

Effect of *Helicobacter pylori* infection on gastric epithelial cell proliferation

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INTRODUCTION

Helicobacter pylori (*H. pylori*) infection is one of the main pathogens of chronic gastritis and duodenal ulcer (DU), and it may be considered as a risk factor in the incidence of gastric cancer^[1]. *H. pylori* infection may lead to the anomaly of gastric epithelial cell proliferation which is closely related to the development of gastric cancer. Vacuolating cytotoxin (VacA) is an important virulence and vacA subtype determines the toxic activity^[2]. According to its signal sequence, it can be grouped into type s1a, s1b, s1c and s2^[3,4]. Strains harboring vacAs1a are more closely related with digestive diseases^[5] and may be the strains with high toxicity. The effect of *H. pylori* infection on gastric epithelial cell proliferation depends on the vacA subtype^[6]. The report of the effect of strains with vacAs1a on gastric epithelial cell proliferation has not been found in China. We particularly study the effect of this strain in order to reveal whether the patients suffering from *H. pylori* infection have accelerated proliferation of gastric epithelium compared with non-infected patients, and whether the strains harboring vacAs1a have more severe effect on it.

MATERIALS AND METHODS

Patients

Patients suffering from dyspepsia underwent diagnostic endoscopy and biopsy. Those taking H₂ antagonists, proton pump inhibitors, non-steroidal anti-inflammatory drugs, antibiotics or bismuth salts were excluded from the study. Patients with gastric ulcer or cancer were also excluded. Eighty-four patients with chronic gastritis (CSG) and 16 patients with duodenal ulcer (DU) with mean age of 46.45 years (22 years - 76 years) entered the

study. Biopsy specimens were taken from the site approximately 2 cm-5 cm from the pylori.

Histology and diagnosis of *H. pylori* infection

Two antral and one corpus biopsy specimens were routinely processed, and stained with haematoxylin and eosin. Examine *H. pylori* by fast urease test, modified Giemsa stain and culture. At least two positive results of test regarded as *H. pylori* infection.

Immunohistochemistry

An antral biopsy specimen was put immediately into RPMI containing bromodeoxyuridine (BrdU, 5 g/L). It was immersed in a waterbath for 60 min at 37°C and then fixed in Carnoy solution. Sections were stained with anti-BrdU antibody by ABC technique. The nuclei of proliferative cell were stained. Five hundred epithelial cells were counted and the number of positively stained epithelial cell nuclei expressed in percentage as labelling index (LI%). All sections were examined by the same person who was unaware of the subject's *H. pylori* status.

Polymerase chain reaction

H. pylori DNA was extracted routinely, vacAs1a amplified by PCR, 50 µL reaction solution contains the following: 1 × reaction buffer, dNTP mixture (0.2mM each), vacAs1a primers (0.2µM each), 1.25 unit Taq DNA polymerase and 4 µL template. PCR program comprises predenaturation at 94°C for 5 min, followed by 37 cycles of 1 min at 94°C, 90s at 52°C, 45s at 72°C, and a final incubation at 72°C for 7 min. PCR products were inspected by electrophoresis on 2% agarose gels stained by ethidium bromide. It is regarded as vacAs1a positive if a clear band can be seen at 190bp. Primers: 5'-GTCAGCATCACACCGCAAC-3', 5'-CTGCTTG-AATG CGCCAAAC-3'^[3].

Statistics

LI% is transformed to arcsin LI% 1/2, *t* test, Chisquare and multi variate linear-regression analysis were used to deal with the data.

RESULTS

The prevalence of *H. pylori* infection in CSG patients was 50%, and that of DU reached 93.75% ($P < 0.01$), but the disparity of vacAs1a proportion between *H. pylori* positive CSG and DU was not significant (43.59% vs 58.33%, $P > 0.05$).

There is significant difference on LI% between

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CSG and DU ($P < 0.05$), but considering the different prevalence of *H. pylori* infection, we got negative result ($P > 0.05$) from comparing the LI% between positive cases of CSG and DU. The results (Table 1) of analyzing the effect of *H. pylori* infection and its different strains on proliferation, showed that patients with *H. pylori* had higher LI% ($6.14\% \pm 1.21\%$) than *H. pylori* negative ones ($2.43\% \pm 0.61\%$, $P < 0.001$). Patients harboring vacAs1a strains had significantly higher gastric epithelial cell proliferation LI% ($n = 24$, $8.00\% \pm 1.46\%$) than those with non-vacAs1a strains ($n = 27$, $4.51\% \pm 0.86\%$, $P < 0.02$) or noninfected patients ($P < 0.001$).

The sections were graded into mild, moderate and severe according to the extent of inflammation and intestinal metaplasia. Statistics shows a close relationship between inflammation and *H. pylori* status ($P < 0.005$), however it is negative on metaplasia ($P > 0.05$). No significant relationship was found between inflammation and vacAs1a genotype ($P > 0.05$).

The results show that inflammation and neutrophil infiltration were closely related to epithelial cell proliferation ($P < 0.001$), but not to metaplasia ($P > 0.05$). Multivariate linear-regression analysis shows that among the factors, such as age, sex, DU, inflammation, neutrophil infiltration, intestinal metaplasia, vacAs1a strains, non-vacAs1a strains and so on, vacAs1a strain and inflammation are the independent factors influencing the epithelial cell proliferation.

Table 1 BrdU LI% of the patients ($\bar{x} \pm s$)

	LI%	case number
H.pylori-positive	6.14 ± 1.21^a	57 [§]
vacAs1a positive	$8.00 \pm 1.46^{a\#}$	24
vacAs1a negative	4.51 ± 0.86^c	27
H.pylori-negative	2.43 ± 0.61	43

^a $P < 0.001$ vs *H. pylori* negative patients; ^c $P < 0.01$ vs *H. pylori* negative patients; ^e $P < 0.02$ vs non-vacAs1a *H. pylori* patients; [§]vacAs1a were not examined in 6 cases because of loss of specimens.

Table 2 Multivariate linear-regression analysis

LI%	coefficient	SD	t	P> t	95%confidence interval
vacAs1a	4.47	1.37	3.27	0.002	1.76 7.19
inflammatory coefficient	3.89	1.21	3.22	0.002	1.49 6.30
	4.50	1.89	2.38	0.019	0.75 8.26

DISCUSSION

The genesis of gastric cancer is the result of long-term effect of multiple factors of environment and host. Epidemiological investigation and histological evidences showed that *H. pylori* infection was related to gastric cancer independently. *H. pylori* infection induced gastric epithelial cell proliferation, increase of mitosis and mutation^[7]. Because of the instability of the genome of the proliferative cell, hyperproliferation increases the

possibility of DNA damage and aneuploidy. Dysplasia may evolve into carcinoma if damaged DNA cannot be repaired on time or fail in promoting the apoptosis system. Accelerated cellular proliferation rate is the property of malignant tissue and has been confirmed in gastric carcinoma^[8].

The genesis of most gastric adenocarcinomas is believed to follow a series of defined histologic steps from normal gastric mucosa to chronic gastritis, atrophic gastritis, intestinal metaplasia, and neoplasia^[9]. It has been postulated that *H. pylori* plays a causative role at the early phases in this chain of malignant progression^[10]. Therefore, we studied CSG and DU patients (part of them had intestinal metaplasia).

The prevalence of *H. pylori* in DU patients (93.75%) was much higher than that in CSG (50%) which supports the conclusion that *H. pylori* is a closely associated pathogen of DU.

It is reported that gastric epithelial cell proliferation in *H. pylori* associated gastritis patients increased prominently compared with normal control subjects and patients with *H. pylori* negative chronic gastritis, and it reduced after *H. pylori* was eradicated^[11-16]. Our results are in agreement with these reports.

No significant difference was found on BrdU LI% between *H. pylori* positive DU and CSG patients which reveals that the existence of DU does not alter the status of proliferation, some factors other than hyperproliferation such as increased apoptosis may play an important role, in the genesis of DU by keeping the dynamic equilibrium of the epithelium^[17-20]. But some CSG patients cannot keep efficiently this equilibrium, in other words, the proliferation increases without corresponding apoptosis, DNA is prone to be attacked by other carcinogens, resulting in canceration.

About 50% population infected by *H. pylori*, gastric cancer or DU only occurred in a small portion of them. This may be associated with many factors, one of the determinants is the virulence of the strain infected.

Compared with the non-vacAs1a strains infected patients, epithelial cell proliferation of the vacAs1a strains infected patients was much higher. So vacAs1a *H. pylori* strains may be able to promote the epithelial cell proliferation. Multivariate linear-regression shows that vacAs1a strain is an independent influencing factor, which further supports the conclusion that vacAs1a strain is of high virulence. In view of the importance of hyperproliferation during the genesis of gastric cancer, vacAs1a strain may play a critical role in it.

There is much difference on the constitution of the vacA subtype of *H. pylori* according to the reports from different areas. The proportion of vacAs1a strains varied greatly^[3,21]. There have been few reports on the genotype of vacA in China. She *et al* reported the relationship between 60 *H. pylori* strains and the alimentary diseases. The relevance

ratios of vacAs1 in gastric cancer, peptic ulcer and chronic gastritis were 87.5%, 78.9% and 9.1% respectively^[22]. There was obvious geographical discrepancy in the distribution of *H. pylori* vacA subtype.

We found that intestinal metaplasia had nothing to do with *H. pylori* infection, this result corresponds with the report of Cahill *et al.* We also found that intestinal metaplasia was not correlated with proliferation, which differs with some other reports in its clinical importance, the relationship with gastric cancer and effect on cell proliferation. Therefore, we cannot draw conclusion that intestinal metaplasia is not associated with proliferation. Further studies will be conducted.

The mechanism that *H. pylori* and its different strains accelerate proliferation is not clear. Ricci *et al* found VacA can inhibit cell proliferation *in vitro*, while cytotoxin-associated gene (CagA) does not affect proliferation^[23]. *H. pylori* can induce proliferation *in vivo*, so *H. pylori* may act by this suggesting that *H. pylori* based on its ability of inciting inflammatory reaction, influencing the gastrin secretion, but not the direct action of virulences to exact the effect on cell proliferation.

Inflammation and neutrophil filtration are both associated with *H. pylori* infection. That means *H. pylori* infection can arouse acute and chronic inflammation. Accelerated proliferation is related to the extent of inflammation, and the latter is highly related to *H. pylori* infection. This points out that *H. pylori* infection may promote proliferation by inflammation, which was once reported by Lynch *et al*^[11,12]. It is also shown that inflammation acts on proliferation as an independent factor. *H. pylori* infection affects proliferation at least partly by inflammatory action. On the contrary patients harboring vacAs1a strains have similar inflammatory response to those with non-vacAs1a strains, but their ability of inducing proliferation differed. Thereby *H. pylori* may promote proliferation by inflammation, and vacAs1a strains may act by the mechanism other than inflammation, such as the increase of ammonia^[24], gastrin^[25], and the decrease of ascorbic acid concentration^[26,27].

We found that *H. pylori* infection was closely related to gastric epithelial cell proliferation, and the vacAs1a strains had higher activity. The vacAs1a strain and extent of inflammation affect proliferation independently. But the effect of *H. pylori* and its different strains on apoptosis is not clear and needs further studies. Besides, *H. pylori* can be typed into m1 and m2 strains according to vacA middle sequence, and positive or negative cagA. They may have different effects on proliferation and apoptosis, these may play important roles in the pathogenicity of *H. pylori*.

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