to our hospital for close examination and therapy. He had received cholecystectomy for gallbladder stones at 40 years of age. 

The physical examination and laboratory data including serum anti-parietal cell antibody, anti-Hp, Cre, AST, ALT, ALP, γGT, LDH, BUN, and Creatinine showed no abnormalities (Table).

### Table. Laboratory Data on Admission.

<table>
<thead>
<tr>
<th>Normal Range</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cell count (μL)</td>
<td>3.590-9.640</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.2-17.2</td>
</tr>
<tr>
<td>Platelet count (× 10^12/μL)</td>
<td>148-339</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>6.7-8.3</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.8-5.3</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>0.3-1.2</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>12-41</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>7-45</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>90-298</td>
</tr>
<tr>
<td>γGT (IU/L)</td>
<td>4-50</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>90-230</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>8-21</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.8-1.3</td>
</tr>
</tbody>
</table>


The incidence of HPNGC differs among previous reports (2-6), most likely due to the various definitions of tomography, endoscopic submucosal dissection (ESD) was performed. ESD was successfully performed, and the histological findings of the resected tissues demonstrated a well-to moderately-differentiated adenocarcinoma (Fig. 2A-C), which invaded into the submucosa (SM1, 300 μm, Fig. 2D) and lymphovascular lumens (ly1) (Fig. 2E). Lymphovascular invasion was not detected by additional D2-40 immunostaining using continuous sections (Fig. 2F), however, we judged the invasion of lymphovascular lumens to be positive according to the findings of hematoxylin and eosin staining (Fig. 2E). Undifferentiated cancer cells were not found by the additional pathological evaluation using continuous sections. Moreover, no atrophic and dysplastic changes, intestinal metaplasia, inflammatory cell infiltration, or Hp infection by Giemsa staining were detected in the mucosa around the cancer. According to the Japanese classification system, the final pathological diagnosis was [M, Post., 0-IIc, 13×8 mm in 40×37 mm, well- to moderately-differentiated adenocarcinoma (tub1, tub2), SM1 (300 μm), UL(-), ly1, v0, pHM0, pVM0] (Fig. 2G). The tumor cells were positive for mucin (MUC5AC and partially positive for MUC6 and MUC2 (Fig. 3) according to the immunohistochemistry analysis, thus the mucin phenotype was considered to be the mixed type. Additionally, immunostaining of pepsinogen (PG) I and H/K+-ATPase was negative for the tumor cells (Fig. 3, indicating that this tumor was not derived from the fundic gland. In situ hybridization for the EBV genome (EBV-ISH) was also negative (Fig. 3). Due to the presence of lymphovascular involvement, additional fundic resection was performed followed by the 2010 Japanese gastric cancer treatment guidelines, Ver.3. The surgical specimen revealed no residual cancer or lymph node metastasis. No postoperative intragastric recurrence or distant metastasis was observed.

### Discussion

The incidence of HPNGC differs among previous reports (2-6), most likely due to the various definitions of...
In some reports, the Hp status was assessed only by a histologic examination or serum antibody titer. Matsuo et al. (3) and Ono et al. (2) used the most stringent definitions of HPNGC, and in their reports, the prevalence of HPNGC was reported to be less than 1%. In the present case, serum antibodies against parietal cells and Hp and UBT were all negative, and no atrophic change was found in the non-tumorous mucosa by the histological and endoscopic examinations. Additionally, the possibility of a gastric fundic carcinoma or EBV-related cancer was excluded by pathological evaluation. Therefore, the final diagnosis of Hp-unrelated differentiated adenocarcinoma developed from the normal gastric mucosa was made.

In comparison with Hp-positive gastric cancers, strictly-defined HPNGC are diagnosed at an earlier age [mean age: 56.5 years (3)] with no gender predominance (2, 3). In the endoscopic appearance, the depressed lesion was characteristic for HPNGC (3), which is consistent with the present case. Previous studies demonstrated that approximately 70% of HPNGC was pathologically diagnosed as poorly-differentiated or signet ring cell adenocarcinoma (3, 5). In the report from Ono et al. (2) 1 case (0.42%) was diagnosed as HPNGC in 240 cases of gastric cancer. Similarly, the report from Matsuo et al. (3) showed that 21 of 3161 gastric cancer patients (0.66%) were HPNGC while less than 7 cases had well- to moderately-differentiated HPNGC. Therefore, the present case is considered to be an extremely rare, differentiated adenocarcinoma in HPNGC which arose from the non-atrophic mucosa in the gastric body.

Adenocarcinoma arising from the fundic gland (the chief cell predominant type) was recently proposed as a new and rare variant that may be involved in previous HPNGC cases. This type of gastric adenocarcinoma is confirmed by positive immunostaining against PG I or H/K-ATPase. The submucosal tumor-like shape, whitish color, dilated vessels with branching architecture, and non-atrophic background mucosa were reported to be common endoscopic features (7). Therefore, when we encounter HPNGC, immunostaining of PG I and H/K-ATPase should be added to exclude this type of tumor. However, in the present case, the tumor did not originate from the gastric fundic gland.

In the former case, approximately one-half of all gastric cancer cells simultaneously expressed intestinal mucin despite the development from normal gastric mucosa without intestinal metaplasia (3-5). This finding suggests that intestinal metaplasia is not essential for the acquisition of the intestinal mucus phenotype. Yoshikawa et al. (8) revealed over 40% of early-stage Lauren’s intestinal-type carcinomas consist of mainly gastric-type cancer cells, and a phenotypic shift from the gastric to intestinal type was evident with the

Figure 1. Pre-treatment endoscopic examination. (A): A regular endoscopic examination revealed a slightly depressed white area, approximately 10 mm in size, with no deep invasion signs and no atrophic background mucosa in the posterior wall of the gastric middle body (yellow circle). A regular arrangement of collecting venules was seen in the tumor-adjacent mucosa. (B): Indigo-carmine staining. (C): NBI light endoscopy showed that the tumor had a villous pattern. (D): Magnified endoscopy with NBI revealed the borderlines of the lesion could be detected clearly.
progression. The tumor cells in this case might acquire the intestinal phenotype with the progression and dedifferentiation.

*H. pylori* infection and atrophic gastritis have been regarded as risk factors for gastric cancer. The combination of serum PG and *H. pylori* antibody (ABC stratification) has been suggested to serve as a useful predictive marker for patients with gastric cancer (9, 10). In these studies, an individual showing normal PG levels (PG I level ≤70 ng/mL and PG I/PG II ratio ≤3) and negative *H. pylori* antibody is classified into group A, which is judged as the absence of *H. pylori* infection and atrophic change. Although the serum PG levels were not measured on admission, the present case would be presumably included in group A. It was additionally reported that the incidence of gastric cancer development stratified by age (under 60 years old), sex (male), and ABC stratification (group A), was 0.1%/year (10). The present case demonstrates that gastric cancer is presumably in the group A individuals and should not be ignored, although the frequency is very rare. Further studies are needed to examine the prevalence and clinicopathological features of gastric cancer from group A patients.

In the present case, an atypical submucosal invasion pattern was found. Although the cancer cells were histologically well- or moderately-differentiated, the mode of invasion appeared to be similar to that found in poorly- or undifferentiated cancer. We included an additional histological examination, however, we could not detect poorly-differentiated or undifferentiated cancer components. The mechanism of invasion of gastric carcinoma is not currently
clear. However, cell adhesion is one of the important steps in invasion and metastasis (11). It has been previously reported that the aberrant expression of proteins associated with cell adhesion is a possible marker of submucosal invasion in differentiated early gastric cancer and may be a useful predictor of lymph node metastasis (12). The breakdown of cell-cell contact may be associated with the invasion of gastric cancer cells into the submucosa and lymphovascular

**Figure 3.** Immunohistochemical and in situ hybridization analyses of the tumor cells. The adenocarcinoma cells were positive for MUC5AC and partially positive for MUC6 and MUC2, but negative for CD10, pepsinogen I, H⁺/K⁺-ATPase, and EBV genome.
lumens.

Compared with advanced gastric cancer, lymph node metastasis is the only independent prognostic factor for early gastric cancer (13, 14). In this case, we performed gastrectomy and lymph node dissection, however, no local recurrences and lymph node metastases were observed in the resected specimen. Careful screening is necessary because the clinical course and recurrence rate of HPNGC are unclear.

In summary, we herein reported a rare case of differentiated HPNGC. Because the prevalence of persistent \textit{Hp} infection and the related gastric cancer is estimated to be decreased in Japan due to the establishment of an early screening system and \textit{Hp} eradication strategy (15), HPNGC will most likely become a more frequent type of gastric cancer in the future. Therefore, the accumulation of HPNGC cases is important to clarify the clinicopathological characteristics of HPNGC.

The authors state that they have no Conflict of Interest (COI).

References


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