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ORIGINAL ARTICLE

Expression of p53-MDM2 feedback loop related proteins in different gastric pathologies in relation to *Helicobacter pylori* infection: Implications in gastric carcinogenesis

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Summary
Aim: To explore the association of p53-MDM2 feedback loop related proteins with gastric pathologies in relation to *Helicobacter pylori* infection.
Methods: Gastric biopsies were obtained from 157 *H. pylori*-negative and positive patients, including normal gastric mucosa (NGM), chronic gastritis (CG), intestinal metaplasia (IM), dysplasia (Dys), and gastric cancer (GC). The expression of mutant p53, MDM2, Bax and PUMA in gastric tissues was detected by immunohistochemistry.
Results: Overall expression of MDM2 and Bax is progressively increased from NGM to GC. PUMA expression is increased in CG but subsequently decreased after the development of IM. *H. pylori* infection is associated with increased mutant p53 and Bax expression but decreased PUMA expression in IM, and increased MDM2 expression in Dys.
Conclusions: These results suggest that different p53-MDM2 feedback loop related proteins are distinctly expressed in the various stages of gastric carcinogenesis; their roles in gastric carcinogenesis in the presence of *H. pylori* infection need to be further investigated.

Introduction

Helicobacter pylori, which was first described in 1983, is recognized as the pathogen of several gastroduodenal diseases, such as active and chronic gastritis, peptic ulcer, and mucosa-associated lymphoid tissue lymphoma [1—4]. Moreover, it has been classified as a class I (definite) carcinogen for gastric cancer by the International Agency for Research of Cancer (IARC), based on epidemiological, animal and clinical studies [5]. *H. pylori* infection plays a critical role in the development of gastric carcinoma through a multistep process from chronic superficial gastritis to chronic atrophic gastritis, intestinal metaplasia, dysplasia and finally gastric carcinoma [6].

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H. pylori infection also increases apoptosis and proliferation in human gastric tissue [7—10]. It has been postulated that aberrantly increased apoptosis in gastric epithelium induced by *H. pylori* infection may be the initial trigger in gastric carcinogenesis [11]. Thus, elucidation of apoptosis related proteins involved in *H. pylori* induced apoptosis in gastric epithelium are critical for understanding the initial steps of gastric carcinogenesis.

p53, a tumor suppressor gene, up-regulates apoptosis and inhibits cell growth [12]. Mutation of this gene is an early event in the atrophy-metaplasia-carcinoma process of gastric carcinogenesis [13,14]. *Murine double minute gene 2* (MDM2), an oncogene, is a negative regulator of the p53, and has also been implicated in carcinogenesis [15]. On one hand, MDM2 can inhibit p53 bioactivity by blocking the transcriptional activity of p53 and promoting p53 protein degradation [16]. On the other hand, p53 can also regulate the synthesis of MDM2 [17]. This so-called p53-MDM2 auto-regulatory feedback loop plays a crucial role in carcinogenesis [17]. p53 up-regulated modulator of apoptosis (PUMA) and Bcl-2-associated X protein (Bax) are the important downstream effectors of p53 [17]. However, little is known on the roles of PUMA and Bax proteins in the p53-MDM2 feedback loop. PUMA is a pro-apoptotic protein that is up-regulated by p53. In turn, PUMA can displace p53 from Bcl-xL, allowing p53 to induce mitochondrial permeabilization, cytochrome C release and apoptosis [18]. Bax is also a pro-apoptotic protein that translocates from the cytosol to the mitochondria following a pro-apoptotic stimulus, and initiates apoptosis [19,20]. Bax also binds to the mitochondria membrane and mediates apoptosis through the mitochondrial pathway [21,22]. Previous studies have explored the mechanism by which *H. pylori* infection may contribute to gastric cancer. However, there are controversies on the relationship between p53 and *H. pylori* infection [23—26]. Moreover, the role of the p53-MDM2 feedback loop in the *H. pylori*-associated process of gastric carcinogenesis infection has not been elucidated. Therefore, the aim of the present study was to determine the expression of mutant p53, MDM2, PUMA and Bax proteins and their correlations in the different stages of the development of gastric carcinoma with or without *H. pylori* infection, in order to explore the roles of p53-MDM2 feedback loop related proteins in different gastric pathologies in relation to *H. pylori* infection.

Methods

Patients

Gastric samples of the patients who underwent upper gastroduodenoscopy from January 2007 to September 2009 at the first affiliated hospital of Nanchang University were retrospectively reviewed and examined. A total of 157 patients (67 females and 90 males, with a mean age of 53.3 [$\pm$12.9] years) were enrolled in this study including 20 with normal gastric mucosa (NGM, all *H. pylori*-negative), 20 with chronic gastritis (CG, all *H. pylori*-positive), 40 with intestinal metaplasia (IM, 20 *H. pylori*-positive and 20 *H. pylori*-negative), 37 with dysplasia (Dys, 19 *H. pylori*-positive, and 18 *H. pylori*-negative), and 40 with gastric cancer (GC, 20 *H. pylori*-positive and 20 *H. pylori*-negative) (Table 1). There was no significant difference in the age and gender distribution among these groups. All patients had not been treated with any regimens aiming at *H. pylori* eradication.

The study design was approved by the Institute review board of the first affiliated hospital, Nanchang University. All patients gave written informed consent for participating in the study.

Detection of *Helicobacter pylori* infection

An ''in-house'' rapid urease test (RUT) and modified Giemsa staining were employed for the detection of *H. pylori* infection. The effectiveness of RUT is more than 95% (data not shown). The modified Giemsa staining was carried out in a double-blind fashion. *H. pylori* infection was diagnosed as positive only if both of the methods produced positive results. An *H. pylori*-negative diagnosis was confirmed if both of the methods yielded negative results.

Histological examinations of gastric samples

Gastric samples were obtained from the patients who underwent endoscopy of the upper gastrointestinal tract. All biopsies were taken from the gastric antrum and the location of lesions of individual patients. The tissues used for histological analysis were fixed in 10% formaldehyde in Ca^{2+} and Mg^{2+} free phosphate-buffered saline (PBS) overnight at 4 °C before paraffin embedding. Paraffin sections of 4 μm were cut with a microtome and stored at room temperature. Pathologic diagnosis and classification were made according to the criteria of the World Health Organization [27] and the updated Sydney system [28].

Immunohistochemical detection of mutant p53, MDM2, Bax and PUMA proteins

Primary antibodies used in this study were mouse monoclonal anti-human mutant p53, MDM2, and Bax proteins (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and rabbit polyclonal anti-human PUMA protein (Cell Signaling biotechnology, Danvers, MA, USA). Anti-human mutant p53, anti-human MDM2 and anti-human Bax antibodies were diluted to 1:100, and anti-human PUMA antibody was diluted to 1:200.

Paraffin sections were mounted on slides and dewaxed in xylene and sequentially dehydrated in 100, 95 and 85% ethanol. Sections were stained using the PV-9000 Polymer Detection System (Zhongshan Goldenbridge, Beijing, PRC) staining protocol. They were washed in PBS and endogenous peroxidase was blocked using 3% H_2O_2. After the specimens were incubated with the primary antibody overnight at 4 °C, they were washed with PBS, followed by incubation with polymer helper for 30 min and polyperoxidase-anti-mouse or rabbit IgG for 30 min. After the sections were washed with PBS, they were incubated with 3,3-diaminobenzidin (DAB, Zhongshan Goldenbridge). Control sections incubated with PBS, instead of primary antibodies, were used as negative controls. Sections were counterstained with hematoxylin.