Original article

Association of *Helicobacter pylori* with colorectal cancer development

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Background: *Helicobacter pylori* (*H. pylori*) may be associated with colorectal cancer. However, the underlying mechanisms are still unclear.

Objectives: Explore the serostatus of *H. pylori* cytotoxicity-associated gene A product (CagA) in patients with colorectal carcinoma, and assess the association of *H. pylori* with colorectal cancer via c-Myc and MUC-2 proteins at tumor tissues.

Methods: *H. pylori* CagA IgG antibodies were screened using enzyme-linked immunosorbent assay (ELISA) in 30 patients with colorectal carcinoma and 30 cancer-free control subjects. Paraffin-embedded blocks were examined for the expression of c-Myc and MUC-2 protein by immunohistochemistry.

Results: *H. pylori* CagA seropositivity increased significantly among colorectal cancer patients ($p < 0.05$). The expression of c-Myc and MUC-2 in colorectal carcinoma patients was over-expressed (80%), and down-expressed (63%) in resection margins ($p < 0.05$). c-Myc over-expression and MUC-2 down-expression were associated with CagA-positive rather than CagA-negative *H. pylori* patients. In 16 CagA seropositive vs. 14 CagA seronegative patients, the expression rate was 97.3% vs. 64.2% and 33.3% vs. 78.5% for cMyc and MUC-2, respectively. CagA IgG level was significantly higher in positive than in negative c-Myc patients ($p = 0.036$), and in negative than in positive MUC-2 patients ($p = 0.044$). c-Myc and MUC-2 were positively and inversely correlated with CagA IgG level ($p < 0.05$).

Conclusions: CagA-seropositive *H. pylori* is most probably associated with colorectal cancer development. Part of the underlying mechanism for such association might be via alterations in expression of MUC-2, which depletes the mucous protective layer in the colo-rectum, and c-Myc, which stimulates the growth of cancerous cells.

Keywords: *Helicobacter pylori*, colorectal cancer, Cag-A, MUC-2

Helicobacter pylori (*H. pylori*) is the only bacterium so far to be recognized as a human carcinogen [1, 2]. Cytotoxicity-associated gene A product (CagA) protein is a highly immunogenic outer-membrane protein of variable molecular mass, 120-140 kDa [3]. Individuals infected with *H. pylori* CagA-positive strains develop circulating antibodies

to CagA protein, which can be used as a marker for diagnosis [1, 2].

The main extra-gastric organ affected with *H. pylori* tumor formation was found to be the colorectum. Many studies showed that colorectal cancer or even adenoma is associated with increased sero-prevalence of *H. pylori* [4-6]. Moreover, it was shown that CagA-positive rather than CagA-negative *H. pylori* are associated with colorectal cancer [7, 8]. This indicated that the pathogenicity island of *H. pylori*, CagA, might play a central role in the induction or propagation of colorectal cancer.

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Mechanisms behind the association between CagA-positive *H. pylori* and colorectal cancer have not been clarified yet. Nevertheless, it is prudent to exploit the abundant research on *H. pylori* association with gastric cancer for disclosing the possible mechanisms. It was found that *H. pylori* in gastric cancer patients may influence cell cycle progression and carcinogenesis through post-translational effects on specific genes expression [9]. Among the most implicated gene expressions, there has been strong evidence for the role of c-Myc protein [9, 10] and MUC-2 protein [11, 12] in the carcinogenic potential of *H. pylori* in gastric cancers. In addition, c-Myc and MUC-2 proteins are associated with 70-90% of colon cancer cases [10, 13].

These two proteins have surprisingly not been investigated for their role in the association of colorectal cancer with *H. pylori* in general and CagA-positive *H. pylori* in particular. It was suggested that c-Myc protein might regulate the rate of growth required for cell cycle progression and cell division [14, 15]. It is also likely to affect cell life-span, apoptosis, morphology, and DNA integrity. C-Myc protein can also promote tumorigenesis [16]. On the other hand, mucins are major components of the mucous layer that lubricates the gastrointestinal tract and protects its epithelium from mechanical, chemical, enzymatic, and microbial damage. MUC-2 protein is the typical secretory mucin that predominates in colorectal goblet cells [17]. Hence, MUC-2 protein may be a major target in the colorectal mucosa of colorectal cancer patients.

There was no single report available in scientific literature to explore the association of the expression of both c-Myc and MUC-2 with *H. pylori* CagA seropositive colorectal cancer cases. In this study, we explored the relationship between *H. pylori* CagA serostatus and colorectal cancer, and assessed the possible relationship between the expression of both c-Myc and MUC-2 and *H. pylori* CagA serostatus in colorectal cancer patients.

Materials and methods

Patients

This study included 30 consecutive sporadic colorectal carcinoma patients (17 men and 13 women) ranged between 51 and 82 years (mean: 66.8 years). The patients, who attended hospitals to undergo elective surgical resection of their colorectal cancer, were eligible for this study.

This study was conducted between 2006 and 2008 on Middle Eastern patients of Arabian ethnicity in Iraq. Ethical clearance to conduct the research was obtained from the Biomedical Research Ethics Committee as well as from the hospitals where patients were retrieved. Consents were obtained from all participants in this study.

Selection of patients was accomplished with the assistance of surgeons in the hospitals. Data were collected through direct interview with the patient. Moreover, patients' hospital records and previous medical reports were all retrieved. The exclusion criteria were peptic ulcer disease, treatment with H₂ blockers or proton pump inhibitors, previous gastric surgery, colorectal cancer other than carcinoma, malignancy at another site, and inflammatory bowel diseases to avoid the possibility of mucin depletion due to the inflammatory response characteristic of these diseases [18]. The involved colorectal cancer patients were not biased to certain stage of disease; 19% of patients were presented at B1 and B2, 28% at C1 and C2, and 53% at D.

Thirty age- and sex-matched controls of the same ethnic group were selected randomly from subjects undergone colonoscopy who revealed negative colorectal cancer status. Individuals with peptic ulcer disease or treatment with H₂ blockers or proton pump inhibitors were excluded from this group.

Serology

Blood samples were obtained from patients preoperatively as well as from controls. Sera were kept frozen during the period of sample collection. Sera were screened for the presence of *H. pylori* CagA IgG antibodies by enzyme-linked immunosorbent assay (ELISA) (Genesis Diagnostics, Nottingham, UK). The cutoff value was calculated and compared with that provided by the manufacturer. The cases with serum levels above the cutoff value were only regarded as CagA positive.

Immunohistochemistry

Slide preparation. Paraffin-embedded blocks of both tumor and resection margin were retrieved for each patient. Hematoxylin and eosin slides were prepared and examined by a histopathologist. For histological typing, WHO classification of colorectal carcinoma was adopted. Thereby, carcinomas were classified as mucinous, either the extracellular or the intracellular mucin (signet-ring cells) is more than 50%

of tumor bulk [19]. Because of the legal and ethical impracticality of obtaining colorectal tissue specimens from normal individuals of the control group, sections of resection margins were considered as malignant-free-controls for immunohistochemistry analysis.

Immunohistochemical staining. Paraffin-embedded blocks were sectioned at 5 µm thickness and mounted on Fisherbrand Superfrost/Plus. Slides then left overnight at room temperature to dry. Dewaxing was carried out by heating the slides in hot air oven overnight at 65°C and dipping them in xylene containing jars. Rehydration was carried out by dipping the slides in descending grades of alcohol. Non-specific reactions were blocked by the addition of protein blocking reagent provided by UniMAK staining kit (InnoGenex, San Ramon, USA). Sections were incubated with diluted primary antibody for one hour at 37°C in humid chamber. Best results were obtained when dilution of c-Myc (mouse mAb 9E10; InnoGenex, San Ramon, USA) and MUC-2 (mouse mAb; CCP58, InnoGenex, San Ramon, USA) was 1:100 and 1:200, respectively. Then, the sections were incubated with biotinylated secondary antibody and streptavidin-alkaline phosphatase conjugate followed by the addition of BCIP/NBT substrate where

bluish purple color is developed. Sections were counterstained with nuclear fast red and mounted.

Immunohistochemical scoring. Two blinded readers examined the IHC staining, one from our research team and another one, a histopathologist. Both of them yielded very close results that confirmed and validated the IHC results. The expression of c-Myc was evaluated by counting the number of tumor cells with bluish-purple nuclear staining under light microscopy (400X). Percentages of stained tumor cells were obtained for each section. A scoring system that included an evaluation of both the staining intensity and the percentage of stained tumor cells was employed (**Table 1**). Cancers were regarded as c-Myc positive when their immunoreactivity scores were ≥ 1 . While cancers with immunoreactivity scores of zero were regarded as c-Myc negative.

For MUC-2, the same previous method was used to calculate the percentages of tumor cells with cytoplasmic bluish-purple staining under light microscopy (400X). The percentages were then converted into semi-quantitative scores (**Table 2**). Cancers were regarded as MUC-2 positive when the score was ≥ 3 [21, 22].

Table 1. Scoring system for evaluation of c-Myc immunostaining.

(%) stained tumor cells	Score	Stain intensity	Score	Multiplication value*	Immuno-reactivity Score	c-Myc
0	0	No staining~	0	0, 1	0	Negative
<5	1	Weak	1	2, 3	1	Positive
5-20	2	Moderate	2	4, 6	2	
21-50	3	Strong	3	8, 9, 12	3	
>50	4					

*Multiplication value= (percentage score) $\times$ (intensity score). ~As in normal colonic epithelia.

Table 2. Semi-quantitative scoring system for MUC-2 immunostaining.

% stained cells	Score	MUC-2
0	0	Negative
1-5	1	
6-30	2	
31-60	3	Positive
>60	4	

Statistical analysis

After being tested by normality tests, Kolmogorov-Smirnov test, Student’s *t* test of significance was used for two-group comparison of parametric quantitative data. On the other hand, chi-square (χ^2) test of significance and Fisher’s exact test, when the use of chi test is inapplicable, were adopted for the comparison and calculation of association in the categorized qualitative data. Correlation coefficient (*r*) was used as a qualitative indicator to express the correlation between variables, while the regression coefficient (*b*) was used to express the quantitative nature of each association and to measure the dependence of one variable on the other. Means were described with standard errors (SE) to indicate the variability of the data and the precision of the estimated sample means. Probability values of *p* <0.05 were considered statistically significant.

The CagA IgG levels in U/ml for the control group (n=30) were used to calculate the cut-off value, and to compare it with that given by the kit manufacturer. The calculations were made as follows:

99% Confidence interval (cut-off value)
= mean + 2.57 × SE_{mean},

where SE_{mean} is standard error of the mean, and 2.57 is taken from the table of student’s *t*-test under the *p*=0.01 for the 29 degrees of freedom. As the upper limit was considered as the cut-off value, all readings above were considered positive. This allows our

decision as for positive or negative cases to be accurate with a margin of error less than 0.5%.

Results

Association of CagA and colorectal cancer

It was found that CagA seropositivity was much higher in colorectal cancer patients (53.3%) than in control group (20.0%) (*p* <0.01) (Fig. 1).

A significant difference was observed when the serum levels of CagA IgG antibodies of both patients and controls were analyzed based on the student’s *t* test of significance; where the mean of patients (12.78±3.75 U/mL) was about three folds that of the controls (3.82±0.88 U/mL) (*p* <0.05) (Table 3). Based on student’s *t* test, there was no significant difference (*p* >0.05) in serum levels of CagA IgG antibodies between males and females of colorectal cancer patients and among different stages of the disease.

The correlation coefficient between CagA IgG level and age in the control group was negative and not significant (*r*=-0.212). The regression coefficient was very small (*b*=-0.016 U/mL/year), which might be taken for no cause and effect between age and CagA IgG level. Similarly, the correlation coefficient between CagA IgG level and age of the patients group was calculated and found to be negative, small and not significant (*r*=-0.126). This indicates a very low and weak relationship between CagA IgG level and age. To quantify this relation, the regression coefficient (*b*) of CagA IgG level on age (years) was calculated and found to be -0.172 U/mL per year.

Fig. 1 *H. pylori* CagA serostatus by ELISA in colorectal cancer patients and controls (cut-off point = 6.25 U/mL).

Table 3. *H. pylori* CagA IgG measurements (U/ml) by ELISA in colorectal cancer patients and controls.

Group	Mean	Median	SE*	95% CI [†]	99% CI	t test P-value
Controls (n=30)	3.82	2	0.88	(2.10-5.53)	(1.56-6.07 [‡])	0.026
Patients (n=30)	12.78	5.50	3.75	(5.43-20.12)	(3.12-22.43)	

*Standard error (SE). [†]Confidence interval (CI). [‡]Calculated cut-off point.

Role of c-Myc and MUC-2 in CagA-positive *H. pylori* colorectal cancer patients

Expression of c-Myc was positive in tumor sections of 80% of patients which was much higher than in resection margins where there was complete negative staining of c-Myc (0%) ($p < 0.05$) (**Table 4**).

The immunostaining of c-Myc was nuclear and heterogenous with different staining intensities seen in the malignant cells of colorectal tissue (**Fig. 2**).

It was found that the mean level of CagA IgG in colorectal cancer patients with positive expression of c-Myc (13.74 ± 1.9 U/mL) was significantly higher than in patients with negative staining (8.92 ± 2.53 U/mL) ($p < 0.05$) (**Table 4**). However, there was borderline difference between the positive expression of c-Myc in CagA seronegative, 9/14 (64.2%), and that in CagA seropositive patients, 15/16 (93.7%) ($p = 0.07$) (**Table 5**). By using Pearson correlation (r) between the serum level of CagA IgG and the expression percentage of c-Myc in colorectal cancer tissues, it was found that both c-Myc and CagA IgG were positively correlated, $r = +0.53$.

MUC-2 expression was positive in tumor sections of 63% (19/30) of patients which was lower than in resection margins where the positive staining for MUC-2 was 100% (30/30) ($p < 0.05$) (**Table 5**). The MUC-2 immunostaining was primarily in goblets cells with a perinuclear cytoplasmic localization both in normal (resection margin) and malignant colorectal tissues (**Fig. 3**).

MUC-2 expression was significantly associated with the mucinous type of colorectal carcinoma ($p < 0.05$). The mean level of CagA IgG in patients with negative expression of MUC-2 (14.24 ± 1.64 U/mL) was higher than that of positive staining (10.25 ± 2.12 U/mL) ($p < 0.05$) (**Table 4**). Nevertheless, the positive expression of MUC-2 in CagA seropositive patients (50%), versus that in CagA seronegative patients (78.5%), revealed no significant difference ($p > 0.05$) (**Table 5**).

However, MUC-2 is known to be secreted exclusively high in **mucinous types** of colorectal cancer while it is secreted much lower in all other types of colorectal cancer; therefore, including the four cases of mucinous colorectal cancer would definitely lead to bias in the results which in turn will not reflect the real impact of MUC-2 on the carcinogenesis of colorectal cancer in the presence of *H. pylori*. For this reason, four cases of mucinous colorectal cancer were excluded because of their biasness toward MUC-2 positive expression. Accordingly, the difference between the positive expression of MUC-2 in CagA seropositive patients (33.3%), and that in CagA seronegative patients (78.5%), was largely increased ($p < 0.05$) (**Table 4**). Accordingly, the current study revealed that the seropositivity of *H. pylori* CagA in colorectal cancer patients was found to be significantly associated with lower expression of MUC-2 and higher expression of c-Myc. Pearson correlation coefficient between MUC-2 and CagA IgG, $r = -0.66$, confirmed the inverse relationship between MUC-2 and CagA IgG level in colorectal cancer patients.

Table 4. CagA IgG level between c-Myc positive/negative and MUC-2 positive/negative expression in colorectal cancer patients.

Variables	CagA mean $\pm$ SE* (U/mL)	P-value
c-Myc positive	13.74 ± 1.90	0.036
c-Myc negative	8.92 ± 2.53	
MUC-2 positive	10.25 ± 2.12	0.044
MUC-2 negative	14.24 ± 1.64	

*Standard error (SE).

Fig. 2 Immunohistochemical staining of c-Myc in colorectal tissue. Immunostaining of c-Myc protein by BCIP/NBT (bluish-purple) counterstained with nuclear fast red. **(A)** Colorectal adenocarcinoma (CRC), percentage score (3) x intensity score (3) = multiplication value (9), immunoreactivity score (3). **(B)** CRC with percentage score 2. **(C)** CRC with percentage score 1. **(D)** Signet-ring cell adenocarcinoma showing negative staining for c-Myc (arrow). **(E)** High power magnification of (B) depicts c-Myc nuclear staining of malignant cells with strong stain intensity (arrow). **(F)** High power magnification of (C) with moderate stain intensity (arrow). **(G)** CRC, depicts weak stain intensity (arrow). **(H)** High power magnification of normal colonic epithelium (resection margin) adjacent to (B) showing no staining of c-Myc. Magnification power for A-C (x100), D-H (x400).

Table 5. c-Myc and MUC-2 positive expression in *H. pylori* CagA seropositive and seronegative colorectal cancer patients.

Variables	C-myc (+) ve N (%)	MUC-2 (+) ve N (%)	MUC-2 (+) ve N (%) <i>Excluding 4 mucinous cases</i>
Colorectal patients	24/30 (80)	19/30 (63.3)	-
Control subjects	0/30 (0)	30/30 (100)	-
P-value	<0.00000001	<0.001	-
CagA seropositive	15/16 (93.7)	8/16 (50.0)	4/12 (33.3)
CagA seronegative	9/14 (64.2)	11/14 (68.7)	11/14 (78.5)
P-value	0.07	0.14	0.044

Fig. 3 Immunohistochemical staining of MUC-2 in colorectal tissue. Immunostaining of MUC-2 apomucin by BCIP/NBT (bluish-purple) counterstained with nuclear fast red. (A) Colorectal adenocarcinoma (CRC) used as negative control by replacing the primary antibody with PBS buffer. (B) CRC with score 2. (C) CRC with score 2. (D) Mucinous colorectal adenocarcinoma with score 4. (E) High power magnification of (A) positively stained for MUC-2. (F) High power magnification of (B) depicts perinuclear cytoplasmic staining of MUC-2 (arrow). (G) High power magnification of (C). (H) Signet-ring cell adenocarcinoma, depicts MUC-2 staining of signet-ring cell (arrow). Magnification power of A-D (x100), E-H (x400).

Discussion

The present study related the expression of c-Myc and MUC-2 with *H. pylori* CagA serostatus in colorectal cancer. The serostatus of *H. pylori* CagA was significantly higher in colorectal carcinoma patients than in cancer-free controls. Unfortunately, no similar regional studies were available for comparison. However, the current study is in agreement with a study conducted in Poland, which showed that *H. pylori* IgG seroprevalence, especially that expressing CagA, was significantly higher in colorectal cancer patients (53%) than in controls (20%) [8]. Similarly, a significant association between

H. pylori CagA seropositivity and colorectal as well as gastric cancer was confirmed by another study [7] which showed that CagA seropositive rate was about two times higher in colorectal cancer patients (82%) than in controls (41%) and the difference was statistically significant. Although other various studies did not investigate specifically CagA serostatus, however they showed that colorectal cancer or even adenoma is associated with increased seroprevalence of *H. pylori* [4-6]. In contrast to the findings of the current study, a study conducted on Finnish male smokers aged between 50-69 years found no association between *H. pylori* CagA seropositivity and

incident colorectal adenocarcinoma [8]. The inconsistency of results might be due to variation in the geographical distribution of *H. pylori* as well as in the frequency of its *cagA*-positive strains for a certain population. The significant increase of CagA seropositivity among the patients of the current study might be explained chronologically according to two possibilities. First, CagA-positive *H. pylori* infection preceded the development of cancer. Thus, it might be incriminated as one of the risk factors that lead to cancer. The second possibility, infection came after cancer development because of an attenuated immune response that accompanies cancer development.

The present study showed that the correlation coefficient between CagA level and age was negative and not significant in both patients and controls. However, a point to be noted is that the negative regression coefficient in colorectal cancer patients was much higher ($b = -0.172$ U/ml/year) than that of controls ($b = -0.016$ U/mL/year). This might be attributed to the immune impairment with age. Therefore, in the scope of the current study, immune impairment with age appears more obvious in colorectal cancer patients than in cancer-free controls. Hence, impairment of the immune response that may accompany carcinogenesis of any tumor is a possibility that cannot be ignored for the higher level of CagA positive strains in colorectal cancer patients. In this framework, higher *H. pylori* CagA-positive strains should be involved in other cancers. Shmueli and coworkers included in their study colorectal and non-gastrointestinal cancers in addition to cancer-free controls [7]. They found that the seroprevalence of CagA in colorectal adenocarcinoma was much higher than in both non-gastrointestinal cancers and cancer-free control groups ($p < 0.001$) providing evidence that the high level of CagA-positive *H. pylori* in colorectal cancer is most probably related to cause and effect rather than immune impairment.

The current study found that c-Myc and MUC-2 were overexpressed and downexpressed respectively in colorectal cancer tissues when compared to resection margin tissues. Moreover, the current study examined the expression of c-Myc in relation to *H. pylori* CagA serostatus. It was found that the mean level of CagA IgG in patients with positive expression of c-Myc was significantly higher than in patients with negative staining and the positive c-Myc expression was just higher in CagA-seropositive than in CagA-seronegative patients. Furthermore, both c-Myc and

CagA IgG were positively correlated. Unfortunately, studies addressing the relationship between *H. pylori* and the expression of c-Myc in colorectal cancer patients were very scarce, if any. However, this relationship was more studied on human gastric cancer cell line (AGS). It was demonstrated that a significant upregulation of *c-myc* proto-oncogene was observed in AGS cell line upon infection with *H. pylori* [24]. This finding was consistent with another study that demonstrated enhanced prevalence of c-Myc expression in patients with gastric atrophy who were chronically infected with *H. pylori* type I strains [25]. Additionally, another study showed that c-Myc overexpression in precancerous gastric conditions of patients infected with *H. pylori* compared to the same conditions but without infection gave the chance for gastric carcinogenesis [26]. Although the current study did not investigate serum level of gastrin, however, hypergastrinemia induced by *H. pylori* infection could be an alternative pathway that justifies the high percentage of c-Myc expression among CagA seropositive patients of colorectal cancer. The latter explanation is based on the fact that gastrin is a growth factor for colorectal malignancy, and was shown to mediate proliferation via both endocrine and autocrine/paracrine mechanisms including up regulating c-Myc [27]. Thus, it would be recommended conducting a similar, but expanded, study to include other parameters like serum gastrin, growth factors, and inflammatory cytokines.

MUC-2 expression was found positive in all resection margins (100%), while it was significantly lowered in colorectal cancer tissues (63%), providing evidence that the lower expression of MUC-2 is a sign of carcinogenesis in colorectal mucosal tissue. In this regard, the current study tried to investigate the possible impact of infection with *H. pylori* CagA positive strains on the expression level of MUC-2 in colorectal cancer patients. The results of the current study demonstrated that, after the four cases of mucinous colorectal cancer cases were excluded, only 33% of CagA seropositive patients showed positive expression of MUC-2 versus 79% in CagA seronegative patients. The higher rate of MUC-2 negative expression among CagA seropositive patients might be due to the impact of *H. pylori* infection, which was supported in the current study by the inverse correlation found between MUC-2 and CagA IgG and the significant higher level of CagA IgG in MUC-2 negative than MUC-2 positive expression

cases. Keeping with our observation, the only two cases that gave the lowest score for MUC-2 immunostaining (score 1) were exclusively of CagA seropositive group. These interesting observations were in agreement with the outcome of a previous study correlating *H. pylori* infection with mucus secretion. This study showed that prolonged presence of viable *H. pylori* inhibited baseline mucus secretion from colonic cell line HT29-C1.16E, which is known to express *MUC2* and *MUC3* genes [28]. In addition, *H. pylori* did not only inhibit total mucin synthesis but also suppressed *MUC1* and *MUC5AC* gene expression in a human gastric cell line and these tissue alterations in gastric mucins were reversible after eradication of *H. pylori* infection [29].

Conclusion

A significant association between colorectal cancer and CagA-positive *H. pylori* infection was confirmed. CagA IgG level was not associated with age, sex, or stage of the colorectal cancer disease. Overexpression and downexpression of c-Myc and MUC-2 respectively were confirmed in colorectal cancer versus resection margin tissues. Most importantly, c-Myc and MUC-2 seem to be related, maybe in cause effect manner, with *H. pylori* CagA infection in that c-Myc tends to be positively correlated with CagA IgG level where CagA IgG level was higher in positive than in negative c-Myc expression cases while MUC-2 tends to be expressed less and inversely correlated with CagA seropositivity where CagA IgG level was higher in negative than in positive MUC-2 expression cases. Collectively, CagA-positive *H. pylori* appear to have an obvious relationship with the development of colorectal cancer via different mechanisms including growth induction by over-expressing c-Myc and depletion of the mucin protective layer by downexpressing MUC-2. Nevertheless, further larger studies are recommended in order to implicate other virulence factors, such as VacA cytotoxin and BabA adhesion, to shed more light on their possible role in colorectal carcinogenesis with *H. pylori* infection.

Authors declare no conflict of interest (scientific or financial).

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