Trefoil Peptides, E-cadherin, and β-catenin Expression in Sporadic Fundic Gland Polyps

Further Evidence Toward the Benign Nature of These Lesions

Anna D'Odorico, MD, Mauro Cassaro, MD,† Sabina Grillo, MD,* Roberta Lazzari, PhD,* Andrea Buda, MD,* Pietro Cardellini, PhD,‡ Carlo Sturniolo Giacomo, MD,* and Massimo Rugge, MD†*

Abstract: Sporadic fundic gland polyps (FGP) are the most common type of gastric polyps and their pathogenesis is still unclear, although a β-catenin gene mutation has been described. They are regarded as benign lesions but low-grade dysplasia has been observed, arising more debate on their potential progression to a malignant phenotype. We investigated in FGP the role of factors involved in cell integrity, proliferation, and intercellular adhesion: trefoil peptides (TFF1, TFF2), MIB1, E-cadherin, and β-catenin. We selected randomly 24 patients with FGP, 24 with normal gastric mucosa and 12 with atrophic gastritis with diffuse intestinal metaplasia (IM-gastritis), all *Helicobacter pylori* negative. The expression of all factors was examined by immunohistochemistry. In polyps and normal mucosa, TFF1 is expressed only in foveolar compartment whereas in IM-gastritis the signal is reduced in all the compartments. TFF2 is expressed in polyps and normal mucosa, in proliferative and basal compartment, whereas in IM-gastritis the expression is reduced or absent. E-cadherin is expressed in the entire zone: with a medium signal in normal mucosa and polyps, and weaker in IM-gastritis. The β-catenin's signal in normal mucosa and polyps is moderate-to–intense in proliferative and basal compartments, whereas in IM-gastritis signal is significantly reduced in all the compartments. MIB1 in normal mucosa and polyps is expressed only in proliferative compartment, whereas its expression is stronger in IM-gastritis and involves also basal compartment. In conclusion all the factors considered were normally expressed in FGP and this, especially considered against the findings in IM-gastritis, supports the benign nature of FGP.

Key Words: β-catenin, E-cadherin, fundic gland polyps, MIB1, trefoil peptides

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From the Departments of *Surgical and Gastroenterological Sciences; †Oncology and Surgical Science; and ‡Biology, University of Padua, Padova, Italy.

Reprints: Anna D'Odorico, MD, Azienda Ospedaliera di Padova, U.O. Gastroenterologia, Via Giustiniani 2, 35128 Padova, Italia (e-mail: anna.dodorico@unipd.it).

Fundic gland polyps (FGP) constitute the most common gastric polyps (47%), arising in 2 distinct clinicopathologic scenarios: sporadic and syndromic. Sporadic FGP are encountered in 0.9% to 1.9% of patients who undergo upper gastrointestinal (GI) endoscopic evaluation, and are most frequent in middle-aged women.[1,2]

Macroscopically and histologically, FGP are typically small polyps (2 to 5 mm) located on the gastric body and fundus, and may be single or multiple.[3,4] Most commonly, the surface epithelium of FGP closely resembles the normal gastric surface epithelium. The nature of FGP is still debated: most authors have considered them to be hamartomatous lesions, others as a peculiar form of hyperplastic polyps.[5] Recently, the World Health Organization classification[6] described FGP as a type of polyps consisting of a localized hyperplasia of the deep epithelial compartment of the fundic mucosa, specially of mucous neck cells with a variable degree of cystic dilatation. The frequency of dysplasia in FGP is closely associated with the setting in which they arise: in fact, dysplasia is common in familial adenomatous polyposis-associated FGP (25% to 53%), but rare in sporadic forms (2% to 3%).[7–10] Although sporadic forms are generally assumed to be nonneoplastic lesions, the recent observation of dysplasia in these polyps has reawakened the debate on their potential for progression to a malignant phenotype.

Several cellular factors, such as trefoil peptides (TFFs), E-cadherin, and β-catenin, which have been shown to regulate mucosal integrity, intercellular adhesion, and cell proliferation, have been studied on GI diseases for their role in the early steps of cancerogenesis.

TFFs are a family of small peptides bearing 1 or more trefoil structural motifs (P-domain) and they are synthesized and secreted by mucin-secreting epithelial cells lining the GI tract.[11–13] In normal gastric mucosa, TFF1 is localized in the surface/foveolar epithelium and TFF2 is expressed in mucous cells of the neck zone of the oxyntic mucosa and in the antral glands.

TFF peptides have a pivotal cytoprotective role in maintaining the surface integrity of GI mucosa, forming stable gel complexes with mucins that can withstand the mechanical stress and GI proteases.[14] In response to

injury, they are rapidly upregulated near sites of mucosal lesions and this induction is not tissue-specific.[15,16]

TFF1 is also an important player in regulating the balance between GI cell proliferation, differentiation, and death: dysregulation of this sensitive equilibrium may easily contribute to cancer progression. In fact, TFF1 and TFF2 are frequently downregulated in primary gastric cancer.[17–19]

The normal expression of intercellular adhesion molecules is a key factor for the maintenance of tissue architecture and homeostasis. The E-cadherin/catenins complex is the main component of adherent junctions: E-cadherin binds to the cytosolic catenin family proteins (α, β, γ catenin), which link to the actins to form the cell cytoskeleton.[20–22] The β-catenin may be expressed on the membrane, in the cytoplasm, or in the nucleus, performing different roles; in particular, when it is transferred to the nucleus, it stimulates transcription of target genes which activate cell cycle.

Changes or dysfunctions in this complex give rise to a loss of cell adhesion and polarity, like in carcinomas, permitting or enhancing cell invasion of adjacent tissue.[23]

A somatic β-catenin mutation has recently, been described in sporadic FGP (91%), but it is not associated with abnormal expression or subcellular localization.[24]

To better-characterized sporadic FGP, we investigate the expression of these cellular factors that have been shown to regulate cell integrity, intercellular adhesion, and cell proliferation.

MATERIALS AND METHODS

Patients

From January 2002 to June 2004, among 5328 patients who underwent upper GI endoscopy at the GI endoscopy unit of Padua Hospital, we found 229 (4.3%) with sporadic FGP. FGP were most frequently found in women (70%), with a mean age of 54 years, and in 3.24% of patients, polyps were associated at *Helicobacter pylori* infection (33% in patients without FGP; $P < 0.0001$). None of the sporadic FGP evaluated had dysplasia.

Sixty patients, all *H. pylori* negative, were randomly selected from this database and were divided into the following groups: 24 patients with sporadic FGP (60% female, average age 53 ± 12.8 y), 24 patients with normal gastric mucosa (75% female, average age 46 ± 12 y), and 12 patients (75% female, average age 67 ± 8 y) with atrophic gastritis with diffuse intestinal metaplasia (IM) of the glands (according to modified Sydney System).[25]

Immunohistochemistry

Immunohistochemistry for TFF1, TFF2, E-cadherin, β-catenin, and MIB-1 was performed on paraffin-embedded tissue samples. Formalin-fixed sections (5 mm thick) were deparaffinized, rehydrated, and washed in phosphate buffered saline (pH 7.4).

Endogenous peroxidase activity was inhibited by treatment with 3% hydrogen peroxide for 10 minutes, and then sections were heated in microwave oven for 20 minutes at 750 W in 10 mM citrate buffer (pH 6.0). After incubation with 5% normal blocking serum for 20 minutes, sections were incubated with primary antibodies (TFF1, 1:100 dilution, Zymed Laboratories, San Francisco, CA; TFF2, 1:25 dilution, NovoCastra Laboratories, Newcastle upon Tyne, UK; E-cadherin and β-catenin, 1:250 diluition, BD Transduction Laboratories, San Jose, CA; MIB-1, 1:150 dilution, Dako Cytomation Denmark) overnight at 4°C. After rinsing with phosphate buffered saline, slides were incubated with biotinylated secondary antibody and then with peroxidase substrate solution (R.T.U. VECTASTAIN Elite Universal ABC kit, Vector Laboratories, Burlingame, CA).

Staining was developed by reaction with 3,3′-diaminobenzidine substrate-chromogen solution (Dako-Cytomation, Denmark A/S Glostrup, Denmark), and followed by counterstaining with Mayer's hematoxylin solution. All immunoreactions were manually performed.

Normal gastric mucosa was used as negative control and atrophic gastritis with IM as positive control.

Determination of Labeling Index

Only sections with a full thickness of mucosa (epithelium + lamina propria + muscularis mucosae) were considered.

The immunoreaction was tested considering the gastric glands divided into 3 zones: zone 1 (isthmus ie, proliferative zone), zone 2 (gland base), and zone 3 (gastric pit).

For all immunoreactions, the number of positive staining cells (nuclei or cytoplasm) in every 500 epithelial cells was counted in each zone and expressed as a percentage.

A semiquantitative score was used:

0 = absent.
1 = positivity till 30% of examined cells.
2 = positivity between 31% and 60% of examined cells.
3 = positivity more than 61% of examined cells.

Statistical Analysis

Statistical analyses were performed with the Pearson-χ^2 test, and a P value of less than 0.05 was regarded as statistically significant.

RESULTS

TFF Peptides

In normal gastric mucosa and in FGP, TFF1 is expressed only in the foveolar compartment, with a strong cytoplasmatic signal, whereas in atrophic gastritis with IM we found a weaker expression and anomalies in the glandular localization of TFF1: it is more weakly expressed in the foveolar compartment ($P < 0.00001$), but it is also unusually found in the basal and proliferative compartments ($P < 0.001$) (Figs. 1–3).

TFF2 is expressed in normal gastric mucosa and in FGP, in the proliferative and basal compartments, with

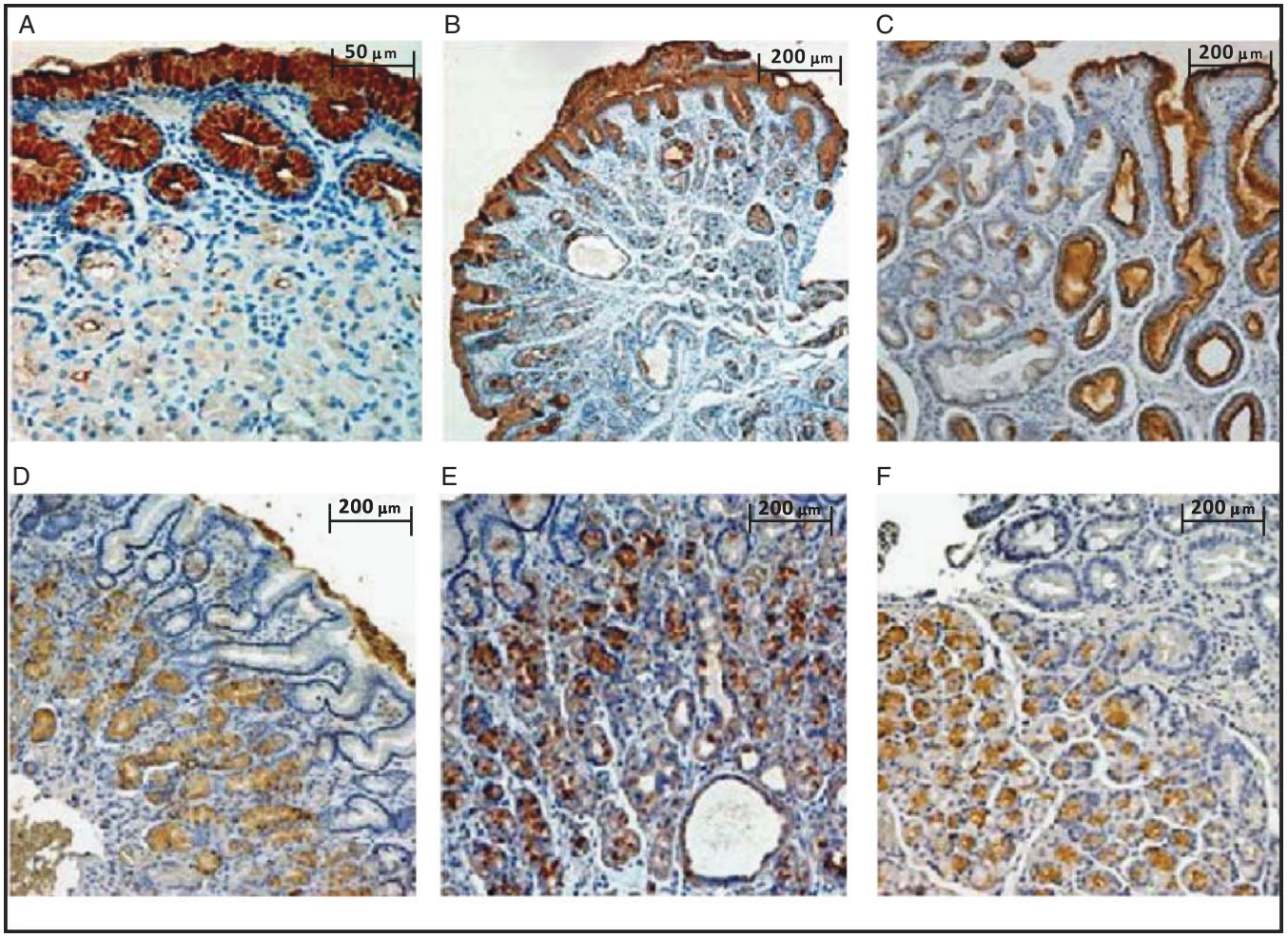

FIGURE 1. Expression of trefoil peptides (TFF1 and TFF2): TFF1 only occurs in the foveolar compartment of normal gastric mucosa (A) and the same pattern FGP (B). In atrophic gastritis with IM (C), the signal is significantly reduced in the foveolar compartment (C). Original magnification: 200 × for normal gastric mucosa, 50 × for FGP and IM-gastritis. TFF2 is found in the basal and proliferative compartments of normal gastric mucosa (D) and FGP (E). In atrophic gastritis with IM (F), the signal is significantly reduced or absent throughout the gastric gland. Original magnification: 50 × for all the samples. FGP indicates fundic gland polyps; IM, intestinal metaplasia.

a cytoplasmatic localization, and it is absent in foveolar pits. In atrophic gastritis, in contrast, TFF2 is absent in all the compartments ($P < 0.0001$) (Figs. 1–3).

E-cadherin/β-catenin

E-cadherin is characterized by a different pattern of expression in the tissue studied: in normal mucosa and FGP it shows a membrane expression in all the compartments whereas in atrophic gastritis with IM E-cadherin's immunostaining is weaker along the whole gastric gland ($P < 0.01$) (Figs. 2, 3).

In normal mucosa and FGP, β-catenin is always expressed in the proliferative and basal compartments, whereas in the foveolar region it is found only in 32% of cases. In atrophic gastritis with IM, the signal is significantly reduced in all the compartments studied (atrophic gastritis vs. normal gastric mucosa: Z1 and Z2 $P = 0.0002$; Z3 $P < 0.01$). There is no detectable nuclear staining of β-catenin in any examined section (Figs. 2, 3).

MIB1

In normal mucosa and FGP, MIB-1 signal is present only in the proliferative compartment, with a weaker signal in FGP ($P = 0.03$), whereas in atrophic gastritis with IM the expression is stronger and involves also basal compartment ($P < 0.001$) (Figs. 2, 3).

DISCUSSION

In our population the frequency of FGP is 4.3% in accordance with the literature, more common in women, with low prevalence of *H. pylori* infection.[3,26]

The origin and the risk of progression of FGP remain uncertain: several factors have been studied for their possible role in their pathogenesis as proton pump inhibitors, hormonal factors, and β-catenin alteration.

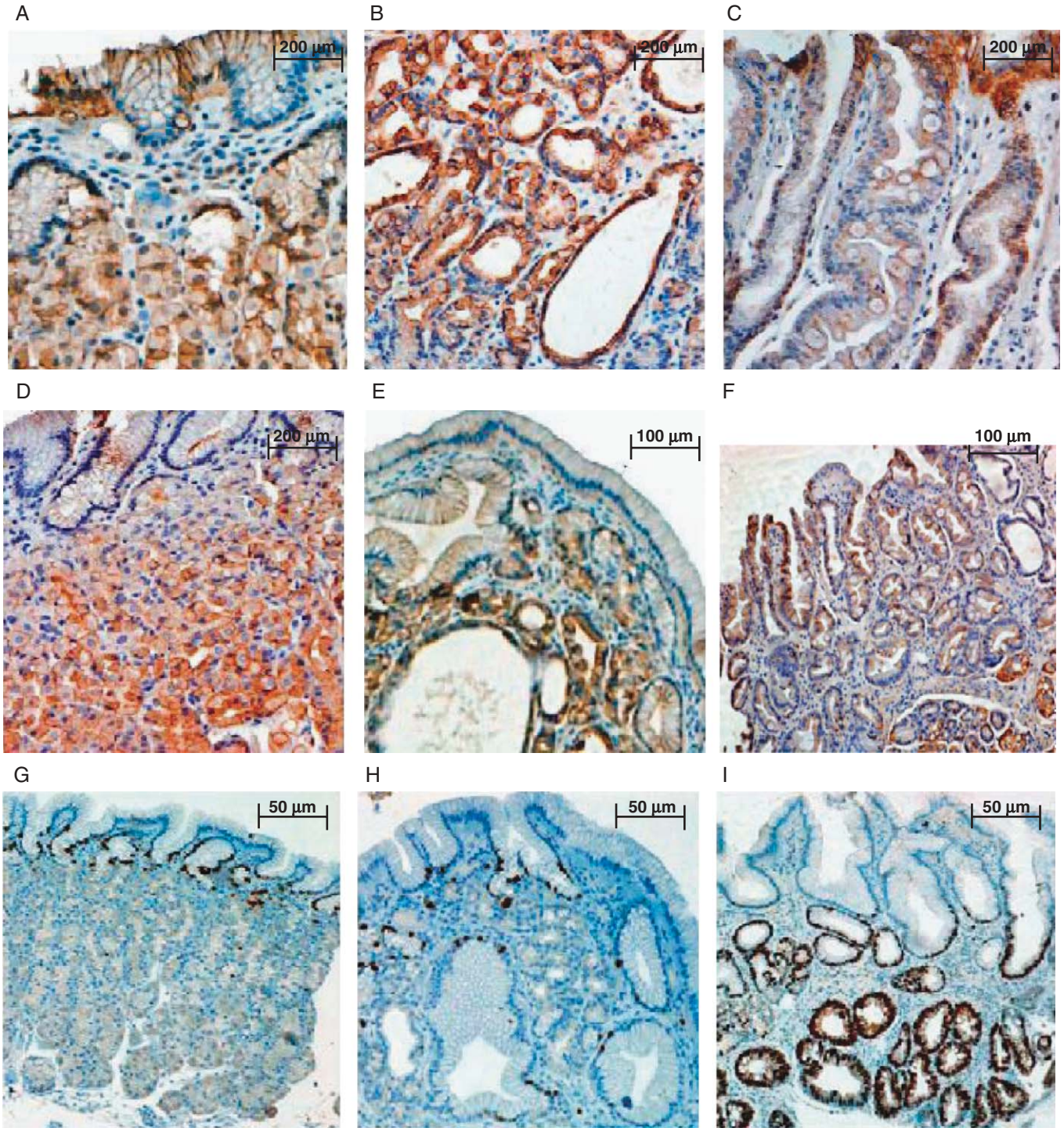

Figure 2. E-cadherin is expressed in all the compartments of normal gastric mucosa (A) and FGP (B). The detail of atrophic gastritis with IM shows a reduced E-cadherin expression in all compartments (C). Original magnification: 200 × for all the samples. The β-catenin is expressed in all compartments of normal gastric mucosa (D) and FGP (E). In atrophic gastritis with IM (F), the signal is significantly reduced in all compartments. Original magnification: 200 × for normal gastric mucosa, 100 × for FGP and IM-gastritis. MIB1 is only expressed in the proliferative compartment of normal gastric mucosa (G) and FGP (H), whereas in atrophic gastritis with IM (I) the signal is stronger and also involves the basal compartment. Original magnification: 50 × for all the samples. FGP indicates fundic gland polyps; IM, intestinal metaplasia.

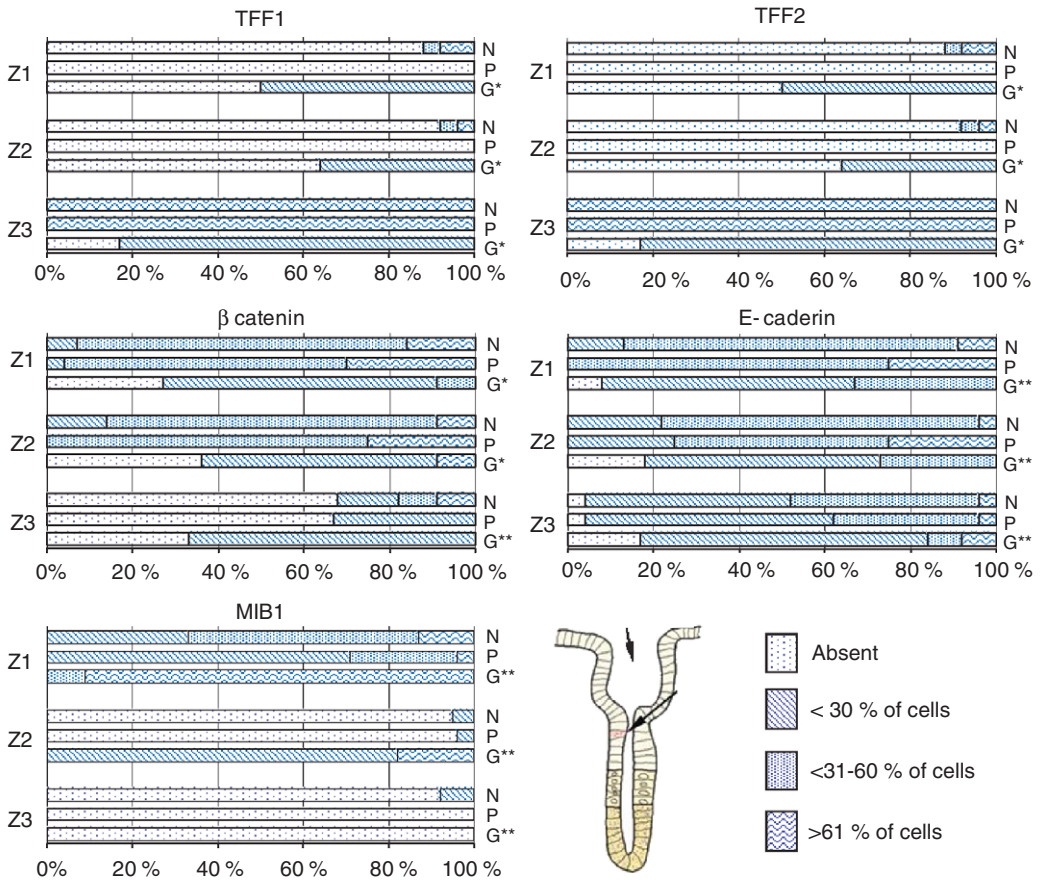

FIGURE 3. Signal intensity is expressed using a semiquantitative score index for each gland zone. Trefoil peptide (TFF1) *P<0.0001 GAM versus FGP and normal mucosa. TFF2 *P<0.0001 GAM versus FGP and normal mucosa. The β-catenin *P<0.0001 GAM versus FGP and normal mucosa, **P<0.01 GAM versus normal mucosa; E-cadherin **P<0.01 GAM versus FGP and normal mucosa; MIB1 **P<0.01 GAM versus FGP and normal mucosa, **P<0.01 FGP versus normal mucosa. FGP indicates fundic gland polyps; G, atrophic gastritis; GAM, gastric mucosa; N, normal gastric mucosa; P, fundic gland polyps; Z1, proliferative zone; Z2, gland base; Z3, gastric pit.

FGP have generally been regarded as nonneoplastic lesions; however, dysplasia has been recently demonstrated.[7–9,27]

To clarify their pathogenesis and characteristics we compared the expression of TFF1, TFF2, β-catenin, E-cadherin and MIB1 in FGP, normal gastric mucosa, and atrophic gastritis with IM (type of gastritis which could evolve into carcinoma)[25]; all cases were *H. pylori* negative, to exclude possible interferences related to the infection.

In FGP, we found a normal expression of TFF1 (in foveolar region) and TFF2 (in the basal compartment), suggesting integrity of mucoprotective function and cellular proliferation, regulated by TFF peptides. In atrophic gastritis with IM, in contrast, the expression of TFF peptides was significantly reduced in all compartments ($P < 0.0001$), probably due to a decrease in the number of gastric gland cells producing TFFs owing to atrophy.

To our knowledge, only 1 study has evaluated the expression of TFF2 in gastritis, in which Hu et al[28] demonstrated increased levels of TFF2 in both chronic superficial gastritis and chronic atrophic gastritis. This finding is only apparently in contrast with our results: the stronger expression of TFF2 in chronic atrophic gastritis than in normal gastric mucosa, described by Hu et al, is probably due to the high prevalence of *H. pylori* infection (60%) among the subjects considered. It is well known that the inflammatory injury, caused by the infection, induces an increase in TFF2 expression, which would explain the higher TFF2 signal in *H. pylori* positive cases of atrophic gastritis. To avoid any interferences between *H. pylori* infection and TFF2 expression, we only considered atrophic gastritis with IM samples from *H. pylori* negative patients. For this reasons we believed that, in our study, the lower TFF2 expression found in atrophic gastritis with IM is more likely to be related to the degree of cell atrophy.

The E-cadherin/β-catenin complex plays a pivotal role in the maintenance of normal tissue architecture and in the suppression of cancer invasion: in fact recently a downregulation of this complex has been demonstrated in early gastric cancer.[29,30]

We found a normal expression and location of β-catenin in FGP: immunostaining shown diffuse membranous positivity and no nuclear staining was apparent in any of the cases. Abraham et al demonstrated a high frequency of β-catenin gene mutation in sporadic FGP, suggesting that these polyps are clonal lesions and arise through genetic alterations rather like those associated with some adenomatous polyps of the GI tract. Despite this genetic mutation, however, they found no nuclear accumulation of β-catenin at immunohistochemistry, suggesting that the adenomatosis polyposis coli /β-catenin signaling pathway is not completely deregulated in FGP, despite β-catenin gene mutation.[20,31]

In addition, E-cadherin expression, studied here for the first time in FGP, proved to be normal in all cases: these observation further suggesting the integrity of the tissue architecture and normal E-cadherin/β-catenin-mediated cellular adhesion. In atrophic gastritis with IM, immunostaining of the complex was significantly reduced in all compartment studied, suggesting that an altered expression of this complex plays an important role in tumorogenesis.[22,29,30]

To date, the etiopathogenesis of FGP is not well known, but cell proliferation is bound to be a key point in the polyp's development. MIB1 signal in FGP is confined to the proliferative compartment, as in normal gastric mucosa, with a weaker signal ($P = 0.03$). In literature adenomatous and hyperplastic polyps are characterized by enhanced proliferative activity.[22,32–34]

MIB1 immunostaining in atrophic gastritis IM was significantly increased in the proliferative and basal compartments: in literature the overexpression of cell proliferation is generally used as a reliable marker of a premalignant evolution and tumor progression.[35]

From our results, we can suggest that cell proliferation in FGP only increases in the earlier step, subsequently returns to basal levels.

In conclusion, in FGP the normal expression of all factors studied suggests the integrity of mechanism of GI protection and intercellular adhesion, and the reduced proliferative activity would confirm the benign nature of these polyps.

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