

# Detection of *H.pylori* DNA in gastric epithelial cells by *in situ* hybridization

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## Abstract

**AIM:** To investigate the presence of *H.pylori* DNA within gastric epithelial cells in patients with *H.pylori* infection and its possible carcinogenic mechanism.

**METHODS:** Total 112 patients, with pathologically confirmed chronic superficial gastritis, chronic atrophic gastritis, intestinal metaplasia, atypical hyperplasia or gastric cancer were studied. Among them, 28 were *H.pylori* negative and 84 *H.pylori* positive. *H.pylori* DNA in gastric epithelial cells was detected by GenPoint catalyzed signal amplification system for *in situ* hybridization.

**RESULTS:** In the *H.pylori* positive group, zero out of 24 chronic superficial gastritis (0.0%), four out of 25 precancerous changes (16.0%) and thirteen out of 35 gastric cancers (37.1%) showed *H.pylori* DNA in the nucleus of gastric epithelial cells, the positive rates of *H.pylori* DNA in the nucleus of gastric epithelial cells were progressively increased in chronic superficial gastritis, precancerous changes and gastric cancer groups ( $\chi^2=12.56$ ,  $P=0.002$ ); One out of 24 chronic superficial gastritis (4.2%), eleven out of 25 precancerous changes (44.0%) and thirteen out of 35 gastric cancers (37.1%) showed *H.pylori* DNA in the cytoplasm of gastric epithelial cells ( $\chi^2=10.86$ ,  $P=0.004$ ). In the *H.pylori* negative group, only one patient with gastric cancer was found *H.pylori* DNA in the nucleus of gastric epithelial cells; Only two patients, one patient with precancerous changes and another with gastric cancer, showed *H.pylori* DNA in the cytoplasm of gastric epithelial cells. Furthermore, *H.pylori* DNA must have been in the cytoplasm as long as it existed in the nucleus of gastric epithelial cells.

**CONCLUSION:** *H.pylori* DNA exists both in the nucleus and the cytoplasm of gastric epithelial cells in patients with *H.pylori* infections. The pathological progression from chronic superficial gastritis, precancerous changes to gastric cancer is associated with higher positive rates of *H.pylori* DNA presence in the nucleus of gastric epithelial cells.

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## INTRODUCTION

Gastric cancer is the second most common fatal malignancy in the

world and is the cause of more than 750000 deaths annually<sup>[1-3]</sup>. Many studies have showed that *H.pylori* infection is closely related with gastritis, peptic ulcer and gastric cancer, and may play a causative role at the early phases of this chain<sup>[3-16]</sup>. *H.pylori* was classified as a class I carcinogen by the International Agency for Research on Cancer in 1994. So far the mechanism of *H.pylori* carcinogenesis has not been illuminated<sup>[3-7]</sup>. The *H.pylori* DNA must invade gastric epithelial cells first, and then exists chronically in gastric epithelial cell in an unknown manner before integration. The *in situ* hybridization technique has become an essential tool for detecting the localization of DNA transcripts on tissue sections<sup>[17-19]</sup>. The aims of our study are to investigate whether there is *H.pylori* DNA within gastric epithelial cells and the possible carcinogenic mechanism by GenPoint catalyzed signal amplification system for *in situ* hybridization in patients with *H.pylori* infections.

## MATERIALS AND METHODS

### Clinical data

By *H.pylori* diagnostic criteria, 112 patients, including 28 *H.pylori* that were negative and 84 *H.pylori* that were positive, were studied. There were 24 cases with chronic superficial gastritis, 25 cases with precancerous changes and 35 cases with gastric cancer among the *H.pylori* positive group, and 10, 16 and 2 cases, respectively among the *H.pylori* negative group. Histological examination was by routine haematoxylin and eosin stain, and by the *H.pylori* methylene blue staining kit as suggested by the manufacturer (Fujian Sanqiang, China). Rapid urease tests (Digestech, China), <sup>13</sup>C-urea breath tests (Isodiagnostak, Canada) or <sup>14</sup>C-urea breath tests (Shenzhen Headway, China), and serological *H.pylori*-IgG tests (Orion Diagnostica, Finland) were carried out as suggested by each manufacturer. The positive criteria were randomly for two positive items among the following three items: (1) histology (routine haematoxylin and eosin stain, methylene blue stain), (2) urease dependent tests (rapid urease tests, <sup>13</sup>C- or <sup>14</sup>C- urea breath tests), and (3) serological *H.pylori*-IgG tests. Negative criteria were met if three items were negative.

### In situ hybridization

The size of biotinylated Long DNA Probe for *H.pylori* M<sub>2</sub>6000 Protein Gene (Maxim Biotech, USA.) is 303 base pair. 5 $\mu$ m sections were coated on single well slides and hot plated for 12h before deparaffination and rehydration with ethanol. The sections were treated sequentially with 2g·L<sup>-1</sup> Triton-X100 for 15min, 1mM HCl for 10min and digested with 10g·L<sup>-1</sup> pepsin (Sigma, USA) for 15min, and 20mg·L<sup>-1</sup> proteinase K (Meack, Germany) for 30min. The enzyme was inactivated by treatment with 2g·L<sup>-1</sup> glycine in pH7.4 phosphate-buffered saline (PBS) for 5min. Then the sections were incubated sequentially with 10g·L<sup>-1</sup> RNase at 37°C for 30min, 3ml·L<sup>-1</sup> H<sub>2</sub>O<sub>2</sub> in methanol for 30min and prehybridized with hybridization buffer solution at 37°C for 60min. To denature the probe and cellular DNA, the sections were heated with hybridization buffer solution containing 1mg·L<sup>-1</sup> of *H.pylori* probe at 95°C for 15min. The sections were hybridized at 42°C in a humidified chamber for 60min, then treated with Dako GenPoint catalyzed signal amplification system

for *in situ* hybridization as suggested by the manufacturer. The sections were counterstained with hematoxylin and examined under a light microscope.

### Catalyzed signal amplification system for *in situ* hybridization

The Dako GenPoint system created an additional level of amplification for biotin detection. After an initial binding of streptavidin-peroxidase to the biotinylated probe, the peroxidase catalyzed the oxidation of biotinyl-tyramide, which immediately formed covalent bonds with aromatic groups in the specimen. This reaction deposited large amounts of biotin at the site of hybridization. The additional biotin was then used to capture more streptavidin-peroxidase. The signal was finally developed by adding the chromogenic indicator dye diaminobenzidine (DAB), which was oxidized by the precipitate at the site of hybridization.

## RESULTS

In the *H.pylori* positive group, zero out of 24 chronic superficial gastritis (0.0%), four out of 25 precancerous changes (16.0%) and thirteen out of 35 gastric cancers (37.1%) showed *H.pylori* DNA in the nucleus of gastric epithelial cells, the positive rates of *H.pylori* DNA in the nucleus of gastric epithelial cells were progressively increased in chronic superficial gastritis, precancerous changes and gastric cancer groups ( $\chi^2=12.56$ ,  $P=0.002$ ); One out of 24 chronic superficial gastritis (4.2%), eleven out of 25 precancerous changes (44.0%) and thirteen out of 35 gastric cancers (37.1%) showed *H.pylori* DNA in the cytoplasm of gastric epithelial cells ( $\chi^2=10.86$ ,  $P=0.004$ ). In the *H.pylori* negative group, only one patient with gastric cancer was found *H.pylori* DNA in the nucleus of gastric epithelial cells; Just two patients, one patient with precancerous changes and another with gastric cancer, showed *H.pylori* DNA in the cytoplasm of gastric epithelial cells. Furthermore, *H.pylori* DNA must have been in the cytoplasm as long as it existed in the nucleus of gastric epithelial cells.

## DISCUSSION

*H.pylori* has been acknowledged as a possible carcinogen<sup>[3-16]</sup>. Genome integration is the most important carcinogenic mechanism in some microorganisms<sup>[19-21]</sup>. However, there is no reliable evidence of integration of the *H.pylori* DNA in the human genome at this time<sup>[22-24]</sup>. Parsonnet proposed that *H.pylori* has a similar mechanism as virus carcinogenesis<sup>[4]</sup>, viz, *H.pylori* DNA integrated into the gastric epithelial cells genome, which may induce transformation or malignancy of the normal cell. The *H.pylori* DNA must invade the gastric epithelial cells first, and then exists chronically in gastric epithelial cell in an unknown manner before integration. We found that the *H.pylori* DNA can exist both in the nucleus and cytoplasm of gastric epithelial cells in patients with *H.pylori* infections, predominately in cases with precancerous changes and gastric cancer by *in situ* hybridization technique that has been proved a powerful tool for *in situ* gene localization in individual cell<sup>[17-19]</sup>. *H.pylori* DNA can invade into host cells, even into the nucleus, and exist chronically within cells, which is indicated *H.pylori* DNA and genome of host cell would affect each other, even *H.pylori* DNA integrated into genome of host cell. Chiou and his colleagues<sup>[25]</sup> demonstrated that *H.pylori* infection caused an alteration of gene expression in AGS cells and identified 21 overexpressed genes and 17 suppressed genes from the cDNA expression arrays. Some other studies have also showed the alteration of gene expression, cell proliferation and apoptosis<sup>[14-16,26-38]</sup> in patients with gastric cancer or *H.pylori* infection.

We collected 84 *H.pylori* positive cases, including 25 precancerous changes and 35 gastric cancers, and significantly

increased sensitivity by Dako GenPoint catalyzed signal amplification system. The rates of *H.pylori* DNA in the nucleus of gastric epithelial cells was progressively increased in chronic superficial gastritis, precancerous changes and gastric cancer, at 0.0%, 16.0% and 37.1%, respectively in the *H.pylori* positive group. So the progression from chronic superficial gastritis to precancerous changes and to gastric cancer was associated with the presence of *H.pylori* DNA in the nucleus of gastric epithelial cells. This may indicate that *H.pylori* DNA and the genome of the host cell may affect each other, as *H.pylori* DNA is integrated into genome of the host cell. As a result this may change the structure and function of the host cell genome, and thus destroy the stability of the genome. We also found there was *H.pylori* DNA in the *H.pylori* negative group. High sensitivity of *in situ* hybridization<sup>[17-19]</sup> and previous *H.pylori* infection may explain the result. *H.pylori* DNA was also located in the cytoplasm of gastric epithelial cells. *H.pylori* can be seen to invade gastric mucosa by electron or immunoelectron microscopy<sup>[39-42]</sup>. *H.pylori* was able to destroy the junction of cells, and even invade into the cytoplasm of stromal cells in the lamina propria. Yang and his colleagues<sup>[41]</sup> found that *H.pylori* could be engulfed and degraded by the human gastric cancer cell line SGC-7901 using transmission electron microscopy. Once the *H.pylori* DNA invaded the gastric epithelial cells, it could enter the nucleus when the karyotheca disappears during the metaphase of mitosis. If the *H.pylori* DNA was found in nucleus, it was also found in the cytoplasm in our study. In the *H.pylori* positive group, the positive rates of finding *H.pylori* DNA in cytoplasm in precancerous changes and gastric cancer were higher than that in chronic superficial gastritis, while there was no statistic significance in the rate between precancerous changes and gastric cancer.

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