Epithelial cell turnover in relation to ongoing damage of the gastric mucosa in patients with early gastric cancer: increase of cell proliferation in paramalignant lesions

Akiko Shiotani1, Hiroyasu Ishi2, Shingo Ishiguro2, Masaharu Tatsuta2, Yukinori Nakae3, and Juanita L. Merchant4

1Health Administration Center, Wakayama University, 930 Sakaedani, Wakayama 640-8510, Japan
2Department of Gastrointestinal Oncology, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan
3Aishinkai Nakae Hospital, Wakayama, Japan
4Departments of Internal Medicine and Molecular and integrative Physiology, University of Michigan, Ann Arbor, MI, USA

Background. Gastric cancer is typically an end result of Helicobacter pylori-associated chronic gastritis. The pathogenesis is thought to involve effects on gastric mucosal epithelial cell turnover. In this study, we aimed to compare apoptosis and proliferation in the noncancer-containing mucosa of H. pylori-positive patients with early gastric cancer with these phenomena in H. pylori-positive controls. Methods. Two specimens each were obtained from the greater and lesser curvatures of the corpus and from the greater curvature of the antrum. The histopathological grading used was the updated Sydney System. Apoptotic epithelial cells were detected using the terminal deoxy nucleotidyl transferase-mediated deoxy-uridine triphosphate (dUTP) biotin nick-end labeling (TUNEL) method. The expression of Ki 67 was evaluated by immunostaining. Results. Forty-five H. pylori-positive patients with endoscopic mucosal resection for early gastric cancer and 52 H. pylori-positive controls were studied. Gastric cancer was associated with a higher frequency of incomplete intestinal metaplasia (IM; odds ratio [OR], 19.1; 95% confidence interval [CI], 6.9–53.2; P < 0.001). The apoptotic index (AI) in the greater curvature of the corpus and the proliferation index (PI) in each part were significantly higher in cancer patients than in the control group. The median PI in the antrum was significantly higher in the incomplete IM group than that in the complete IM group (17.6 vs 12.6; P = 0.009). The PI and the AI in the greater curvature of the corpus correlated with the activity score, and the PI correlated with the IM score. Conclusions. In the cancer patients, H. pylori-induced gastritis was associated with increased cell proliferation and apoptosis compared with mucosal findings in the controls. IM seems to be one of the most important factors affecting cell proliferation and may be one of the components of carcinogenesis that results in proliferation-dominant cell kinetics.

Key words: intestinal metaplasia, gastric cancer, proliferation, apoptosis, Helicobacter pylori

Introduction

The evolution of Helicobacter pylori-associated gastric cancer begins with superficial gastritis that progresses to chronic gastritis, atrophy with islands of intestinal metaplasia (IM), development of dysplasia, and, finally, frank carcinoma.1–4 There is strong evidence that H. pylori infection alters the kinetic pattern of the gastric glandular epithelium. Effects of the infection on gastric mucosal epithelial cell turnover are thought to contribute to the process of carcinogenesis, with disturbances in the balance between cellular proliferation and apoptosis predisposing to either cell loss with mucosal damage or cell accumulation and cancer development.

Recent studies suggest that H. pylori infection induces cell apoptosis, a genetically regulated form of programmed cell death.5–9 Moss et al.9 reported that gastric epithelial apoptosis was enhanced in duodenal ulcer patients with H. pylori infection and that the increased number of apoptotic cells returned to normal after H. pylori eradication. On the other hand, other studies have reported that H. pylori infection is not associated with significant changes in gastric epithelial apoptosis, because there is wide variability in the apoptotic indices of both H. pylori-positive and negative patients.10

The aim of this study was to compare apoptosis and proliferation in the non-cancer-containing mucosa of patients with early gastric cancer with these phenomena...
in *H. pylori*-positive controls. We also assessed the relationship between cell turnover and histological findings.

**Methods**

This was a case-control study of patients with a history of endoscopic mucosal resection (EMR) for early gastric cancer. The study was performed in Japan, at the Osaka Medical Center for Cancer and Cardiovascular Diseases and at Aishinkai Nakae Hospital. Patients were enrolled for the study between November 2001 and June 2003.

**Patients**

*H. pylori*-positive patients with a medical history of EMR for early stage, noncardiac intestinal type of gastric cancer without lymph node metastasis, and *H. pylori*-positive, age- and sex-matched control patients were eligible for participation. Patients were excluded if they had had eradication therapy for *H. pylori* infection; used anti-secretory drugs or nonsteroidal anti-inflammatory drugs (NSAIDs); or had hemorrhagic diseases, insulin-dependent diabetes mellitus, cirrhosis, or renal failure. Demographic data collected at study entry included age, sex, smoking habits, alcohol consumption, and drug treatments, including the use of anti-secretory drugs. Drinking and smoking were defined as regular intake when consumption was more than 35 g of ethanol or 5 cigarettes or more per day, respectively. The study was approved by the Osaka Medical Center for Cancer and Cardiovascular Diseases Ethics Committee, and informed consent was obtained from each patient.

**Histopathological grading**

Endoscopies were performed by experienced endoscopists after the patients had had a 12-h fast. Two specimens each were obtained from the greater curvature of the antrum and the greater and lesser curvature of the corpus. The specimens were stained with hematoxylin and eosin, and additionally stained with Giemsa to score *H. pylori* density. A visual analogue scale, graded from 0 (absent/normal) to 3 (maximal intensity) was used for the following parameters: activity of gastritis (granulocytic infiltration), inflammation (lymphocytic and plasma cells infiltration), glandular atrophy, IM, and *H. pylori* density, according to the updated Sydney classification.11 Well-oriented tissue sections with gastric pits and glands cut longitudinally and visible along the entire isthmus zone were selected for quantitative analysis. Histopathological grading was determined twice for each slide, by a single pathologist who was blinded to the previous histological scores and other experimental results. Average histological scores were used to analyze the data.

**Diagnosis of *H. pylori* infection**

Venous blood samples were analyzed for specific IgG *H. pylori* antibodies with an enzyme-linked immunosorbent assay (ELISA) kit, using the E plate test (Eiken Kagaku, Tokyo, Japan). Patients were considered to be infected with *H. pylori* if the serum test was positive or if chronic gastritis/atrophy with *H. pylori* was histopathologically present.

**Subtyping IM**

Samples were also stained with Alcian blue (AB)/high iron diamine (HID) to categorize IM as complete type (type I) or incomplete type (types II and III). The slides were immersed in HID solution for 20 h at room temperature, then rinsed with deionized water and stained with 1% AB (pH 2.5) for 2 min.

**Immunohistochemistry**

Three adjacent sections, 4-µm-thick, were cut onto each of three polylysine-coated glass slides. The sections were deparaffinized, washed in phosphate-buffered saline (PBS), and autoclaved for 10 min in sodium citrate buffer for antigenic retrieval. For immunostaining, sections were incubated with mouse monoclonal anti-human Ki-67 (DAKO; Carpinteria, CA, USA) at a dilution of 1:100 at room temperature for 1 h. After a washing in PBS, the sections were incubated for 1 h with an anti-mouse immunoglobulin conjugated to horseradish peroxidase dextran-labeled polymer, using an EnVision peroxidase mouse system (DAKO). The sections were washed in PBS, stained in 0.05% diaminobenzidine hydrochloride (DAB) solution for 5 min, then counterstained with hematoxylin. After dehydration with xylene, the sections were mounted under a glass coverslip. More than 500 cells along the proliferation zone were counted, and Ki-67-positive cells were expressed as a percentage of the total number of cells, as the proliferative index (PI).

**Detection of apoptotic cells**

Apoptotic cells were detected by the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate (dUTP) biotin nick-end labeling (TUNEL) method, using Apoptag fluorescein direct in situ apoptosis detection kits (ApopTag; Intergen, New York, NY, USA) according to the manufacturer’s pro-