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• *BRIEF REPORTS* •

Relationship between β-catenin expression and epithelial cell proliferation in gastric mucosa with intestinal metaplasia

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Abstract

AIM: To investigate β-catenin expression in patients with intestinal metaplasia, and to look for a possible relationship between β-catenin expression and either epithelial proliferation values or *Helicobacter pylori* (*H pylori*) infection.

METHODS: Twenty patients with complete type intestinal metaplasia were studied. β-Catenin expression and epithelial cell proliferation in antral mucosa were assessed using an immunohistochemical analysis. *H pylori* infection was detected by histology and a rapid urease test.

RESULTS: Reduced β-catenin expression on the surface of metaplastic cells was detected in 13 (65%) out of 20 patients. Moreover, in eight (40%) patients intranuclear expression of β-catenin was found. When patients were analyzed according to *H pylori* infection, the prevalence of both β-catenin reduction at the cell surface and its intranuclear localization did not significantly differ between infected and uninfected patients. Cell proliferation was higher in patients with intranuclear β-catenin expression as compared to the remaining patients, although the difference failed to reach the statistical significance (36±8.9 *vs* 27.2±11.4, $P = 0.06$). On the contrary, a similar cell proliferation value was observed between patients with reduced expression of β-catenin on cell surface and those with a normal expression (28.1±11.8 *vs* 26.1±8.8, $P = 0.7$). *H pylori* infection significantly increased cell proliferation (33.3±10.2% *vs* 24.6±7.4%, respectively, $P = 0.04$).

CONCLUSION: Both cell surface reduction and intranuclear accumulation of β-catenin were detected in intestinal metaplasia. The intranuclear localization of β-catenin increases cell proliferation. *H pylori* infection does not seem to play a direct role in β-catenin alterations, whilst it significantly increases cell proliferation.

Key words: β-Catenin; Intestinal metaplasia; Proliferation; *Helicobacter pylori*

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INTRODUCTION

Catenins are a family of transmembrane proteins, which play a pivotal role in epithelial intercellular adhesion[1]. Moreover, β-catenin participates in the regulation of cell proliferation, being a critical component of the surface-to-nucleus WNT signal transduction pathways[2,3]. Alterations of β-catenin expression have been shown to be involved in cancer development[4]. Indeed, such alterations have been detected in gastric cancer, showing a correlation with tumor type, degree of differentiation, and poor survival of patients[5-9]. On the other hand, scanty data are available regarding β-catenin expression in precancerous conditions. Although alterations of some adhesion molecules have been detected in patients with intestinal metaplasia[10], no significant remarks emerged from the studies regarding β-catenin expression in these patients[10,11]. However, such studies were based on gastrectomy specimens of patients with gastric cancer and, therefore, only an advanced step of the carcinogenic process was evaluated.

Gastric carcinogenesis is a multistep process consisting of a cascade of alterations starting with chronic active gastritis and progressing to atrophy, metaplasia, and dysplasia[12]. In particular, intestinal metaplasia is widely recognized as being the most prevalent precursor of intestinal type gastric carcinoma[13]. Among environmental factors involved in carcinogenesis of the stomach, *Helicobacter pylori* (*H pylori*) infection appears to play an important role. Indeed, epidemiological studies have clearly demonstrated a significant association between this infection and gastric cancer development. Moreover, several changes involved in gastric carcinogenesis such as epithelial cell hyperproliferation,

free oxygen radical formation, ascorbic acid reduction, genetic alterations have been described in the gastric mucosa of infected patients[14-16].

The present study was designed in order to assess β-catenin expression in patients with intestinal metaplasia but with neither dysplasia nor gastric cancer, and to look for a possible relationship between β-catenin expression and either epithelial proliferation index of gastric mucosa or *H pylori* infection status.

MATERIALS AND METHODS

Patients

Patients with dyspeptic symptoms consecutively referred for upper endoscopy with presence of histology of intestinal metaplasia in the antrum and without concomitant evidence of either dysplasia in the stomach or neoplastic lesions in the upper gastrointestinal tract were selected. Patients were enrolled irrespectively of *H pylori* status. Patients who received proton pump inhibitors, H_2-receptor antagonists, antibiotics or NSAIDs in the 4 wk preceding the study as well as those previously treated for *H pylori* infection were excluded from the study. Patients with either liver impairment or kidney failure were also excluded.

Endoscopic procedure

After overnight fasting, all patients underwent upper endoscopy and three biopsies were taken from the antrum and three from the gastric body. Two biopsies from the antrum and two from the gastric body were used for histological assessment. Biopsy specimens of the antrum were also used for immunohistochemical analysis. The remaining two biopsies (one each from the antrum and gastric body) were used to carry out a rapid urease test (CP-test, Yamanouchi, Milan, Italy). *H pylori* infection was considered to be present when both the histological assessment on Giemsa staining revealed the presence of bacteria and rapid urease test was positive, as suggested in current guidelines[17].

Immunohistochemical analysis

For β-catenin and proliferation assessment, immunohistochemistry was carried out by the avidin-biotin-peroxidase method. Briefly the sections were deparaffinized in xylene and rehydrated through a graded alcohol series to distillated water. Antigen retrieval was performed by immersing the slides in 10 mol/L citrate buffer (pH 6.0) and heating them in a microwave for 3 cycles, 5 min each, at 750 W. Endogenous peroxidase activity and non-specific bindings were blocked by incubation with 3% hydrogen peroxide and nonimmune serum, respectively. Sections were then incubated with mAbs against β-catenin (Clone 14, 1:500 dilution; Transduction Laboratories, Lexington, KY, USA) and mAbs against Ki-67 (Clone MIB-1, 1:100 dilution YLEM, Italy) for 1 h at room temperature. Immunoreactivity was revealed with the chromogen DAB test and the sections were counterstained with Mayer hematoxylin solution for 7 min. Negative control sections were prepared by substituting primary antibody with buffered saline.

A semiquantitative approach was used for scoring the β-catenin expression according to the method previously described by Mingchao[10]. Briefly, the staining pattern of the intestinal metaplastic areas was compared with that of the adjacent normal gastric mucosa. Expression of β-catenin in metaplastic areas was considered 'normal' when both the intensity and the frequency of the cell membrane stains were equivalent to those found on the bordering nonmetaplastic gastric mucosa, 'reduced' when the staining was less than the adjacent mucosa, and 'negative' in the absence of staining. In addition, when β-catenin stained clearly in the nuclei of more than 10% of gastric epithelial cells, expression was judged to be positive for nuclear staining.

A quantitative approach was used instead for scoring the Ki67 expression. The number of cells was determined by counting the positively-stained nuclei on 10-20 randomly selected fields at 400×.

All immunostaining evaluations were performed blindly and by two independent observers. All sections for which the two observers disagreed were re-evaluated and, after opportune discussion, a final agreement was achieved.

Statistical analysis

Data between patient subgroups were compared using the Student's *t*-test for unpaired data, and the Fisher's exact test with Yate's correction for small numbers. A P value less than 0.05 was considered statistically significant.

RESULTS

Overall, 20 consecutive patients (9 male and 11 female; mean age: 60.8±8.4 years) were enrolled. At endoscopy, no macroscopic alterations of the gastric mucosa were detected, whilst two patients showed erosions in the duodenal bulb. *H pylori* infection was present in 13 (65%) patients and absent at both rapid urease test and histology in the seven remaining patients. Intestinal metaplasia was graded as complete type in all cases.

β-catenin expression in gastric mucosa

No case of completely negative β-catenin immunostaining was observed. A reduced expression of β-catenin on the surface of metaplastic cells as compared to adjacent normal glands was detected in 13 (65%) out of 20 patients. Moreover, in eight (40%) patients an intranuclear expression of β-catenin was detected. Among this group, six (75%) patients also showed reduced β-catenin expression. When patients were analyzed according to *H pylori* infection, β-catenin expression was decreased in 8 out of 13 infected patients as well as in 5 out of 7 uninfected cases ($P = 0.5$). Similarly, the prevalence of intranuclear localization of β-catenin expression did not significantly differ between infected and uninfected patients (4/13 *vs* 4/7, respectively; $P = 0.2$).

Cell proliferation in gastric mucosa

The mean value of Ki67 labeling index proved to be distinctly higher in eight patients with intranuclear β-catenin expression as compared to the remaining 13 patients, although the difference failed to reach statistical significance (36±8.9 *vs* 27.2±11.4, $P = 0.06$). On the contrary, by

excluding those patients with intranuclear localization of β-catenin, a similar cell proliferation value was observed between the seven patients with reduced membranous expression of β-catenin and the five patients with normal expression (28.1±11.8 *vs* 26.1±8.8, $P = 0.7$). As far the role of *H pylori* infection is concerned, data found that patients with infection had a significantly increased cell proliferation value than that of uninfected patients (33.3±10.2% *vs* 24.6±7.4%, respectively, $P = 0.04$).

DISCUSSION

The integrity of the function of adhesion molecules, such as E-cadherin and α, β, γ-catenins, allows the maintenance of normal interactions between cells necessary during embryogenesis, cell growth, and differentiation[1-3,18]. Loss of intercellular adhesiveness plays a role in the early steps of neoplastic transformation, and it is implicated in invasive growth and metastasization[5-9,19]. β-catenin participates in the adhesion process by binding the cytoplasmic domain of E-cadherin and it has been involved in the surface-to-nucleus WNT signal transduction pathways. Its translocation into the nucleus may contribute to accelerated cell proliferation[3]. β-catenin mutations in exon 3, interfering with the GSK-3β phosphorylation domain and leading to intranuclear accumulation of the protein, have been reported in intestinal gastric carcinoma as well as in colorectal cancer[20-23]. Moreover reduced β-catenin expression was recorded both in intestinal type of gastric cancer[5,6,24] and in intestinal metaplasia surrounding cancer lesions[25], although data are controversial[10,11,24]. In order to determine whether β-catenin alterations could be detected early in gastric carcinogenesis, the present study focused on β-catenin expression in intestinal metaplasia not associated with gastric cancer. Unlike previous study[26], in our series a reduction of β-catenin on the epithelial surface was observed in more than half of the patients. Intriguingly, a nuclear accumulation of β-catenin expression in 40% of patients with intestinal metaplasia was also found, and it was associated with reduced immunostaining at the intercellular boundaries in 75% of these cases. A previous study reported a nuclear accumulation of β-catenin in about 10% of 401 gastric carcinomas, all but one exhibiting reduced membranous staining[24]. Similarly, an inverse correlation between decreased membranous and increased nuclear staining of β-catenin was also observed in colorectal cancer[27,28]. Interestingly, we observed that patients with intranuclear β-catenin expression showed higher values of cell proliferation in the gastric mucosa as compared to those without it. This is a noteworthy remark in keeping with previously reported studies focused on the role of nuclear localization of β-catenin[3]. Indeed, in an experimental model, colonocyte hyperproliferation was associated with immunohistochemical alterations in subcellular distribution of β-catenin and with accumulation of the protein in the nuclear compartment[29]. This finding, however, has been not observed in gastric cancer[23,24]. Therefore, it could be hypothesized that the nuclear β-catenin expression may be linked to the early stage of carcinogenesis, as seen in colorectal polyps[30]. Conversely, loss of membranous expression could allow regenerating cells to dedifferentiate and lose cell-cell cohesiveness, properties that would facilitate the process of epithelial regeneration, as reported for E-cadherin expression during the reparative process of peptic ulcer[31]. Similarly, in endometrial glandular cells in the mid-to-late proliferative phase, nuclear accumulation of β-catenin was described, suggesting that it could play a physiological role in the rapid turnover of the cell cycle without gene mutation[32].

As far the role of *H pylori* infection is concerned, we failed to observe a significant difference in β-catenin expression between infected and uninfected patients, suggesting that this infection is not directly implicated in this phenomenon. On the contrary, in agreement with the results of several studies[14,16], a significant increase in the epithelial cell proliferation index was detected in *H pylori*-positive patients as compared to uninfected patients.

In conclusion, in the present immunohistochemical study, we described alterations in β-catenin expression in the gastric mucosa with intestinal metaplasia not associated to other more advanced histological lesions. β-catenin alterations consisted of both a weakness of membrane staining and in an intranuclear accumulation of the protein. Moreover, we observed a distinct increase in cell proliferation in those patients with intranuclear localization of β-catenin expression. *H pylori* infection does not seem to play a role in β-catenin alterations, whilst it significantly increases cell proliferation in gastric mucosa with intestinal metaplasia.

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