BIOCHEMICAL PHARMACOLOGY 73 (2007) 1697–1702

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/biochempharm

Commentary

CagA protein of *Helicobacter pylori*: A hijacker of gastric epithelial cell signaling

Osamu Handa [a,*], *Yuji Naito* [b], *Toshikazu Yoshikawa* [c]

[a] *Department of Biomedical Safety Science, Kyoto Prefectural University of Medicine, Kyoto 602-8566, Japan*
[b] *Department of Medical Proteomics, Kyoto Prefectural University of Medicine, Kyoto 602-8566, Japan*
[c] *Department of Inflammation and Immunology, Kyoto Prefectural University of Medicine, Kyoto 602-8566, Japan*

ARTICLE INFO

Keywords:
Phosphorylation
Mitochondria
DNA damage
Signal transduction
Carcinogenesis

ABSTRACT

Epidemiological study has shown strong correlation between the *Helicobacter pylori* (*H. pylori*) infection and gastric carcinogenesis. However, the mechanism by which *H. pylori* induces gastric carcinogenesis is not known. In this review, we focused on the product of cytotoxin-associated gene A (CagA), one of the important virulence factors of *H. pylori*. *H. pylori* injects CagA protein into the host gastric epithelial cells through its needle-like structure, type IV secretion system. Injected CagA hijacks physiological signal transduction and causes pathological cellular response such as increased cell proliferation, motility, apoptosis and morphological change through different mechanisms. *H. pylori* has been shown to produce reactive oxygen species (ROS) in infected gastric mucosa. Although the main source of ROS production is possibly host neutrophil, we propose novel source of ROS production in this review; CagA itself can induce ROS production in gastric epithelial cell. Excessive ROS production in gastric epithelial cells can cause DNA damage and thus might involve in gastric carcinogenesis. Understanding the molecular mechanism by which *H. pylori*-induced carcinogenesis is important for developing new strategies against gastric cancer.

1. Introduction

In 1994, *Helicobacter pylori* (*H. pylori*) was defined as a group 1 (a definite) carcinogen by International Agency for Research on Cancer (IARC), a part of World Health Organization (WHO). To date, extensive research has been done in many laboratories to clarify the mechanism by which *H. pylori* induces gastric carcinogenesis. One of the candidate proteins is cytotoxin-associated gene A (cagA) protein injected into host gastric epithelial cells by type IV secretion system (T4SS) of *H. pylori*. Injected-CagA protein, a *H. pylori* virulence factor, is thought to hijack the cellular signal transduction and involve in gastric carcinogenesis. In this review, we summarized reported CagA-induced pathological signal transduction pathways and focused on the reactive oxygen species (ROS) production, in *H. pylori*-infected gastric epithelial cells and possible mechanism by which *H. pylori* involve gastric carcinogenesis, since ROS have been shown to involve in tumor initiation and in particular enhance the expression of oncogenes and stimulate cell proliferation.

2. *Helicobacter pylori* (*H. pylori*)

H. pylori in the base of a gastric ulcer was first reported by Marshall and Warren in 1984, which revolutionized the

* *Corresponding author*. Tel.: +81 75 251 5505; fax: +81 75 252 3721.
E-mail address: handao@koto.kpu-m.ac.jp (O. Handa).
0006-2952/$ – see front matter
doi:10.1016/j.bcp.2006.10.022

pathogenesis and the treatment of chronic gastritis, peptic ulcer, and gastric cancer. This small (3.5 μm × 0.5 μm) non-spore-forming and spiral-shaped Gram-negative rod bacteria transmitted from human-to-human possibly by the fecal–oral or oral–oral route [1] and induces infiltration of the gastric mucosa by neutrophils, macrophages, and T and B lymphocytes. However, our immune system and inflammatory response cannot clear the infection of this bacterium, and leaves the host prone to complications resulting from chronic inflammation or ulcer formation to gastric carcinogenesis.

Recent studies of the *cagA* gene, located in the most downstream portion of the *cag* pathogenicity island (*cag*PAI), have demonstrated that the CagA protein is associated with peptic ulcer disease, gastric cancer, and mucosa-associated lymphoid tissue (MALT) lymphoma. In Western countries, persons carrying cagPAI have an enhanced risk for developing atrophic gastritis, non-cardia gastric adenocarcinoma, and peptic ulcerations, but in East-Asian countries, the relationship of cagPAI with disease is more difficult to establish [2].

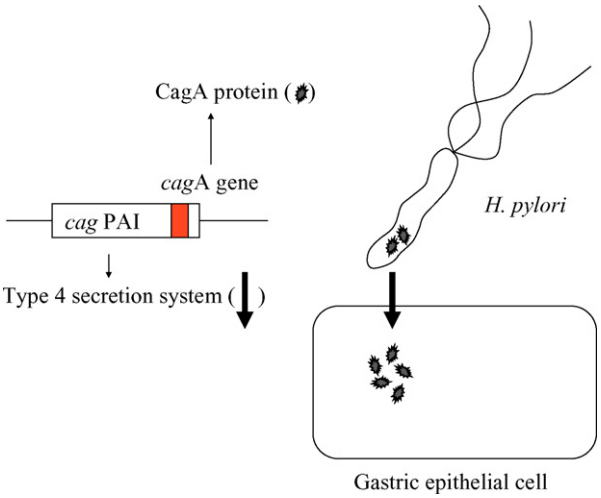

Fig. 1 – *cag*PAI and *cagA* gene.

3. CagA protein

H. pylori strains can be divided into two major sub-types based on their ability to produce a CagA protein (125–145 kDa), a *cagA* gene product of *H. pylori*, which is injected directly from the bacterium into the infected gastric epithelial cell via the type IV secretion system (T4SS) [3,4] (Fig. 1). The *cagA* gene is localized at the most downstream portion of the *cag* pathogenicity island (*cag*PAI) [5], a 40 kb DNA segment that was most likely incorporated into the *H. pylori* genome by a process of horizontal transfer in the same way as many other Gram-negative rods. Approximately, 60% of *H. pylori* strain in Western countries possess *cag*PAI [5], however, almost 90% of those in East Asian countries possess *cag*PAI [6]. The cagPAI segment contains 31 genes, including cagA–cagZ and cagα–cagζ, some of which code components required to form T4SS.

CagA protein can be sub-classified into two main types, East-Asian CagA and Western CagA, based on its polymorphisms in tyrosine phosphorylation sites [7] (Fig. 2). Tyrosine phosphorylation of CagA occurs at the EPIYA motif; a five amino acid sequence (Glu-Pro-Ile-Tyr-Alu) that is present in the carboxy-terminal variable region of the protein. The EPIYA motif is part of four distinct EPIYA sites; EPIYA-A, -B, -C, and -D. Each motif is defined by the amino acid sequence that surrounds the EPIYA sequence. In the Western countries, commonly circulate CagA possess EPIYA-A and EPIYA-B sites followed by one to three times repeated EPIYA-C sites (ABC, ABCC and ABCCC type) in which most prevalent type is ABC type [8]. In the East-Asian countries, on the contrary, most of CagA possesses EPIYA-A, -B and -D (ABD type) [9]. Among these EPIYA sites, EPIYA-C and -D is a major phosphorylation site in each type.

Azuma et al. reported that the prevalence of East-Asian CagA-positive strains appeared to be associated with the rate of gastric cancer mortality worldwide and endemic circulation of *H. pylori* populations with more virulent East-Asian CagA proteins may affect the prevalence of gastric cancer in East-Asian countries [10].

4. CagA as a hijacker of cellular signaling (Fig. 3)

As described above, CagA is injected directly from the bacterium into the infected gastric epithelial cells via the type IV secretion system [3,4]. Since most, but not all, CagA proteins contain tyrosine phosphorylation motifs, majority of injected CagA localizes to the plasma membrane and undergoes tyrosine phosphorylation by the host Src family protein

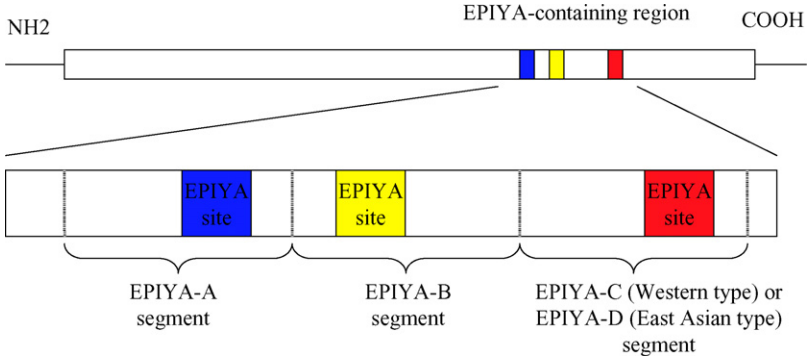

Fig. 2 – EPIYA. EPIYA motif consists of five amino acids: Glu-Pro-Ile-Tyr-Alu.

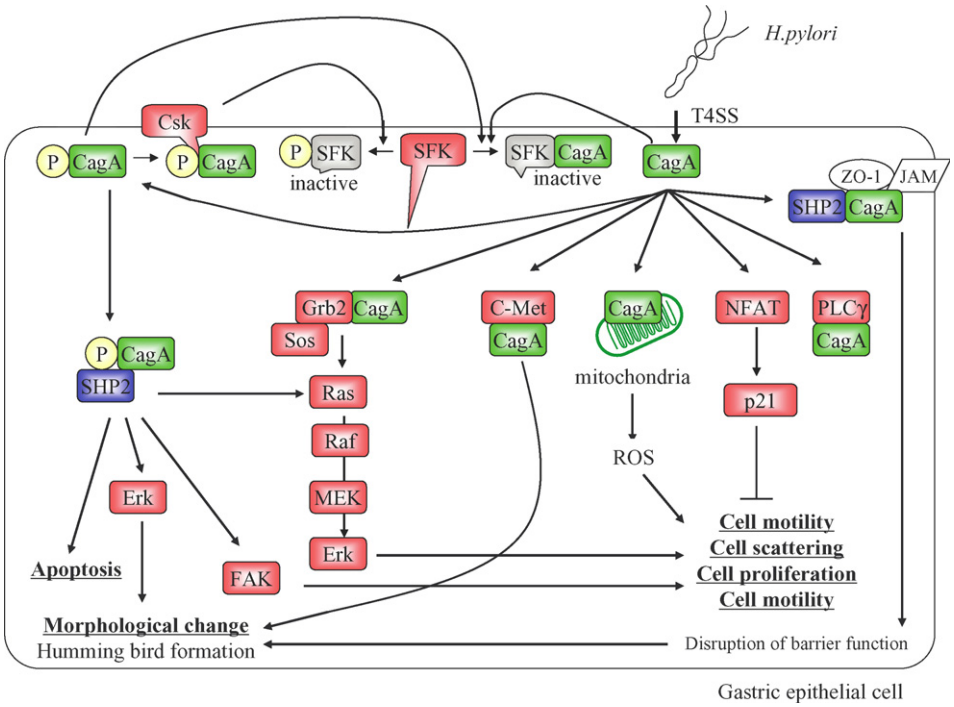

Fig. 3 – CagA-induced pathological signal transduction. CagA protein injected through type IV secretion system (T4SS) of *H. pylori* could interact with several intracellular signaling molecules in both tyrosine phosphorylation-dependent and -independent manners and thus might promote early stage of gastric carcinogenesis. Csk, C-terminal Src kinase; SHP2, Src homology 2 (SH2) domain containing tyrosine phosphatase; Erk, extracellular signal-regulated kinase; FAK, focal adhesion kinase; Grb2, growth factor receptor bound protein 2; JAM, junctional adhesion protein; MEK, mitogen-activated protein kinase kinase; NFAT, nuclear factor of activated T cells; PLCγ, phospholipase C-γ; ROS, reactive oxygen species; SFK, Src family kinase; Sos, son of sevenless; ZO-1, zonula occludens-1.

tyrosine kinases (SFK) such as Src, Fyn, Lyn and Yes [11,12]. Since phosphorylation occurs without any stimuli, SFK are constitutively activated in gastric epithelial cells.

Phosphorylated CagA then binds to two tandem-repeated Src homology 2 (SH2) domain containing tyrosine phosphatase (SHP2) and deregulates phosphatase activity, or binds to other host molecules involved in a variety of signal transduction pathways, which affects host cell gene expression affecting cytokine release, cell cycle and cell structure. EPIYA-D site of East-Asian CagA were reported to have a significantly higher SHP2 binding affinity than EPIYA-C site of Western CagA and thus may correlated with the higher incidence of gastric carcinoma in East-Asian countries [13].

CagA-activated SHP2 stimulates extracellular signal-regulated kinase (Erk) by both Ras-dependent and -independent pathway [14,15]. Induction of hummingbird phenotype by CagA requires SHP2-induced Erk activation, but is independent of Ras or growth factor receptor bound protein 2 (Grb2) activation [16,17]. CagA-activated SHP2 have also been reported to dephosphorylates focal adhesion kinase (FAK) and inhibits kinase activity, which elicits elevated cell motility by reducing active focal adhesion spots [18].

The complex formation between tyrosine phosphorylated CagA and SHP2 has been reported in infected cells *in vitro* as well as in transfected cells with CagA and in biopsy specimens of gastric mucosa (even in atrophic mucosa but not in mucosa with intestinal metaplasia or gastric carcinoma [19]) in CagA positive *H. pylori*-infected patients [20], indicating the importance of the complex formation in the pathogenesis of *H. pylori*. SHP2 has two tandem-repeated SH2 domain (N-SH2 and C-SH2) on its N-terminus and one protein tyrosine phosphatase (PTP) domain on its C-terminus [7,9,21]. Two SH2 domain of SHP2 specifically recognize two tyrosine phosphorylated EPIYA motifs of CagA. Upon binding, conformational change of SHP2 is occurred and results in its continuous protein tyrosine phosphatase (PTP) activation [13]. Recent studies have shown that gain-of-function mutations in *PTPN11*, the gene encoding SHP2, are associated with various human malignancies such as child leukemia and some solid tumors [22,23], indicating that SHP2 is a oncoprotein that is substantially involved in human malignancies.

A fraction of phosphorylated CagA interacts with the carboxy-terminal Src kinase (Csk) (~20%), although with lower affinity than SHP2 (~80%), and forms a complex, which in turn inhibits SFK activity by tyrosine phosphorylation [24]. CagA has also been reported to bind to Src and inhibit its kinase activity regardless of the CagA phosphorylation status [25]. By suppressing excessive CagA phosphorylation and subsequent recruitment of SHP2, *H. pylori* might down-regulates the excessive cytotoxicity of CagA.

Phosphorylation-independent hijackings of signal transduction of CagA has also been reported. CagA interacts with the scaffolding protein zonula occludens-1 (ZO-1) and tight junctional adhesion protein (JAM), by recruiting SHP2 and

other signaling molecules, which causes ectopic assembly of a tight junction at the site of *H. pylori* attachment, ultimately resulting in monolayer leakage [26]. CagA can bind to growth factor receptor bound protein 2 (Grb2) forming a complex with son of sevenless (Sos), and to C-Met hepatocyte growth factor receptor and phospholipase C-γ (PLCγ) [17,27]. Although the complex of CagA with C-Met involves in morphological change of epithelial cells, the biological function of the complex formation of CagA with PLCγ remains to be elucidated. Interestingly, Grb2 binding to CagA occurred independently of CagA phosphorylation, but did require the presence of the CagA phosohorylation sequence. More recently, CagA was found to activate the nuclear factor of activated T cells (NFAT) in gastric epithelial cells in tyrosine phosphorylation-independent manner [28]. One of the NFAT-dependent genes activated by CagA in gastric epithelial cells is $p21^{Cip1}$ cyclin-dependent kinase inhibitor. Thus, although CagA activates a growth promotion signal via SHP2 deregulation, it simultaneously inhibits progression of the cell cycle through NFAT activation.

CagA could interact with several intracellular signaling molecules as described above in both tyrosine phosphorylation-dependent and -independent manners and thus might promote early stage of gastric carcinogenesis.

5. CagA-induced ROS production and gastric carcinogenesis

Reactive oxygen species (ROS) has been shown to play an important role in carcinogenesis by inducing DNA damage. In patients with *H. pylori* infection, production of ROS was found to be enhanced in endoscopic biopsied samples from the duodenum and stomach [29,30]. Davies et al. [29] found a positive association between ROS production and the infective load of *H. pylori*. ROS production was also found in gastric mucosa of *H. pylori*-infected patients with gastric ulcer [30] and chronic gastritis in the absence of peptic ulcer [31]. The main source of ROS production in *H. pylori*-infected gastric mucosa is probably host neutrophils, which are activated by soluble factors of *H. pylori* known as a neutrophil-activating protein (NAP). CagA positivity also affects ROS production in gastric epithelial cells. Suzuki et al. found that ROS production in gastric epithelial cells was significantly enhanced by the infection of *cagA*-positive *H. pylori* species with an extensive accumulation of neutrophils [30,32]. An *in vitro* study demonstrated that *cagA*-positive strains induce an increased oxidative burst in polymorphonuclear neutrophils (PMNs) with higher ROS production [33]. Besides neutrophils, injected CagA itself might induce ROS production in gastric mucosa. By transfecting *cagA* gene in gastric epithelial cells, we found that fraction of expressed CagA protein localizes to mitochondria and produce significant amount of ROS in the cells [34]. In addition, increased ROS production might involve in acceleration of cell cycle and subsequent cell proliferation [35]. The mechanism by which CagA induce ROS production in gastric epithelial cells are not known. However, CagA localize to mitochondria may deregulate the function of mitochondria electron transport chain and produce primarily super oxide ($O_2^{\bullet -}$). To clarify the importance of ROS production in CagA expressed gastric epithelial cells, an in vitro study using CagA transfected normal rat gastric epithelial cells (RGM1) are now underway in our laboratory.

One of the mechanisms by which *H. pylori*-induced carcinogenesis is now believed to be dependent on an accumulative oxidative DNA damage [36]. In gastric carcinoma patients, significantly higher levels of 8-hydroxydeoxyguanosine (8-OHdG), the main oxidative modifying product of DNA, was reported in their tumor-adjacent tissues and tumor tissues than in normal tissues [37]. Farinati et al. stated that *H. pylori* infection is the single most important factor in determining oxidative DNA damage, as assessed by 8-OHdG levels [38]. In addition, accumulated oxidative DNA damage such as 8-OHdG can be only partially repaired through enzyme pathways that may, in turn, cause further DNA damage [39] including DNA mutation and ultimately gastric carcinogenesis. Eradication therapy could not easily repair this DNA oxidative damage and is partially irreversible after eradication [40]. In addition to 8-OHdG production, an accumulation of intracellular ROS can induce point mutation in the DNA, thus disrupting the expression and function of several tumor suppressing genes such as p53, which might contribute to the pathogenesis of gastric cancer [41]. Therefore, in younger patients with cagA-positive *H. pylori*, DNA oxidative damage in the gastric mucosa is accumulated in earlier stage of their life, and may cause more extensive gastric mucosal derangement [40,42].

As described above, CagA induces ROS production in mitochondria. The mitochondrial DNA (mtDNA) is more susceptible to ROS damage than nuclear DNA because of its close proximity to the electron transport chain and its lack of protective histones or DNA-binding proteins [43]. In addition, mtDNA damage is not sufficiently repaired because of little amount of repair enzymes in some cells [44]. As a result, the respiratory enzymes containing the defective mtDNA-encoded protein sub-units may exhibit impaired electron transport function and thereby increase the electron leak and ROS production, which in turn elevate the oxidative stress and oxidative damage to mitochondria. Consequently, CagA might induces oxidative stress to the gastric mucosa, and may damage cellular components, including polyunsaturated fatty acids, proteins and mtDNA, which may enhance nuclear DNA damage, and possibly result in the pathogenesis of gastric carcinogenesis.

6. Concluding remarks

The precise mechanism of gastric carcinogenesis induced by *H. pylori* is still unclear. In this review, we propose a novel pathological signal transduction that CagA itself can induce ROS production in gastric epithelial cell. However, there are many factors involve in gastric carcinogenesis, such as another virulence factors of *H. pylori*, host factors and environmental factors. In addition, different mechanism was proposed by Houghton et al. in which they indicated that malignant cells immigrate to the injury site from the bone marrow [45]. If this is the case, increased turnover of gastric stem cells eventually lead to depletion of the resident stem cell pool and to subsequent recruitment and settlement of bone

marrow-derived cell (BMDC) into gastric mucosa that do not differentiate properly resulting ultimately in carcinogenesis.

We should be aware that the road to carcinogenesis is not through the single way. Moreover, recent reports have indicated that the ratio of eradication therapy-resistant case is increasing in *H. pylori*-infected patients and the effective eradication therapy has been anticipated. Therefore, to understand the signal transduction in *H. pylori*-infected gastric epithelial cells and the molecular mechanism by which *H. pylori*-induced carcinogenesis will be important for deciding subscribe strategies against eradication therapy-resistant *H. pylori* and for developing new strategies against gastric cancer.

REFERENCES

[1] Kelly SM, Pitcher MC, Farmery SM, Gibson GR. Isolation of *Helicobacter pylori* from feces of patients with dyspepsia in the United Kingdom. Gastroenterology 1994;107:1671–4.

[2] Naito Y, Yoshikawa T. Molecular and cellular mechanisms involved in *Helicobacter pylori*-induced inflammation and oxidative stress. Free Radic Biol Med 2002;33:323–36.

[3] Rohde M, Puls J, Buhrdorf R, Fischer W, Haas R. A novel sheathed surface organelle of the *Helicobacter pylori* cag type IV secretion system. Mol Microbiol 2003;49:219–34.

[4] Fischer W, Puls J, Buhrdorf R, Gebert B, Odenbreit S, Haas R. Systematic mutagenesis of the *Helicobacter pylori* cag pathogenicity island: essential genes for CagA translocation in host cells and induction of interleukin-8. Mol Microbiol 2001;42:1337–48.

[5] Censini S, Lange C, Xiang Z, Crabtree JE, Ghiara P, Borodovsky M, et al. Cag, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. Proc Natl Acad Sci USA 1996;93:14648–53.

[6] Maeda S, Yoshida H, Ikenoue T, Ogura K, Kanai F, Kato N, et al. Structure of cag pathogenicity island in Japanese *Helicobacter pylori* isolates. Gut 1999;44:336–41.

[7] Hatakeyama M. Oncogenic mechanisms of the *Helicobacter pylori* CagA protein. Nat Rev 2004;4:688–94.

[8] Covacci A, Censini S, Bugnoli M, Petracca R, Burroni D, Macchia G, et al. Molecular characterization of the 128-kDa immunodominant antigen of *Helicobacter pylori* associated with cytotoxicity and duodenal ulcer. Proc Natl Acad Sci USA 1993;90:5791–5.

[9] Higashi H, Tsutsumi R, Fujita A, Yamazaki S, Asaka M, Azuma T, et al. Biological activity of the *Helicobacter pylori* virulence factor CagA is determined by variation in the tyrosine phosphorylation sites. Proc Natl Acad Sci USA 2002;99:14428–33.

[10] Azuma T, Ohtani M, Yamazaki Y, Higashi H, Hatakeyama M. Meta-analysis of the relationship between CagA seropositivity and gastric cancer. Gastroenterology 2004;126:1926–7 [author reply 7–8].

[11] Selbach M, Moese S, Hauck CR, Meyer TF, Backert S. Src is the kinase of the *Helicobacter pylori* CagA protein in vitro and in vivo. J Biol Chem 2002;277:6775–8.

[12] Stein M, Rappuoli R, Covacci A. Tyrosine phosphorylation of the *Helicobacter pylori* CagA antigen after cag-driven host cell translocation. Proc Natl Acad Sci USA 2000;97:1263–8.

[13] Hatakeyama M, Higashi H. *Helicobacter pylori* CagA: a new paradigm for bacterial carcinogenesis. Cancer Sci 2005;96:835–43.

[14] Maroun CR, Naujokas MA, Holgado-Madruga M, Wong AJ, Park M. The tyrosine phosphatase SHP-2 is required for sustained activation of extracellular signal-regulated kinase and epithelial morphogenesis downstream from the met receptor tyrosine kinase. Mol Cell Biol 2000; 20:8513–25.

[15] Neel BG, Gu H, Pao L. The 'Shp'ing news: SH2 domain-containing tyrosine phosphatases in cell signaling. Trends Biochem Sci 2003;28:284–93.

[16] Higashi H, Nakaya A, Tsutsumi R, Yokoyama K, Fujii Y, Ishikawa S, et al. *Helicobacter pylori* CagA induces Ras-independent morphogenetic response through SHP-2 recruitment and activation. J Biol Chem 2004;279:17205–16.

[17] Mimuro H, Suzuki T, Tanaka J, Asahi M, Haas R, Sasakawa C. Grb2 is a key mediator of *Helicobacter pylori* CagA protein activities. Mol Cell 2002;10:745–55.

[18] Tsutsumi R, Takahashi A, Azuma T, Higashi H, Hatakeyama M. Focal adhesion kinase is a substrate and downstream effector of SHP-2 complexed with *Helicobacter pylori* CagA. Mol Cell Biol 2006;26:261–76.

[19] Zheng PY, Jones NL. *Helicobacter pylori* strains expressing the vacuolating cytotoxin interrupt phagosome maturation in macrophages by recruiting and retaining TACO (coronin 1) protein. Cell Microbiol 2003;5:25–40.

[20] Yamazaki S, Yamakawa A, Ito Y, Ohtani M, Higashi H, Hatakeyama M, et al. The CagA protein of *Helicobacter pylori* is translocated into epithelial cells and binds to SHP-2 in human gastric mucosa. J Infect Dis 2003;187:334–7.

[21] Higashi H, Tsutsumi R, Muto S, Sugiyama T, Azuma T, Asaka M, et al. SHP-2 tyrosine phosphatase as an intracellular target of *Helicobacter pylori* CagA protein. Science 2002;295:683–6.

[22] Loh ML, Vattikuti S, Schubbert S, Reynolds MG, Carlson E, Lieuw KH, et al. Mutations in PTPN11 implicate the SHP-2 phosphatase in leukemogenesis. Blood 2004;103:2325–31.

[23] Tartaglia M, Kalidas K, Shaw A, Song X, Musat DL, van der Burgt I, et al. PTPN11 mutations in Noonan syndrome: molecular spectrum, genotype–phenotype correlation, and phenotypic heterogeneity. Am J Hum Genet 2002;70:1555–63.

[24] Murakami K, Kodama M, Fujioka T. Latest insights into the effects of *Helicobacter pylori* infection on gastric carcinogenesis. World J Gastroenterol 2006;12:2713–20.

[25] Selbach M, Moese S, Hurwitz R, Hauck CR, Meyer TF, Backert S. The *Helicobacter pylori* CagA protein induces cortactin dephosphorylation and actin rearrangement by c-Src inactivation. EMBO J 2003;22:515–28.

[26] Amieva MR, Vogelmann R, Covacci A, Tompkins LS, Nelson WJ, Falkow S. Disruption of the epithelial apical–junctional complex by *Helicobacter pylori* CagA. Science 2003;300:1430–4.

[27] Churin Y, Al-Ghoul L, Kepp O, Meyer TF, Birchmeier W, Naumann M. *Helicobacter pylori* CagA protein targets the c-Met receptor and enhances the motogenic response. J Cell Biol 2003;161:249–55.

[28] Yokoyama K, Higashi H, Ishikawa S, Fujii Y, Kondo S, Kato H, et al. Functional antagonism between *Helicobacter pylori* CagA and vacuolating toxin VacA in control of the NFAT signaling pathway in gastric epithelial cells. Proc Natl Acad Sci USA 2005;102:9661–6.

[29] Davies GR, Simmonds NJ, Stevens TR, Sheaff MT, Banatvala N, Laurenson IF, et al. *Helicobacter pylori* stimulates antral mucosal reactive oxygen metabolite production in vivo. Gut 1994;35:179–85.

[30] Suzuki H, Miura S, Imaeda H, Suzuki M, Han JY, Mori M, et al. Enhanced levels of chemiluminescence and platelet activating factor in urease-positive gastric ulcers. Free Radic Biol Med 1996;20:449–54.

[31] Danese S, Cremonini F, Armuzzi A, Candelli M, Papa A, Ojetti V, et al. *Helicobacter pylori* CagA-positive strains affect oxygen free radicals generation by gastric mucosa. Scand J Gastroenterol 2001;36:247–50.
[32] Suzuki H, Hibi T. Oxidative stress in *Helicobacter pylori*-associated gastroduodenal disease. J Clin Biochem Nutrit 2006;39:56–63.
[33] Zhang QB, Nakashabendi IM, Mokhashi MS, Dawodu JB, Gemmell CG, Russell RI. Association of cytotoxin production and neutrophil activation by strains of *Helicobacter pylori* isolated from patients with peptic ulceration and chronic gastritis. Gut 1996;38:841–5.
[34] Handa O, Naito Y, Ishii T, Tsuboi H, Adachi S, Takagi T, et al. *Helicobacter pylori* Cag A induces mitochondria-dependent production of reactive oxygen species in gastric epithelial cell. Gastroenterology 2006;130((4) Suppl 2):A523.
[35] Handa O, Naito Y, Ishii T, Tsuboi H, Adachi S, Takagi T, et al. Cytotoxin-associated gene product A of *Helicobacter pylori* induced cellular response in rat gastric epithelial cell. Digestion 2006;73(Suppl 2):69.
[36] Nishibayashi H, Kanayama S, Kiyohara T, Yamamoto K, Miyazaki Y, Yasunaga Y, et al. *Helicobacter pylori*-induced enlarged-fold gastritis is associated with increased mutagenicity of gastric juice, increased oxidative DNA damage, and an increased risk of gastric carcinoma. J Gastroenterol Hepatol 2003;18:1384–91.
[37] Lee BM, Jang JJ, Kim HS. Benzo[*a*]pyrene diol-epoxide-I-DNA and oxidative DNA adducts associated with gastric adenocarcinoma. Cancer Lett 1998;125:61–8.
[38] Farinati F, Cardin R, Degan P, Rugge M, Mario FD, Bonvicini P, et al. Oxidative DNA damage accumulation in gastric carcinogenesis. Gut 1998; 42:351–6.
[39] Kuchino Y, Mori F, Kasai H, Nishimura S, Inoue H, Iwai S, et al. Misreading of 8-hydroxydeoxyguanosine-containing DNA in in vitro DNA replication.
In: Proceedings of the Nucleic Acids Symposium Series; 1986. p. 157–8.
[40] Farinati F, Cardin R, Russo VM, Busatto G, Franco M, Rugge M. *Helicobacter pylori* CagA status, mucosal oxidative damage and gastritis phenotype: a potential pathway to cancer? Helicobacter 2003;8:227–34.
[41] Ernst P. Review article: the role of inflammation in the pathogenesis of gastric cancer. Aliment Pharmacol Therapeut 1999;13(Suppl 1):13–8.
[42] Maaroos HI, Vorobjova T, Sipponen P, Tammur R, Uibo R, Wadstrom T, et al. An 18-year follow-up study of chronic gastritis and *Helicobacter pylori* association of CagA positivity with development of atrophy and activity of gastritis. Scand J Gastroenterol 1999;34:864–9.
[43] Wei YH, Lu CY, Lee HC, Pang CY, Ma YS. Oxidative damage and mutation to mitochondrial DNA and age-dependent decline of mitochondrial respiratory function. Ann N Y Acad Sci 1998;854:155–70.
[44] Dobson AW, Xu Y, Kelley MR, LeDoux SP, Wilson GL. Enhanced mitochondrial DNA repair and cellular survival after oxidative stress by targeting the human 8-oxoguanine glycosylase repair enzyme to mitochondria. J Biol Chem 2000;275:37518–23.
[45] Houghton J, Stoicov C, Nomura S, Rogers AB, Carlson J, Li H, et al. Gastric cancer originating from bone marrow-derived cells. Science 2004;306:1568–71.