Low presence of p53 abnormalities in H. pylori-infected gastric mucosa and in gastric adenocarcinoma

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Background. Alterations of the p53 gene and/or its abnormal protein accumulation have been observed in gastric cancer and preneoplastic lesions. Our aim was to assess possible associations between different H. pylori strains and p53 abnormalities in patients with dyspepsia and with gastric cancer. Methods. Seventy-five dyspeptic patients and 40 patients with gastric adenocarcinoma entered the study. H. pylori status was determined by the rapid urease test, histology, and polymerase chain reaction (PCR) analysis. Overexpression of the p53 protein was evaluated by immunohistochemistry. Detection of p53 mutations was done by direct DNA sequencing. Results. Fifty-four of the 75 (72.0%) dyspeptic patients and 27 of the 40 (67.5%) gastric cancer patients showed H. pylori infection. Cytotoxin-associated gene (cagA)-positive strains were found in 31 of the 54 (58%) dyspeptic patients and in 25 of the 27 (92.6%) neoplastic patients. As regards vacA, s2 strains showed the highest prevalence among dyspeptic patients (24 of 54 patients; 44.4%), whereas s1 strains were more expressed among cancer patients (23 of 27; 85.2%). Among the dyspeptic patients, 1 patient with duodenal ulcer showed p53 overexpression. Three mutations were identified by DNA sequencing: one in a patient with normal endoscopic findings and two in patients suffering from gastritis. Among the neoplastic patients, 16 subjects (40%) showed p53 overexpression (9 had diffuse-type and 7 intestinal-type cancer). Four mutations (10%) occurred in patients with intestinal-type gastric cancer. No association between p53 abnormalities (overexpression/mutation) and H. pylori infection was found in either group of patients. Conclusions. These results lead us to hypothesize that H. pylori infection does not affect the p53 pattern in gastric mucosa. Moreover, mutations of the p53 gene do not seem to be a predominant event in gastric carcinogenesis, at least in our populations.

Key words: dyspepsia, H. pylori, gastric cancer, p53

Introduction

Gastric carcinogenesis is a multistep process in which different environmental and genetic factors are involved. Several elements point to the role of Helicobacter pylori in the development of preneoplastic and neoplastic changes.1,2 The relationship could be due mainly to increased cellular proliferation.3,4 In addition, specific virulence determinants of H. pylori strains can influence the outcome of the infection. Among them, the cytotoxin-associated gene (cag) is a marker for a genomic pathogenic island whose presence is associated with a more severe clinical outcome.5,6 Another cytotoxin that injures epithelial cells is encoded by vacA.7 Subgenotypes of vacA have been identified and specific vacA alleles correlate with the presence of the cagA and gastroduodenal pathology.8 The subsequent passage toward cancer is probably caused by other factors, such as the onset of infection or causes which are independent of H. pylori.

In this connection, genetic abnormalities of oncogenes or oncosuppressor genes in patients with H. pylori infection may also account for the different evolution of the infected gastric mucosa. Nevertheless, the association between H. pylori infection and oncogene or oncosuppressor gene modifications has still to be completely elucidated.

Among the components of the oncosuppressor family, the p53 gene plays an important role in the cell cycle.
and apoptosis.\textsuperscript{9} If mutations occur in its genomic structure, its function in cell growth regulation may be altered.

Alterations of the \( p53 \) gene and/or its abnormal protein accumulation have been observed in many kinds of malignant tissue of the kidney, colon, and lung.\textsuperscript{10-12} In gastric mucosa, Yamada et al.\textsuperscript{13} found that \( p53 \) gene mutations were detectable in metastases, being a very late event in carcinogenesis. On the contrary, other authors have shown that \( p53 \) abnormalities may occur in precancerous gastric lesions, such as atrophic gastritis and intestinal metaplasia, as well as adenomatous polyps.\textsuperscript{14,15}

As to any relation between \( H. \textit{pylori} \) infection and \( p53 \) alterations, attempts to find possible links have been made by different researchers, but the results were contrasting.\textsuperscript{16,17} Differences in methods, geographic distribution and patient selection, collection of \( H. \textit{pylori} \), and bacterial genotype may partly account for the different results. Thus, in order to further analyze any connection between \( H. \textit{pylori} \) and oncosuppressor genes, the aims of our study were: (i) to investigate the presence of \( p53 \) gene mutations and/or the accumulation of its protein in patients with \( H. \textit{pylori} \)-infected gastric mucosa and in patients with gastric adenocarcinoma from our population in Southern Italy, and (ii) to evaluate whether any relationship exists between these genetic alterations and the bacterial genotype.

**Patients and methods**

Seventy-five dyspeptic patients (35 men and 40 women; median age, 47 years; range, 20–83 years) undergoing upper endoscopy, and 40 consecutive patients (27 men and 13 women; median age, 66 years; range, 32–87 years) who underwent surgical excision for gastric adenocarcinoma at our Institute entered the study.

**Dyspeptic patients**

All the dyspeptic patients were referred by their primary care physician for a diagnostic upper endoscopy.

Patients were considered to be eligible for the study if they had not received non-steroidal anti-inflammatory drugs, antibiotics, bismuth, antacids, H2-receptor antagonists, omeprazole, sucralfate, or misoprostol in the 2 months prior to the examination and had no previous history of gastric tumors, gastric or duodenal ulcers, or gastric surgery. Informed consent was obtained from all subjects.

All patients underwent endoscopy and biopsy performed by two gastroenterologists, who independently described the endoscopic appearance of the stomach. An Olympus GIF 100 endoscopy (Olympus Lake Success, NY, USA) was used in all patients.

The gastroenterologists classified the endoscopic gastric appearance as normal, endoscopic gastritis (edema and/or exude with visible vascular pattern), hemorrhagic gastritis (punctate ecchymoses or frank bleeding into the lumen), erosive gastritis (flat and/or raised erosions), or peptic ulceration (gastric/duodenal ulcer or ulcer scar).\textsuperscript{18}

At least two antral (about 2 cm from the pylorus) and two body (small curve) biopsy specimens were obtained from each patient for histology, and two antral specimens were taken for intrabiopsy urease activity and polymerase chain reaction (PCR) analysis, respectively.

Two biopsy samples from the antrum and two from the body were routinely and blindly assessed, using hematoxylin-eosin stain and periodic acid-schiff (PAS) stain, by an independent pathologist who was unaware of the clinical history or the endoscopic findings corresponding to each biopsy. The presence and severity of gastritis were classified using the classification of Correa and Yardley.\textsuperscript{19} Other histological changes, such as ulceration, intestinal metaplasia, lymphoid follicle formation, and lymphoepithelial lesions, were also recorded.

Additional Warthin-Starry staining was performed to confirm the presence of \( H. \textit{pylori} \) on the mucosal stream of mucus and on the foveolar epithelium.

**Neoplastic patients**

After surgical excision for gastric adenocarcinoma, the tissue specimens were immediately sent to the pathologist. In accordance with standardized sampling protocols, surgical and deeper portions of tumor, as well as the edges of the lesion, including the adjacent normal gastric mucosa, were obtained from each specimen. The sections, with the exception of those for PCR, were then fixed in 10% buffered formalin and paraffin-embedded. Several slices, 4-μm-thick, were obtained from each block and stained with hematoxylin-eosin (H&E) and PAS for the macroscopic assessment.

In order to differentiate the intestinal type from the diffuse type, Laurèn’s criteria\textsuperscript{20} were followed for the diagnosis. The prevalence of signet ring cells or glandular structures was the criterion for diagnosing diffuse type or intestinal type, respectively. PAS-stained sections were useful to recognize the signet ring cells peculiar to the diffuse type.

Particular attention was paid to the morphological pattern of the invasion front, because the two types of tumors behave differently at this site. When both phenotypes coexisted, tumor classification was based on the most representative histology. Thus, a mixed form category was not used.