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ORIGINAL ARTICLE

Retrospective Study

# Methylation of *DAPK* and *THBS1* genes in esophageal gastric-type columnar metaplasia

Roberto Herrera-Goepfert, Luis F Oñate-Ocaña, José Luis Mosqueda-Vargas, Luis A Herrera, Clementina Castro, Julia Mendoza, Rodrigo González-Barrios

Roberto Herrera-Goepfert, José Luis Mosqueda-Vargas, Department of Pathology, Instituto Nacional de Cancerología (INCan), 10480 México, DF, México

Luis F Oñate-Ocaña, Clinical Research Division, Instituto Nacional de Cancerología (INCan), 10480 México, DF, México

Luis A Herrera, Clementina Castro, Julia Mendoza, Rodrigo González-Barrios, Unidad de Investigación Biomédica en Cáncer, Instituto Nacional de Cancerología (INCan)-Instituto de Investigaciones Biomédicas (IIB), Universidad Nacional Autónoma de México (UNAM), 14080 México, DF, México

Author contributions: Herrera-Goepfert R contributed to study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, statistical analysis, funding, and study supervision; Mosqueda-Vargas JL, Mendoza J and González-Barrios R contributed to acquisition of data, drafted of the manuscript, and technical support; Herrera LA, Oñate-Ocaña LF and Castro C contributed to analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, statistical analysis, and technical support; all authors have read and approved the final version to be published.

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Correspondence to: Roberto Herrera-Goepfert, MD, Department of Pathology, Instituto Nacional de Cancerología (INCan), México, Av. San Fernando 22, Colonia Sección XVI, Delegación Tlalpan, 10480 México, DF, México. rhgoepfert@gmail.com
Telephone: +52-55-47471020-11069

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## Abstract

**AIM:** To explore methylation of *DAPK*, *THBS1*, *CDH-1*, and *p14* genes, and *Helicobacter pylori* (*H. pylori*) status in individuals harboring esophageal columnar metaplasia.

**METHODS:** Distal esophageal mucosal samples obtained by endoscopy and histologically diagnosed as gastric-type (non-specialized) columnar metaplasia, were studied thoroughly. DNA was extracted from paraffin blocks, and methylation status of death-associated protein kinase (*DAPK*), thrombospondin-1 (*THBS1*), cadherin-1 (*CDH1*), and *p14* genes, was examined using a methyl-sensitive polymerase chain reaction (MS-PCR) and sodium bisulfite modification protocol. *H. pylori cagA* status was determined by PCR.

**RESULTS:** In total, 68 subjects (33 females and 35 males), with a mean age of 52 years, were included. *H. pylori cagA* positive was present in the esophageal gastric-type metaplastic mucosa of 18 individuals. *DAPK*, *THSB1*, *CDH1*, and *p14* gene promoters were methylated by MS-PCR in 40 (58.8%), 33 (48.5%), 46

(67.6%), and 23 (33.8%) cases of the 68 esophageal samples. *H. pylori* status was associated with methylation of *DAPK* ($P$ = 0.003) and *THBS1* ($P$ = 0.019).

**CONCLUSION:** DNA methylation occurs in cases of gastric-type (non-specialized) columnar metaplasia of the esophagus, and this modification is associated with *H. pylori cagA* positive infection.

**Key words:** DNA methylation; Esophageal columnar metaplasia; Thrombospondin-1; Death-associated protein kinase; *Helicobacter pylori*; *cagA*



**Core tip:** Columnar metaplasia of the esophagus, whether specialized or not, is a hallmark of gastroesophageal reflux disease. Current information suggests that intestinal metaplasia in the esophagus arises from gastric-type metaplasia. In this study, we have demonstrated that *Helicobacter pylori* (*H. pylori*) *cagA*$^+$ can colonize esophageal gastric-type metaplastic mucosa, and that DNA methylation of tumor suppressor genes could be related to *H. pylori cagA*$^+$ infection, which in turn, may predispose to precancerous lesions, including intestinal metaplasia.

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## INTRODUCTION

Columnar metaplasia of the esophagus is defined as the replacement of the normal settled squamous epithelium in the distal portion of the esophagus with columnar epithelium, either with goblet cells [specialized columnar metaplasia, *i.e*., complete or incomplete intestinal metaplasia, Barrett esophagus (BE)], or without goblet cells (non-specialized columnar metaplasia or gastric-type metaplasia; *i.e*., oxyntocardiac and cardiac mucosa)[1]. The importance of such a distinction is found in the higher risk of developing adenocarcinoma of the distal esophagus in patients harboring specialized columnar metaplasia; the incidence of adenocarcinoma originating in BE has been reported as approximately 0.5% per year, with higher risk when long-segment BE is present[2]. However, it is now recognized that the presence of specialized columnar metaplastic cells (goblet cells) is not an essential requirement for the development of such adenocarcinomas[3]. Columnar metaplasia of the esophagus, whether specialized or not, is a hallmark of gastroesophageal reflux disease (GERD), and current information suggests that intestinal metaplasia in the esophagus arises from gastric-type, oxyntocardiac and/or cardiac (non-specialized) metaplasia[4]. Histologically, the non-specialized metaplastic mucosa of the esophagus may display any of the changes commonly observed in settled gastric mucosa, including the changes observed during *Helicobacter pylori* (*H. pylori*) infection[5].

Neoplastic transformation and progression in BE are related with genetic and epigenetic events that ultimately favor abnormal expression of the genes responsible for the intrinsic control mechanisms regulating cellular proliferation and/or apoptosis. DNA methylation is involved in the epigenetic regulation of gene expression (primarily through gene repression) when it occurs predominantly in regions containing CpG islands, which are often concentrated in the promoter regions of genes. In the case of esophageal adenocarcinoma, abnormal methylation patterns are not only detected in neoplastic tissue but also in premalignant Barrett mucosa. These results suggest that hypermethylation of DNA is an early epigenetic event in the multistep process of esophageal carcinogenesis[6].

Recent reports suggested that *H. pylori* is an initiator of the inflammatory microenvironment which might promote carcinogenesis and progression of gastric cancer[7]; in gastric diseases, chronic inflammation and alterations in DNA and histone methylation, especially at promoter regions, are frequently associated with *H. pylori* infection[8,9]. Such epigenetic alteration could be promoted in part by activation of NF-κB and PI3K/AKT-Sp1-RBP2-Cyclin D1 pathways triggered by *H. pylori* Cytotoxin-associated gene product [cytotoxin-associated gene A (*cagA*)] positive[7,9,10]. In addition to aging, increased methylation of several genes has also been described in chronic gastritis and premalignant stages of gastric carcinoma, irrespective of *H. pylori* status[11]. Moreover, *H. pylori* infection induces an overexpression of DNA methyltransferases (DNMTs), which has been associated with CpG island methylation of multiple gene promoters involved in cell growth, differentiation and tumor suppression, like *Ndrg2*, *p14*, *DAPK* and cadherin-1 (*CDH1*), as in chronic gastritis as in gastric cancer[7,12-14].

Consequently, the aim of this retrospective and descriptive study, was to explore the relationship between methylation of the death-associated protein kinase (*DAPK*), thrombospondin-1 (*THBS1*), *CDH1*, and *p14* genes, and the presence of *H. pylori cagA* positive, in the non-specialized, gastric-type columnar metaplastic mucosa of the distal esophagus, in a group of Mexican patients.

**Table 1 Sequences of specific primers used for determining gene methylation status**

| Primer name | | Forward primer sequence (5'-3') | Reverse primer sequence (5'-3') | Product size (bp) | AT (℃) | No. of cycles |
|---|---|---|---|---|---|---|
| DAPK | M | GGATAGTCGGATCGAGTTAACGTC | CCCTCCCAAACGCCGA | 98 | 60 | 35 |
| | U | GGAGGATAGTTGGATTGAGTTAATGTT | CAAATCCCTCCCAAACACCAA | 98 | 60 | 35 |
| p14 | M | GTGTTAAAGGGCGGCGTAGC | AAAACCCTCACTCGCGACGA | 122 | 60 | 40 |
| | U | TTTTTGGTGTTAAAGGGTGGTGTAGT | CACAAAAACCCTCACTCACAACAA | 132 | 60 | 40 |
| CDH1 | M | TTAGGTTAGAGGGTTATCGCGT | TAACTAAAAATTCACCTACCGAC | 115 | 53 | 35 |
| | U | TAATTTTAGGTTAGAGGGTTATTGT | CACAACCAATCAACAACACA | 97 | 57 | 35 |
| THBS1 | M | TGCGAGCGTTTTTTTAAATGC | TAAACTCGCAAACCAACTCG | 74 | 62 | 40 |
| | U | GTTTGGTTGTTGTTTATTGGTTG | CCTAAACTCACAAACCAACTCA | 115 | 62 | 40 |

M: Methylated sequence; U: Unmethylated sequence; AT: Annealing temperature.

## MATERIALS AND METHODS

### *Patients*

This is a retrospective and descriptive study of consecutive cases with the histopathological diagnosis of non-specialized (gastric-type), but not specialized (complete or incomplete intestinal metaplasia), columnar metaplasia of the distal esophagus. Biopsies of the distal esophagus, as well as gastric biopsies, were retrieved from the files of the Department of Pathology at the Instituto Nacional de Cancerología (INCan) in Mexico City during the period between January 2003 and December 2008. Inclusion criteria were females and males, aged > 18 years. All biopsies were obtained by means of the panendoscopy procedure at the Endoscopy Service outpatient clinic, in patients with upper gastrointestinal complaints and with endoscopic suspicion of columnar metaplasia. Relevant demographic and clinical data for each patient were retrospectively retrieved from their clinical records. Non-specialized columnar metaplasia of the esophagus was operationally defined as the presence of cardiac and/or oxyntocardiac gastric mucosa lacking goblet cells and intermingled with recognizable islets of non-keratinized squamous epithelium and/or immersed duct structures lined by multilayered epithelium. Slides stained with hematoxylin and eosin (HE) from each case were thoroughly reviewed, and histological criteria for gastritis (mononuclear cells and neutrophils) and *H. pylori* density were applied to grade the samples according to the Visual analog scales (VAS) proposed by the Updated Sydney System[15]. Giemsa staining was also performed to confirm the presence of *H. pylori*. Finally, cases with sufficient tissue were selected for morphological and molecular analysis. DNA was extracted from paraffin-embedded tissue samples.

### *DNA extraction and bisulfite modification*

DNA was extracted from histological sections of 20 μm in thickness by phenol/chloroform/isoamyl alcohol. One μg of genomic DNA extracted from samples was subjected to sodium bisulfite modification using a Zymo Kit (EZ DNA Methylation™ Kit; Zymo Research Co., United States) following manufacturer's instructions for small samples. As a control, we employed human lymphocyte DNA, known to be unmethylated in the promoter regions of our genes-of-interest.

### *Methylation-specific polymerase chain reaction*

Bisulfite-modified DNA was amplified with primers specific for either methylated or unmethylated sequences[11] (Table 1). PCR products were subjected to electrophoresis on 3% agarose gels and were then visualized under ultraviolet (UV) illumination using ethidium bromide.

### *H. pylori detection by PCR*

The presence of *H. pylori* was corroborated by PCR using the following primers from the conserved region of the *cagA* gene: forward, 5'-TT CAT GGG CGT GTT TGA TG-3', and reverse, 5'-AGC GAC TCC CTC AAC ATC TAA-3'. The fragments were amplified with 30 cycles utilizing a 55 ℃ annealing temperature.

### *Statistical analysis*

Statistical review of the study was performed by a biomedical statistician. The association of tested variables with the presence of *H. pylori* infection was assessed using the $\chi^2$ test. OR with their corresponding 95%CIs were calculated as a measure of association using logistic regression analysis. Two-sided statistics were used in all cases, and a probability (*P*) value of 0.05 was considered as significant. SPSS ver. 19 software (2010; IBM Corp., Armonk, NY, United States) was used for computations.

## RESULTS

A total of 68 subjects (33 females and 35 males) with a mean age of 52 years (range, 25-88 years) fulfilled the histological criteria and were thus eligible for the study. In 35 subjects, gastric mucosa samples were insufficient for the molecular study, and in the remaining 33 (48.5%) subjects, the gastric samples were processed for final analysis. Endoscopic findings at the distal esophagus included variable degrees of mucosal erosion, salmon-colored mucosal tongues and irregular Z line. Histologically, all cases showed chronic inflammation. In the esophageal samples, the

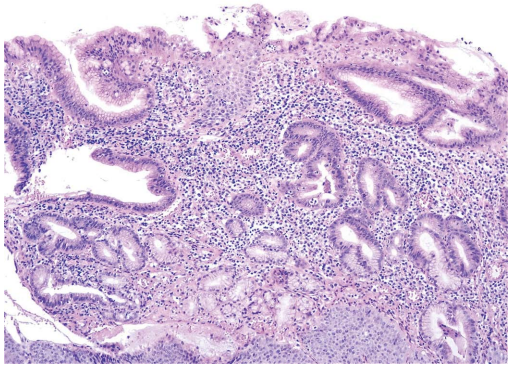

**Figure 1 Islets of non-keratinized squamous epithelium can be observed intermingling with cardiac-type gastric mucosa.** Superficial and glandular gastric epithelial linings demonstrate infiltration by polymorphonuclear leukocytes (Hematoxylin-eosin staining; original magnification × 10).

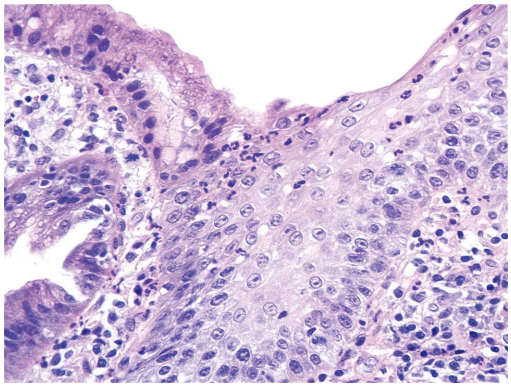

**Figure 2 Histological section of gastric-type columnar mucosa of the esophagus.** Moderate mononuclear infiltrate in the lamina propria and polymorphonuclear leukocytes infiltrating the mucosa layer can be observed (Hematoxylin-eosin staining stain; original magnification × 40).

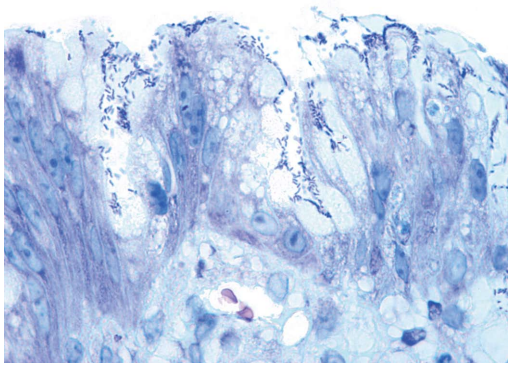

**Figure 3 Numerous rod-shape bacilli can be seen attached to the gastric-type metaplastic columnar epithelium (Giemsa staining; original magnification × 100).**

intensity of the mononuclear infiltrate was moderate in 43 (63.2%) cases, mild in 18 (26.5%), and marked in 7 (10.3%). Forty (58.8%) of the cases displayed polymorphonuclear leukocyte activity in the gastric-type metaplastic mucosa, which was graded as mild in 33 (82.5%) cases, moderate in 5 (12.5%), and marked in 2 (5%) (Figures 1 and 2). In 22 (32.3%) cases, *H. pylori* microorganisms were identified at the luminal surface of the gastric-type metaplastic mucosa. *H. pylori* density was graded as mild in 17 (77.3%) cases, moderate in 3 (13.6%), and marked in 2 (9.1%) (Figure 3). Regarding the 33 gastric biopsies, mononuclear infiltrate intensity was moderate in 16 (48.5%) cases, mild in 14 (42.4%), and marked in 3 (9.1%). Polymorphonuclear leukocyte activity was present in 17 (51.5%) cases and graded as moderate in 8 (47%), mild in 7 (41.2%), and marked in two (11.8%). *H. pylori* microorganisms were found in 17 (51.5%) cases and graded according to density, as moderate in 10 (58.8%), mild in 5 (29.4%), and marked in two (11.8%). In addition, four (12.1%) and two (6%) of the 33 cases displayed mild and moderate complete intestinal metaplasia, respectively. In one (3%) case, there was also mild atrophy of the gastric mucosa.

**Table 2 Association of *Helicobacter pylori cagA*$^+$ infection as detected by PCR, with DNA methylation of the promoter regions of target genes in the esophageal biopsies**

| | | *Helicobacter pylori* infection by PCR | | *P* value |
|---|---|---|---|---|
| | | Negative | Positive | |
| *DAPK* | Unmethylated | 26 | 2 | 0.003[1] |
| | Methylated | 24 | 16 | |
| *THBS1* | Unmethylated | 30 | 5 | 0.019[1] |
| | Methylated | 20 | 13 | |
| *CDH1* | Unmethylated | 19 | 3 | 0.097 |
| | Methylated | 31 | 15 | |
| *p14* | Unmethylated | 36 | 9 | 0.091 |
| | Methylated | 14 | 9 | |

[1]*Helicobacter pylori cagA*$^+$ status was significantly associated with methylation.

*CagA*$^+$ *H. pylori* was detected in the esophageal non-specialized metaplastic mucosa and in the gastric mucosa of 18 (26.5%) of 68 individuals, and in 10 (30.3%) of 33, respectively, by means of Polymerase chain reaction (PCR) (Figure 4A). *DAPK*, *THSB1*, *CDH1*, and *p14* gene promoters were methylated by MS-PCR in 40 (58.8%), 33 (48.5%), 46 (67.6%), and 23 (33.8%) cases of the 68 esophageal samples and in 10 (30.3%), 14 (43.8%), 26 (78.8%), and 10 (31.3%) of the gastric biopsies, respectively. In the remaining cases, these genes were not methylated (Figure 4B). *H. pylori cagA*$^+$ status was significantly associated with methylation of *DAPK* ($P$ = 0.003) and *THBS1* ($P$ = 0.019) in the 68 esophageal samples (Table 2), and bivariate analysis confirmed the significance of this association (Table 3). In the comparative analysis between the 33 gastric and 33 esophageal paired samples, the trend for the association between *H. pylori cagA*$^+$ and methylation of *THBS1* and *DAPK* genes was maintained (Table 4). Methylation of the *CDH1* and *p14* gene promoters did not exhibit statistically significant differences between *H. pylori cagA*$^+$ and *cagA*$^-$ cases, in both the esophageal and

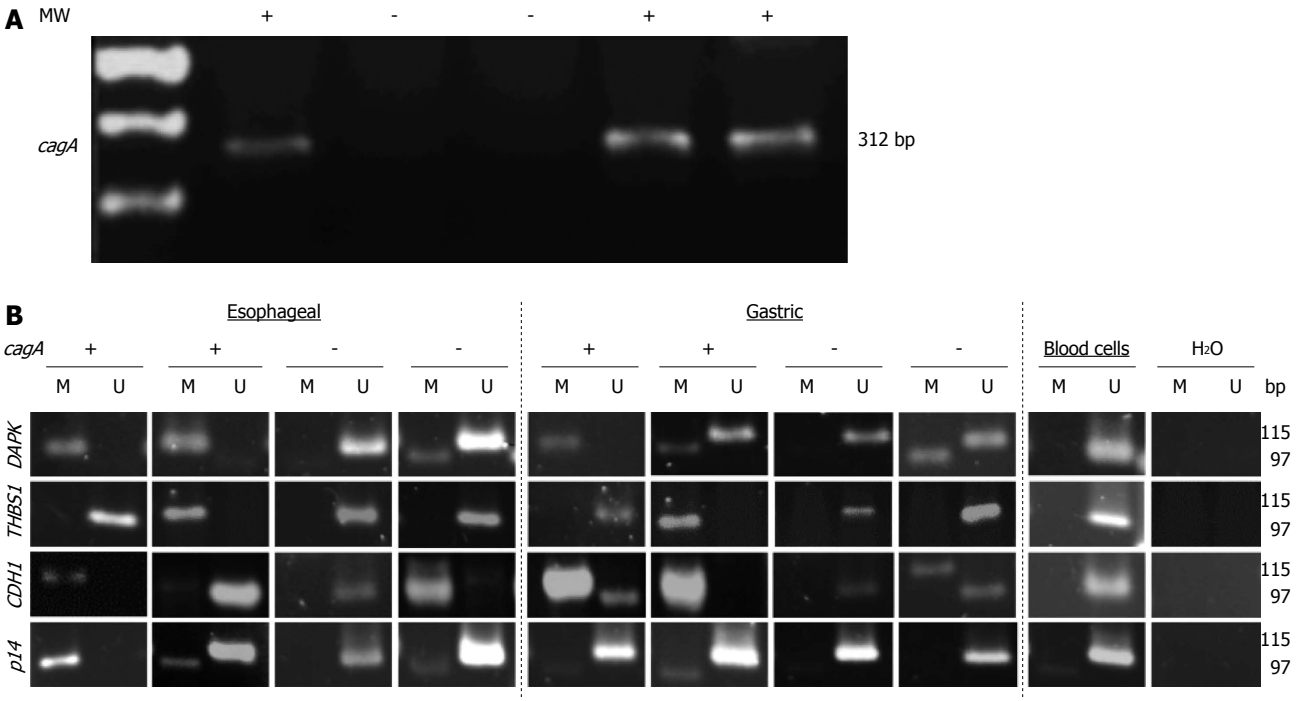


**Figure 4 *cagA* detection and DNA promoter methylation status of *DAPK*, *THBS1*, *CDH1* and *p14* genes in esophageal and gastric mucosa tissue samples.** A: *Helicobacter pylori cagA*$^+$ detection by polymerase chain reaction (PCR) amplification in tissue samples, (+) positive and (-) negative; B: Analysis of methylation status of *DAPK*, *THBS1*, *CDH1*, and *p14* genes promoters in esophageal mucosa and gastric biopsies tissue samples detected by methyl-sensitive PCR. Blood cells were used as control of unmethylated promoter regions. M represents methylated and U represent unmethylated; the status of *Helicobacter pylori cagA* is indicated as (+) positive and (-) negative.

**Table 3 Bivariate analysis of the association between molecular markers and the presence of *Helicobacter pylori cagA*$^+$ infection in esophageal biopsies**

| | OR | 95%CI | *P* value |
|---|---|---|---|
| *DAPK* | 8.67 | 1.80-41.7 | 0.007[1] |
| *THBS1* | 3.90 | 1.20-12.6 | 0.023[1] |
| *CDH1* | 3.06 | 0.78-11.9 | 0.110 |
| *p14* | 0.39 | 0.13-1.18 | 0.096 |

[1]Statistically significant.

**Table 4 Association of *Helicobacter pylori cagA*$^+$ infection as detected by PCR with DNA methylation of the promoter regions of target genes in 33 esophageal and gastric biopsies**

| | | *Helicobacter pylori* infection by PCR | | *P* value |
|---|---|---|---|---|
| | | Negative | Positive | |
| Esophageal biopsies | | | | |
| *DAPK* | Unmethylated | 14 | 0 | 0.004[1] |
| | Methylated | 10 | 9 | |
| *THBS1* | Unmethylated | 15 | 2 | 0.057[1] |
| | Methylated | 9 | 7 | |
| *CDH1* | Unmethylated | 11 | 1 | 0.107 |
| | Methylated | 13 | 8 | |
| *p14* | Unmethylated | 18 | 5 | 0.400 |
| | Methylated | 6 | 4 | |
| Gastric biopsies | | | | |
| *DAPK* | Unmethylated | 17 | 6 | 0.444 |
| | Methylated | 6 | 4 | |
| *THBS1* | Unmethylated | 15 | 3 | 0.062 |
| | Methylated | 7 | 7 | |
| *CDH1* | Unmethylated | 6 | 1 | 0.397 |
| | Methylated | 17 | 9 | |
| *p14* | Unmethylated | 14 | 8 | 0.440 |
| | Methylated | 8 | 2 | |

[1]Statistically significant.

the gastric biopsies. Among the esophageal biopsies, there were no significant differences regarding the age ($P$ = 0.39) or gender ($P$ = 0.34) of the subjects and *H. pylori* status by means of PCR; histopathological variables according to the Updated Sydney System for the classification and grading of gastritis were significantly associated with *H. pylori cagA*$^+$ status (Table 5). On the other hand, *H. pylori* density, mononuclear cells, and neutrophils, as histologically graded according to the Updated Sidney System for the classification of gastritis, did not show correlation with methylation status of the genes-under-study (data not shown).

## DISCUSSION

Allison *et al*[16,17] first described and correctly interpreted the histopathological changes occurring in the distal esophagus of subjects suffering from GERD, and Paull *et al*[18], defined the histological subsets of the columnar lined esophagus. The subsets were then renamed the oxyntocardiac mucosa (formerly the fundic epithelium), cardiac mucosa (formerly the junctional epithelium), and intestinal metaplastic

**Table 5 Clinical and histopathological data of patients depending on the presence of *Helicobacter pylori cagA*$^+$ infection by PCR, in esophageal biopsies**

| | | *Helicobacter pylori* infection by PCR | | *P* value |
|---|---|---|---|---|
| | | Negative | Positive | |
| Age (yr) | ≤ 40 | 16 | 9 | 0.390 |
| | 41-65 | 14 | 4 | |
| | > 65 | 20 | 5 | |
| Gender | Feminine | 26 | 7 | 0.340 |
| | Masculine | 24 | 11 | |
| *Helicobacter pylori* | Negative | 38 | 8 | 0.014[1] |
| (HE) | Positive | 12 | 10 | |
| Sydney | Normal | 38 | 8 | 0.014[1] |
| Classification: | Mild | 11 | 6 | |
| *Helicobacter pylori* | Moderate | 1 | 2 | |
| | Marked | 0 | 2 | |
| Sydney | Normal | 23 | 5 | 0.028[1] |
| Classification: | Mild | 25 | 8 | |
| Neutrophils | Moderate | 1 | 4 | |
| | Marked | 1 | 1 | |
| Sydney | Mild | 17 | 1 | 0.021[1] |
| Classification: | Moderate | 30 | 13 | |
| Mononuclear cells | Marked | 3 | 4 | |

[1]Statistically significant. HE: Hematoxylin-eosin staining.

mucosa (formerly the specialized columnar epithelium) by Chandrasoma *et al*[19].

Although *H. pylori* infection of the gastric mucosa has been proposed as a beneficial factor for GERD and BE[20,21], nearly nothing has been published regarding the potential effects of *H. pylori* colonization on the esophageal non-specialized (gastric-type) metaplastic mucosa. To the best of our knowledge, this study is the first to examine the presence of *H. pylori cagA*$^+$ in columnar metaplastic mucosa of the esophagus and to correlate such bacterial presence with the appearance of early epigenetic events. We selected cases with gastric-type (non-specialized) columnar metaplasia of the esophagus because, in addition to being considered an early morphological change in the evolution of Barrett's esophagus[21], intestinal metaplastic cells are infrequently colonized by *H. pylori*[22]. Given the limited and random sampling of the columnar esophagus, we cannot yet rule out the presence of specialized columnar epithelium in areas other than those analyzed in the present study. However, our goal was to explore the methylation status of select genes and the relationship of methylation with *H. pylori cagA*$^+$ infection in gastric-type metaplastic mucosa of the esophagus.

The majority of these studies, however, have been planned and conducted employing normal settled esophageal mucosa or tissues displaying intestinal metaplasia and not considering non-specialized (gastric-type) metaplastic mucosa. In this study, we explored the methylation status of four genes, *DAPK* (proapoptotic; tumor suppressor), *THBS1* (angiogenesis inhibitor), *CDH1* (cell adhesion), and *p14ARF* (cell cycle regulator; tumor suppressor), in patients harboring gastric-type (non-specialized) columnar metaplasia of the esophagus. Of these, *DAPK* and *THBS1* methylation was significantly associated with *H. pylori cagA*$^+$ infection of the esophageal gastric-type metaplastic mucosa, whereas *CDH1* and *p14* genes methylation was not. In specialized columnar metaplasia of the esophagus (BE), *DAPK*, *CDH1*, and *p14* have been reported to be methylated in 50%, 8%, and 7%, respectively[23], whereas *THBS1* is infrequently methylated[6]. On the other hand, we did not find a statistically significant association between *H. pylori cagA* positive and methylation of any of these genes, in the gastric mucosa. Our findings could be interpreted as the result of the smaller sampling of the gastric mucosa than of the columnar lined esophagus, therefore, with a loss of any potential statistic association. Another plausible explanation is that metaplastic gastric mucosa, in the distal esophagus, is more susceptible to, or predisposes to, the methylation of certain genes than the normal settled gastric mucosa due to disturbances induced by the gastroesophageal reflux, in addition to the *H. pylori* infection.

It is widely recognized that *H. pylori* is able to colonize gastric mucosa along the entire gastrointestinal tract, including areas as proximal as the upper esophagus[24] and as distal as the rectum[25], as well as all sites in between, such as the duodenum[26] and Meckel's diverticulum[27]. Previously, colonization of gastric-type mucosa in BE was also described; however, its clinicopathological significance has been underestimated[5]. Recently, employing a rat experimental model of chronic gastroesophageal reflux, Liu *et al*[28] demonstrated that severity of inflammation and incidence of Barrett esophagus and esophageal adenocarcinoma are increased when *H. pylori* colonizes the esophagus. In the gastric mucosa, *H. pylori* causes a complex immune and inflammatory process that is largely determined by the virulence of strains carrying the cytotoxin-associated gene (*cag*) pathogenicity island (PAI)[29]. Thus, *H. pylori* is responsible for several molecular events that ultimately play a significant role in gastric carcinogenesis. Interestingly, *H. pylori* infection has been suggested as an initiator of gastric carcinogenesis by upregulation of DNA methyltransferase 3B (DNMT3B)[7], and it has been also reported to induce expression of DNMT1 and DNMT3A in gastrointestinal stromal tumors[30]; therefore, *H. pylori* DNMT-induced *de novo* methylation could promote aberrant CpG island methylation, thus increasing the risk of gastric cancer[31,32].

Dysregulation of *DAPK* is implicated in the development and progression of cancer through gene silencing. DAP kinase function is closely related with the *p*53-dependent pathway for apoptosis[33]. In the gastric mucosa, hypermethylation of the *DAPK* promoter has been associated with aging and chronic inflammation[11], as well as premalignant stages of gastric carcinoma[34]. Hypermethylation of the *DAPK* gene in noncancerous gastric and chronic gastritis mucosa has been associated

with the risk of gastric cancer and neutrophil infiltration activity, but not aging, in a *H. pylori*-infected population[10,12,35]. In the esophageal mucosa, decreases in DAPK protein expression correlate with the severity of reflux esophagitis and tumor progression in Barrett carcinogenesis[36].

Moreover, a field effect of *DAPK* has been detected in normal esophageal mucosa of patients with adenocarcinoma and Barrett esophagus[37]. Indeed, possible silencing of *DAPK* by methylation could be an early event in columnar metaplasia of the esophagus, and this silencing remains throughout the process of neoplastic transformation. The association between *DAPK* silencing and *H. pylori* infection has been previously reported in gastric mucosa[12]. It is noteworthy that *H. pylori* infection of the columnar mucosa could be exerting an additive effect on *DAPK* gene promoter methylation, in addition to the reflux.

On the other hand, *THBS1* is an inhibitor of angiogenesis and its expression is regulated by tumor-suppressor genes such as *p*53 and *Rb*. Additionally, *THBS1* possesses tumor suppressive properties *in vivo*. It has been demonstrated that *THBS1* methylation inactivates its expression in several normal and neoplastic cell lines[38].

*DAPK* and *THBS1* have been found to be methylated in the gastric mucosa in patients with diseases ranging from chronic gastritis to carcinoma, with a rising frequency of *DAPK* methylation encountered in advanced stages of carcinogenesis[36]. *THBS1* promoter methylation has been associated with *DAPK* methylation from early-onset sporadic gastric carcinoma[39]. In this way, our findings are in agreement with previous studies that demonstrated that the early steps of Barrett's progression may involve DNA methylation of genes linked with apoptosis and tumor suppressor properties, particularly *DAPK* and *THBS1*. Our findings are also consistent with those reported by Ferrández *et al*[40], who performed a large case-control study and observed that *H. pylori* CagA$^+$ infection does not reduce the risk of BE.

Finally, we are aware that our study has some limitations, regarding its retrospective design, the small number of patients under study, and the inevitable sampling bias due to the varied and random distribution of the histological changes among the columnar lined esophagus. Further prospective studies are warranted to adequately identify patients with GERD with higher risks for developing severe disorders including BE as well as adenocarcinoma and its precursor lesions.

In this study, we showed that CpG methylation occurs in non-specialized, gastric-type columnar metaplasia of the esophagus and is closely related to *H. pylori cagA*$^+$ infection. Given this effect on gastric-type metaplastic mucosa, a conscious search for *H. pylori* and its eradication may be essential for halting the early mechanisms potentially involved in BE development and BE-associated carcinogenesis, among subjects suffering from GERD.

## COMMENTS

### Background

Gastroesophageal reflux disease (GERD) is a highly prevalent condition among worldwide population, and gives rise to esophageal columnar metaplasia, among others. Nowadays, columnar metaplasia of the esophagus is classified into non-specialized (gastric-type) and specialized [intestinal-type; Barrett's esophagus (BE)] neoplastic transformation and progression in BE, are related to genetic and epigenetic events that ultimately favor abnormal expression of the genes responsible for the intrinsic control mechanisms regulating cellular proliferation and/or apoptosis.

### Research frontiers

Current information suggests that intestinal metaplasia arises from gastric-type, non-specialized metaplasia. In the stomach, *Helicobacter pylori* (*H. pylori*) infection has been associated with methylation of multiple gene promoters involved in cell growth, differentiation and tumor suppression, as in chronic gastritis as in gastric cancer. In this study, the authors report the methylation of two genes and its relation to *H. pylori cagA* status, in gastric- type columnar metaplasia of the esophagus.

### Innovations and breakthroughs

DNA methylation of the promoter regions of genes containing CpG islands is involved in the epigenetic regulation of gene expression (predominantly through gene repression), and it is an early event in the multistep process of esophageal carcinogenesis. The authors performed the first study to assess the methylation of some genes involved in neoplastic transformation and progression in several organs, and its correlation with *H. pylori cagA*$^+$ infection, in the esophageal gastric-type metaplastic mucosa.

### Applications

This study provides evidence that CpG methylation occurs in non-specialized columnar metaplasia of the esophagus and is closely related to *H. pylori cagA*$^+$ infection. Given this effect on gastric-type metaplastic mucosa, a conscious search for *H. pylori* and its eradication may be essential for halting the early mechanisms potentially involved in BE development and BE-associated carcinogenesis, among subjects suffering from GERD.

### Peer-review

Herrera-Goepfert *et al* explored gene methylation in esophageal columnar metaplasia, and correlated these findings with the status of *H. pylori cagA*$^+$. It is well written and contains information which readers may be interested. Because it is suggested that intestinal metaplasia in the esophagus arises from gastric-type metaplasia, authors should include intestinal metaplasia in the study.

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