Review

Correspondence
Masatsugu Moriyama
mmoriyam@med.oita-u.ac.jp

Helicobacter pylori virulence and the diversity of gastric cancer in Asia

Lam Tung Nguyen,^{1,2} Tomohisa Uchida,^{1,3} Kazunari Murakami,² Toshio Fujioka² and Masatsugu Moriyama¹

¹Department of Molecular Pathology, Faculty of Medicine, Oita University, Oita 879-5593, Japan

²Department of Gastroenterology, Faculty of Medicine, Oita University, Oita 879-5593, Japan

³Department of Human Environmental and Social Medicine, Faculty of Medicine, Oita University, Oita 879-5593, Japan

Infection with *cagPAI* positive strains of *Helicobacter pylori* is recognized as being associated with an increased risk of gastric cancer. This article reviews the current knowledge on the structures and pathological functions of *cagPAI* and the CagA protein, focusing especially on the molecular mechanism through which CagA may be involved in gastric carcinogenesis. The possible link between the geographical distribution of *cagPAI* and *cagA* variations and gastric cancer diversity in Asia is also discussed.

Introduction

Despite a worldwide trend of decline over past decades, gastric cancer remains a significant health problem in Asia (Parkin, 2001; Parkin *et al.*, 2005). The association between gastric cancer and *Helicobacter pylori* has been well-established (Forman *et al.*, 1991; Uemura *et al.*, 2001) and an estimated 77% of the world's non-cardia cancer is attributable to *H. pylori* infection (Parkin, 2006). In spite of a general consensus that this relationship exists, there is still controversy as to how *H. pylori* might contribute to the large geographical variations in the gastric cancer incidence rate within Asia, which has long been known as the 'Asian enigma' or 'Asian paradox' (Miwa *et al.*, 2002; Singh & Ghoshal, 2006).

From multiple epidemiological research studies, it is now clear that infection with CagA-producing strains of *H. pylori* further increases the risk of gastric cancer (Brenner *et al.*, 2004; Huang *et al.*, 2003). Recent studies have shed new light on the mechanism through which CagA intrudes into the host cell cytosol, dysregulates various cellular signal transduction pathways and thus may play an important role in gastric carcinogenesis (Backert *et al.*, 2000; Hatakeyama, 2004, 2006; Odenbreit *et al.*, 2000). Meanwhile, molecular epidemiological data have gradually revealed more details of the geographical distribution of *H. pylori* genetic variations (Falush *et al.*, 2003; Ghose *et al.*, 2002; Kersulyte *et al.*, 2000; Yamaoka *et al.*, 2002). In this review, we summarize new insights into the pathological activities of CagA and its possible role in gastric oncogenesis. The hypothetical relationship between *cagPAI* and CagA polymorphisms and gastric cancer diversity in Asia will also be discussed in the light of these new discoveries.

cag pathogenicity island

cagPAI is an approximately 40 kb cluster of genes that has been acquired through horizontal transmission from an unknown extraneous source and integrated into the *H. pylori* chromosome relatively recently in evolution (Censini *et al.*, 1996; Covacci *et al.*, 1999). *cagPAI* is divided into two segments, the upstream *cag II* and the downstream *cag I*, consisting of about 14 and 16 genes, respectively (Censini *et al.*, 1996; Tomb *et al.*, 1997). In many strains, an insertion sequence called IS605 is interposed between the two segments. Detailed analysis has revealed that *cagPAI* harbours seven genes homologous with *virB4*, *virB7*, *virB8*, *virB9*, *virB10*, *virB11* and *virD4*, which encode component proteins of the type IV secretion system (TFSS) in *Agrobacterium tumefaciens* (Backert & Meyer, 2006; Censini *et al.*, 1996) (Table 1).

It is well-known that *cagPAI* has at least three important pathological functions. First, it contains the *cagA*, a gene responsible for producing CagA protein, which is believed to have oncogenic potential (Hatakeyama, 2004, 2006). Second, *cagPAI* encodes TFSS, a syringe-like structure specialized in the transfer of bacterial components such as CagA protein and peptidoglycan into host cells (Backert *et al.*, 2000; Odenbreit *et al.*, 2000; Viala *et al.*, 2004). Third, *cagPAI* is implicated in the release of various inflammatory cytokines from the host cells including interleukin 8 (IL-8) (Censini *et al.*, 1996; Yamaoka *et al.*, 1997). By systematic mutagenesis, Fischer *et al.* (2001) demonstrated that 17 and 14 out of 27 examined genes in *cagPAI* are indispensable for CagA translocation and full induction of IL-8, respectively (Table 1). These findings indicate the importance of an intact *cagPAI* for the assembly of a TFSS that is fully functional for CagA delivery and stimulation of IL-8-secretion. *cagPAI* is usually subject to internal disruptions

Table 1. Contribution of the constituent genes of *cagPAI* to CagA translocation and IL-8 induction

Name of <i>cag</i> gene according to:		Involved in:*	
Tomb <i>et al.</i> (1997)	Censini <i>et al.</i> (1996)	CagA translocation	IL-8 induction
HP547	<i>cagA</i>	++	—
ND	<i>cagB</i>	ND	ND
HP546	<i>cagC</i>	++	++
HP545	<i>cagD</i>	+	+
HP544	<i>cagE</i> (<i>virB4</i>)	++	++
HP543	<i>cagF</i>	—	—
HP542	<i>cagG</i>	++	—
HP541	<i>cagH</i>	++	++
HP540	<i>cagI</i>	++	—
HP539	<i>cagL</i>	++	++
HP538	<i>cagN</i>	+	+
HP537	<i>cagM</i>	++	++
ND	<i>cagO</i>	ND	ND
HP536	<i>cagP</i>	—	—
HP535	<i>cagQ</i>	—	—
ND	<i>cagR</i>	ND	ND
HP434	<i>cagS</i>	—	—
HP532	<i>cagT</i> (<i>virB7</i>)	++	++
HP531	<i>cagU</i>	++	++
HP530	<i>cagV</i>	++	++
HP529	<i>cagW</i> (<i>virB8</i>)	++	++
HP528	<i>cagX</i> (<i>virB9</i>)	++	++
HP527	<i>cagY</i> (<i>virB10</i>)	++	++
HP526	<i>cagZ</i>	+	+
HP525	<i>cagx</i> (<i>virB11</i>)	++	++
HP524	<i>cagβ</i> (<i>virD4</i>)	++	—
HP523	<i>cagγ</i>	++	++
HP522	<i>cagδ</i>	++	++
HP521	<i>cagε</i>	—	—
HP520	<i>cagζ</i>	—	—

*Fischer *et al.* (2001).

(Kausser *et al.*, 2004), and even partial deletions within this locus may have an impact on bacterial virulence, and thus also on clinical outcome.

With regard to the role of *cagPAI* in *H. pylori*-host cell interaction, microarray transcriptional analysis has revealed that *cagPAI* is essential for altered expression of 92 % of the genes in epithelial cells infected with *H. pylori* (El-Etr *et al.*, 2004). In contrast, the *cagPAI* negative strain evokes very little transcriptional response in comparison with uninfected cells (Guillemin *et al.*, 2002). These findings once again emphasize the importance of *cagPAI* in the pathogenesis of *H. pylori*-associated diseases.

CagA protein

CagA is structurally characterized by the presence of repeated 5 amino acid sequences (Glu-Pro-Ile-Tyr-Ala),

designated EPIYA motifs, located at the C terminus of the protein (Hatakeyama, 2004, 2006; Higashi *et al.*, 2002a). Functionally, EPIYA motifs are the binding targets of many host cell proteins, especially for Src homology 2 (SH2) domain-containing tyrosine phosphatase (SHP-2) (Higashi *et al.*, 2002b; Higashi *et al.*, 2005; Selbach *et al.*, 2002; Stein *et al.*, 2000, 2002; Tsutsumi *et al.*, 2003). Four different EPIYA motifs (EPIYA-A, EPIYA-B, EPIYA-C and EPIYA-D) have been defined based on the distinctive amino acid sequences flanking each of them. From the alignment of these EPIYA motifs, two major types of CagA protein have been identified. The Western type, which represents the CagA of *H. pylori* strains prevalent in Europe, America, Australia and Africa, contains EPIYA-A and EPIYA-B, followed by up to five repeated sequences of EPIYA-C. The Eastern type, which represents the CagA of *H. pylori* strains circulating in Japan, Korea and China, also possesses EPIYA-A and EPIYA-B, but the third motif is EPIYA-D instead of EPIYA-C (Hatakeyama, 2004, 2006; Higashi *et al.*, 2002a). On rare occasions, various types of untypical combinations among the four kinds of EPIYA motif may be observed including: AB, AAB, ABB, AABC, AABCC, AAABC, AAC, ABBC, ABCABC, ABCB, ABCBCC, ABABC, AC, ACC, ACCC, AABD, AAABD, AAAD, ABBD, ABDABD, ABDDBD, ABABD, AD, BD, BBD, BABD etc. (Fig. 1).

A recent study has uncovered another important domain of CagA termed the CagA multimerization (CM) motif

Fig. 1. Classification of CagA protein. CagA is categorized based on the alignment of different EPIYA motifs. Western CagA has EPIYA-A and EPIYA-B, followed by 1–5 EPIYA-C. Eastern CagA contains EPIYA-A, EPIYA-B and 1–2 EPIYA-D. Rarely, various types of untypical arrangements among the EPIYA motifs can be observed.

(Ren *et al.*, 2006). This domain is located downstream of EPIYA-C or EPIYA-D and contains a conserved sequence of 16 amino acids. Through as yet unclear mechanisms, the CM motif plays a critical role in CagA multimerization, which is a prerequisite for subsequent formation of the CagA-SHP-2 complex, and dysregulation of the β -catenin signal (Kurashima *et al.*, 2008; Ren *et al.*, 2006).

Biological activity of CagA protein inside the host cell

Membrane-associated activities of CagA

Upon delivery into epithelial cells via the TFSS, CagA anchors to the inner surface of cell membrane (Higashi *et al.*, 2005). At these sites, it recruits and modifies the distribution of zonula occludens-1 (ZO-1) and junctional adhesion molecule (JAM), which are normally located at cell junctions. By interaction with ZO-1 and JAM, CagA alters the structure and function of the apical-junctional complex (Amieva *et al.*, 2003; Saadat *et al.*, 2007). Apical-junctional dysfunction may result in loss of control over cytoskeletal architecture, cell polarity, proliferation and differentiation, which is characteristic of oncogenic transformation (Bagnoli *et al.*, 2005; Jamora & Fuchs, 2002).

CagA is then phosphorylated by several Src family kinases (SFKs) on the tyrosine residues of all the EPIYA motifs albeit with much weaker affectivity at EPIYA-A and EPIYA-B than at EPIYA-C and EPIYA-D (Higashi *et al.*, 2002a; Naito *et al.*, 2006; Selbach *et al.*, 2002; Stein *et al.*, 2002). This is a very important process since nearly 80 % of host cell transcriptional changes elicited by CagA are phosphorylation dependent (El-Etr *et al.*, 2004). Moreover, the number of EPIYA motifs is proportional to the phosphorylation level and biological activity of CagA protein, and accordingly is associated with gastric cancer (Argent *et al.*, 2004; Naito *et al.*, 2006).

Phosphorylation-dependent activities of CagA

After phosphorylation, the phosphorylated CagA binds to SHP-2 via the EPIYA-C or EPIYA-D motif (Higashi *et al.*, 2002a, b). Both the N-SH2 and C-SH2 domains of SHP-2 are required for stable complex formation with CagA, signifying the importance of the preceding CagA multimerization mediated by the CM motif (Higashi *et al.*, 2002a; Ren *et al.*, 2006). SHP-2-CagA interaction correlates not only with the number of phosphorylated sites but also with CagA types. In this regard, Eastern CagA has strong affinity for SHP-2 due to a 6 amino acid sequence overlying EPIYA-D (Y-A-T-I-D-F), which is identical to the consensus ligand binding motif for the SH-2 domain of SHP-2 (De Souza *et al.*, 2002). In contrast, the corresponding sequence surrounding EPIYA-C in Western CagA (Y-A-T-I-D-D) differs from the consensus motif in 1 terminal amino acid (D versus F/W) and thus exhibits a weaker binding ability (Higashi *et al.*, 2002a).

Activated by phosphorylated CagA, SHP-2 inhibits focal adhesion kinase (FAK), an enzyme that modulates cell

adhesion, migration and survival (Parsons, 2003; Tsutsumi *et al.*, 2006). A decline in FAK activity triggers a cytoskeletal rearrangement phenomenon called the 'hummingbird phenotype', which is characterized by the elongation and spreading of the host cells (Backert *et al.*, 2001; Segal *et al.*, 1999; Tsutsumi *et al.*, 2006). CagA-activated SHP-2 also stimulates extracellular signal-regulated kinase (Erk) through both Ras-dependent and -independent pathways (Higashi *et al.*, 2004; Neel *et al.*, 2003). Corresponding to its stronger binding affinity for SHP-2, Eastern CagA is reported to exhibit a greater ability to induce the hummingbird phenotype and to have more influence on Erk activity as well as cell growth than the Western type (Fu *et al.*, 2007; Higashi *et al.*, 2002a). Therefore, Eastern CagA is considered to be pathologically more virulent than its Western counterpart (Hatakeyama, 2004, 2006).

Although the majority of phosphorylated CagA binds to SHP-2, a smaller proportion interacts with the C-terminal Src kinase (CSK) (Tsutsumi *et al.*, 2003). Activation of CSK by interaction with CagA in turn inhibits SFKs. Therefore, together with direct suppression of SFKs by phosphorylated CagA (Selbach *et al.*, 2003), the indirect inhibitory pathway via CSK forms dual feedback loops that down-regulate SFK phosphorylation activity. As a result, these two negative feedback loops may mitigate the phosphorylation-related cytotoxicity of CagA. Additionally, CagA-mediated inhibition of SFKs favours the dephosphorylation of other host cell proteins such as vinculin, ezrin and cortactin, resulting in impaired adhesion of host cells to extracellular matrix and disrupted cellular actin organization, which partially contributes to the hummingbird phenotype (Moese *et al.*, 2007; Selbach *et al.*, 2003, 2004) (Fig. 2).

Phosphorylation-independent activities of CagA

Cag A is able to dysregulate several other signalling cascades in a phosphorylation-independent manner. CagA interacts with hepatocyte growth factor (HGF) receptor c-Met, thereby stimulating cell growth, motility and invasiveness (Churin *et al.*, 2003). CagA binds to growth factor receptor-bound protein 2 (Grb-2) and activates the Ras/Erk pathway via son of sevenless (SOS) leading to cell scattering and proliferation. The interaction between CagA and Grb-2 relies strictly on the presence of EPIYA motifs but does not require tyrosine phosphorylation (Mimuro *et al.*, 2002). Notably, both c-Met and Grb-2 have been implicated in malignant transformation (Cheng *et al.*, 1998; Peruzzi & Bottaro, 2006).

Recently, Murata-Kamiya *et al.* (2007) found that by interacting with E-cadherin, CagA disturbs the E-cadherin/ β -catenin complex, leading to cytoplasmic and nuclear accumulation of β -catenin. This is consistent with a previous study in which β -catenin nuclear translocation in gastric mucosa infected with cagPAI positive *H. pylori* was shown to increase in a CagA-dependent manner

Fig. 2. Biological activity of CagA inside the host cell. After translocation into the host cell via the TFSS, CagA is able to dysregulate several cellular signalling cascades. In the phosphorylation-dependent pathway, CagA is phosphorylated by SFKs at EPIYA sites. The phosphorylated CagA then binds to and activates SHP-2, which in turn inhibits FAK and stimulates Erk. Via dual negative feedback loops, phosphorylated CagA downregulates the activity of SFKs directly, and also through activation of CSK. The decline of the activity of SFKs accelerates the dephosphorylation of vinculin, ezrin and cortactin. In the phosphorylation-independent pathway, CagA binds to Grb-2 and activates the Ras/Erk/NF- κ B/IL-8 signalling cascade via SOS. The E-cadherin/ β -catenin complex is disturbed by CagA leading to cytoplasmic and nuclear accumulation of β -catenin. CagA also associates with c-Met, a receptor of HGF. Interactions of CagA with JAM and ZO-1 result in the disruption of tight junctions. The enhanced dephosphorylation of vinculin, ezrin and cortactin, reduced activity of FAK, activation of Erk and c-Met, and disruption of tight junctions all contribute to changes in cell morphology, motility and invasiveness, which include the hummingbird phenotype.

(Franco *et al.*, 2005). Deregulated β -catenin signalling is believed to drive transcriptional upregulation of genes implicated in oncogenic transformation (Fodde & Brabletz, 2007; Hoppler & Kavanagh, 2007). Furthermore, strong nuclear and cytoplasmic expression of β -catenin in various types of gastric neoplasm has also been reported (Tsukashita *et al.*, 2003). These findings raise the possibility that CagA causes aberrant expression of β -catenin, which in turn may be involved in gastric tumorigenesis.

Formerly, CagA was thought to have no role in the induction of pro-inflammatory responses such as IL-8 production based on observations that *cagA*-knockout strains still retain a considerable ability to induce IL-8 production in gastric epithelial cells (Censini *et al.*, 1996; Fischer *et al.*, 2001). However, this general concept was challenged by Brandt *et al.* (2005) who demonstrated that CagA indeed induced IL-8 release via the Ras/Raf/Mek/Erk/

NF- κ B signalling pathway independently of SHP-2 and c-Met. These findings prompted a not-yet-proven assumption that CagA-Grb-2 interaction, which was reported to activate the Ras/Erk pathway, might be an upstream event of CagA-induced NF- κ B stimulation. The explicit involvement of CagA in the NF- κ B/IL-8-activating pathway signifies its direct role in the chronic mucosal inflammation that precedes, and is thought to be linked with, cancer (Balkwill & Mantovani, 2001; Coussens & Werb, 2002; Karin, 2006).

Recently, Matsumoto *et al.* (2007) discovered that activation-induced cytidine deaminase (AID) was upregulated via the NF- κ B-dependent pathway in gastric epithelial cells infected with *cagPAI* positive *H. pylori*. Aberrant expression of AID, a DNA- and RNA-editing enzyme, induces cumulative mutations of somatic genes such as p53 inside host cells. Although not directly implicated, CagA

may have some relationship to that mechanism for a number of reasons. Firstly, CagA is involved in the majority of *cagPAI*-dependent activities (El-Etr *et al.*, 2004). Secondly, CagA itself is able to activate NF- κ B (Brandt *et al.*, 2005). Finally, a *cagE* knockout mutation strain, which failed to translocate CagA into epithelial cells, reportedly had no effect on p53 mutation, as was observed in the *cagPAI* negative strain (Matsumoto *et al.*, 2007).

cagPAI, CagA and gastric cancer diversity in Asia

Gastric cancer is still one of the most common malignant diseases. Overall, the estimated worldwide incidence of gastric cancer in 2002 was 934 000 cases, ranking this cancer fourth behind cancers of the lung, breast and colorectum. Gastric cancer mortality accounted for about 10.4 % of all cancer deaths, with 700 000 cases annually, the second highest rate after only lung cancer (Parkin *et al.*, 2005). About 56 % of newly diagnosed gastric cancers arise in East Asia, of which 42 % are reported from China and 12 % from Japan [GLOBOCAN 2002: Cancer Incidence, Mortality and Prevalence Worldwide, version 2.0, CancerBase no 5 (<http://www-dep.iarc.fr/>); International Agency for Research on Cancer]. In Asia, gastric cancer distribution is geographically characterized by a wide variation, the difference in the incidence rate between regions of high and low prevalence reaching up to 40-fold (GLOBOCAN 2002). Based on the age-standardized incidence rate of gastric cancer, Asian countries can be categorized as high risk (Japan, Korea, China), intermediate risk (Vietnam, Singapore) or low risk (Thailand, India, Bangladesh etc.) (Fig. 3).

One intriguing phenomenon is the 'Asian paradox', whereby the *H. pylori* infection rate among the general population is very high in areas with little risk of gastric cancer such as South-Central Asia (India, Pakistan, Bangladesh) and South-Eastern Asia (Thailand,

Indonesia), but lower in Eastern Asia, where the gastric cancer rate is the highest in the world (Japan, Korea) (Parkin, 2004, 2006). Thus, despite the undeniable role of *H. pylori* in gastric cancer, this paradox suggests that any explanation should not focus only on bacterial prevalence. As a multifactorial model of gastric malignant transformation is currently accepted, other aspects such as bacterial virulence, host genetic susceptibility and diet should also be taken into consideration. In this article we analyse the possible links between *cagPAI* disruption, the CagA genotype and the diversity of gastric cancer in Asia.

cagPAI intactness is thought to be an important determinant of *H. pylori* virulence and clinical outcomes. Nilsson *et al.* (2003) argued that infection with *H. pylori* containing a complete *cagPAI* conferred a fivefold increased risk of developing severe gastroduodenal diseases. An intact *cagPAI* has been detected in 86 % of strains isolated from gastric cancer versus 7 % of those from non-ulcer dyspepsia (Ali *et al.*, 2005). Meanwhile, precancerous lesions have been frequently found in the stomachs colonized by strains with the full *cagPAI* but rarely in those harbouring strains that lack parts of *cagPAI* (Ali *et al.*, 2005). A study in Mongolian gerbils has shown that intact *cagPAI* facilitates *H. pylori* colonization in the body (corpus) of the stomach, resulting in atrophic corpus-predominant gastritis, a well-known precancerous condition (Rieder *et al.*, 2005). Consequently, enhanced carcinoma risk has been observed in 71 % of animals inoculated with an intact *cagPAI* strain compared to 9 % of those infected with a partially deleted *cagPAI* strain (Rieder *et al.*, 2005). In humans, intact *cagPAI* has been detected in 95 % of isolates from gastric ulcers and gastric cancer, both characterized by corpus-predominant gastritis, versus only 6.9 % of those from duodenal ulcer, which is characterized by antrum-predominant gastritis and reduced risk of gastric cancer. The authors concluded that in comparison with partially deleted *cagPAI* strains, those with intact *cagPAI* would

Fig. 3. Age-standardized incidence rate of gastric cancer in selected Asian countries. Data shown as cases per 100 000 by sex (GLOBOCAN 2002).

increase the risk of gastric carcinoma 10-fold in infected subjects (Ali *et al.*, 2005). In Japan, where the gastric cancer incidence rate is high, *cagPAI* is very well-conserved with nearly all *H. pylori* strains carrying an intact island (Ikenoue *et al.*, 2001). Conversely, in India, where the gastric cancer incidence rate is about 10 times lower, 63–87 % of strains have a *cagPAI* that is partially deleted (Ali *et al.*, 2005; Kauser *et al.*, 2004). Surprisingly, the deletions usually occur in the promoter region of *cagA*, which would lead to abolition of gene transcription as reported by Maeda *et al.* (1999). Thus, although Indian *H. pylori* strains often accommodate *cagA* (Ali *et al.*, 2005; Chattopadhyay *et al.*, 2002; Chaudhuri *et al.*, 2003; Datta *et al.*, 2003), many of them might not produce CagA protein and could be regarded as functionally *cagA* negative. Similarly, disrupted *cagPAI* and deletion of the promoter region of *cagA* are also very common in *H. pylori* isolates from Turkey (Salih *et al.*, 2007), possibly corresponding to the low gastric cancer rate there. Taken together, it can be speculated that the differences in the prevalence of intact *cagPAI* strains contribute partly to gastric cancer diversity in Asia.

As discussed above, Eastern CagA is considered more virulent and thus associated with a more severe clinical outcome than its Western counterpart. Azuma *et al.* (2004) demonstrated that in both the antrum and body of the stomach, the grades of inflammation, activity and mucosal atrophy were significantly higher in chronic gastritis patients infected with Eastern *cagA* positive strains than in those infected with Western *cagA* positive strains. As expected, the relationship between Eastern CagA, and gastric cancer was also found to be stronger. In Okinawa, where both genotypes of *cagA* are present, 85 % of *H. pylori* isolates from gastric cancer patients carried Eastern *cagA* whereas only 15 % carried the Western genotype (Satomi *et al.*, 2006). The same authors noted that Eastern *cagA* strains were considerably more prevalent in a region with a high gastric cancer rate (Fukui prefecture) than in a region with a lower rate (Okinawa prefecture) (Azuma *et al.*, 2004; Satomi *et al.*, 2006). A similar result was also obtained by Vilaichone *et al.* (2004) who reported that 85 % of *H. pylori* isolates from ethnic Chinese people living in Thailand harboured Eastern *cagA*, whereas only 18 % of those isolates from indigenous Thais carried such a gene. Accordingly, gastric cancer was found to be common among Chinese Thais but rare among the indigenous Thais (Vilaichone *et al.*, 2004). From these studies, it is tempting to speculate that the prevalence of Eastern *cagA* strains may partly correlate with gastric cancer risk. A growing body of molecular epidemiological data shows that in Korea, China and Japan (except Okinawa) almost all *H. pylori* strains have Eastern *cagA* (Azuma *et al.*, 2004; Choi *et al.*, 2007; Kanada *et al.*, 2008; Uchida *et al.*, 2007; Yamaoka *et al.*, 2002; Zhou *et al.*, 2004a, b), corresponding well to the high incidence rate of gastric cancer in this region. In contrast, the fact that nearly all *H. pylori* isolates from India, Pakistan and Turkey bear Western *cagA* is likely to reflect

the reduced risk of gastric cancer in these populations (Mukhopadhyay *et al.*, 2000; Saribasak *et al.*, 2004; Yamaoka *et al.*, 2002). Therefore, the striking difference in the geographical distribution of *cagA* genotypes may partially contribute to the wide variation of gastric cancer among the countries, and to a larger extent, among Asian countries.

It should be noted that other virulence factors of *H. pylori* may also account for some of the differences in gastric cancer incidence. For example, variations in the signal region(s) (which may be type s1 or s2), and the middle region (m) (which may be type m1 or m2) of the vacuolating cytotoxin-encoding gene (*vacA*) have been shown to correlate with *H. pylori* cytotoxin activity (Atherton *et al.*, 1995, 1997). In this respect, *vacA* s1/m1 is more toxic than *vacA* s1/m2 and *vacA* s2/m2, and has been found to be strongly associated with gastric cancer (Kidd *et al.*, 1999; Miehlke *et al.*, 2000). In East Asia (Japan, Korea), nearly all *H. pylori* strains carry *vacA* s1/m1 while in countries with a low incidence of gastric cancer, such as Thailand and Turkey, this virulence genotype is carried in less than 60 % of strains (Chomvarin *et al.*, 2008; Erzin *et al.*, 2006; Yamaoka *et al.*, 2002). Recently, a new *H. pylori* vacuolating cytotoxin determinant has been described and termed the intermediate region (i). Two i regions have been identified, i1 and i2, and the i1 region has been shown to be associated with gastric cancer (Basso *et al.*, 2008; Rhead *et al.*, 2007). Interestingly, the great majority of *H. pylori* isolates in East Asia have *vacA* i1 (Ogiwara *et al.*, 2008). Therefore, the combination of many virulence factors including an intact *cagPAI*, Eastern *cagA* and *vacA* s1/m1/i1 in most of the *H. pylori* strains isolated from East Asia might be an important contributor to the high incidence of gastric cancer in this region.

Concluding remarks

New data suggest that previous studies using a single serological method to detect *H. pylori* infection might have considerably underestimated the relationship between this bacterium and gastric cancer. Ekstrom *et al.* (2001) demonstrated that the adjusted odds ratio (OR) for non-cardia gastric cancer among *H. pylori*-positive subjects increased from 2.2 to 21 when immunoblot analysis with anti-CagA antibody was added to the conventional ELISA method. Another population-based study, in which both ELISA and Western blotting were used, has shown that the OR for non-cardia gastric cancer rose from 3.7 to 18.3 for any *H. pylori* infection and from 5.7 to 28.4 for *cagA* positive *H. pylori* infection (Brenner *et al.*, 2004). These results suggest that the correlation between *H. pylori* infection and gastric cancer might be changed if additional factors were to be integrated into a large population-based study. Accordingly, the *H. pylori*–gastric cancer relationship should be reinvestigated in the context of *cagPAI* rearrangement and *cagA* polymorphisms because serological positivity for *H. pylori* alone does not fully reflect the

true risk of gastric cancer. With more comprehensive epidemiological data on the trinity of host genetic susceptibility, lifestyle and bacterial virulence factors, the Asian paradox may prove to be not as enigmatic as it seems.

References

- Ali, M., Khan, A. A., Tiwari, S. K., Ahmed, N., Rao, L. V. & Habibullah, C. M. (2005). Association between *cag*-pathogenicity island in *Helicobacter pylori* isolates from peptic ulcer, gastric carcinoma, and non-ulcer dyspepsia subjects with histological changes. *World J Gastroenterol* **11**, 6815–6822.
- Amieva, M. R., Vogelmann, R., Covacci, A., Tompkins, L. S., Nelson, W. J. & Falkow, S. (2003). Disruption of the epithelial apical-junctional complex by *Helicobacter pylori* CagA. *Science* **300**, 1430–1434.
- Argent, R. H., Kidd, M., Owen, R. J., Thomas, R. J., Limb, M. C. & Atherton, J. C. (2004). Determinants and consequences of different levels of CagA phosphorylation for clinical isolates of *Helicobacter pylori*. *Gastroenterology* **127**, 514–523.
- Atherton, J. C., Cao, P., Peek, R. M., Jr, Tummuru, M. K., Blaser, M. J. & Cover, T. L. (1995). Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. Association of specific *vacA* types with cytotoxin production and peptic ulceration. *J Biol Chem* **270**, 17771–17777.
- Atherton, J. C., Peek, R. M., Jr, Tham, K. T., Cover, T. L. & Blaser, M. J. (1997). Clinical and pathological importance of heterogeneity in *vacA*, the vacuolating cytotoxin gene of *Helicobacter pylori*. *Gastroenterology* **112**, 92–99.
- Azuma, T., Yamazaki, S., Yamakawa, A., Ohtani, M., Muramatsu, A., Suto, H., Ito, Y., Dojo, M., Yamazaki, Y. & other authors (2004). Association between diversity in the Src homology 2 domain – containing tyrosine phosphatase binding site of *Helicobacter pylori* CagA protein and gastric atrophy and cancer. *J Infect Dis* **189**, 820–827.
- Backert, S. & Meyer, T. F. (2006). Type IV secretion systems and their effectors in bacterial pathogenesis. *Curr Opin Microbiol* **9**, 207–217.
- Backert, S., Ziska, E., Brinkmann, V., Zimny-Arndt, U., Fauconier, A., Jungblut, P. R., Naumann, M. & Meyer, T. F. (2000). Translocation of the *Helicobacter pylori* CagA protein in gastric epithelial cells by a type IV secretion apparatus. *Cell Microbiol* **2**, 155–164.
- Backert, S., Moese, S., Selbach, M., Brinkmann, V. & Meyer, T. F. (2001). Phosphorylation of tyrosine 972 of the *Helicobacter pylori* CagA protein is essential for induction of a scattering phenotype in gastric epithelial cells. *Mol Microbiol* **42**, 631–644.
- Bagnoli, F., Buti, L., Tompkins, L., Covacci, A. & Amieva, M. R. (2005). *Helicobacter pylori* CagA induces a transition from polarized to invasive phenotypes in MDCK cells. *Proc Natl Acad Sci U S A* **102**, 16339–16344.
- Balkwill, F. & Mantovani, A. (2001). Inflammation and cancer: back to Virchow? *Lancet* **357**, 539–545.
- Basso, D., Zambon, C. F., Letley, D. P., Stranges, A., Marchet, A., Rhead, J. L., Schiavon, S., Guariso, G., Ceroti, M. & other authors (2008). Clinical relevance of *Helicobacter pylori* *cagA* and *vacA* gene polymorphisms. *Gastroenterology* **135**, 91–99.
- Brandt, S., Kwok, T., Hartig, R., Konig, W. & Backert, S. (2005). NF- κ B activation and potentiation of proinflammatory responses by the *Helicobacter pylori* CagA protein. *Proc Natl Acad Sci U S A* **102**, 9300–9305.
- Brenner, H., Arndt, V., Stegmaier, C., Ziegler, H. & Rothenbacher, D. (2004). Is *Helicobacter pylori* infection a necessary condition for noncardia gastric cancer? *Am J Epidemiol* **159**, 252–258.
- Censini, S., Lange, C., Xiang, Z., Crabtree, J. E., Ghiara, P., Borodovsky, M., Rappuoli, R. & Covacci, A. (1996). *cag*, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. *Proc Natl Acad Sci U S A* **93**, 14648–14653.
- Chattopadhyay, S., Datta, S., Chowdhury, A., Chowdhury, S., Mukhopadhyay, A. K., Rajendran, K., Bhattacharya, S. K., Berg, D. E. & Nair, G. B. (2002). Virulence genes in *Helicobacter pylori* strains from West Bengal residents with overt *H. pylori*-associated disease and healthy volunteers. *J Clin Microbiol* **40**, 2622–2625.
- Chaudhuri, S., Chowdhury, A., Datta, S., Mukhopadhyay, A. K., Chattopadhyay, S., Saha, D. R., Dhali, G., Santra, A., Nair, G. B. & other authors (2003). Anti-*Helicobacter pylori* therapy in India: differences in eradication efficiency associated with particular alleles of vacuolating cytotoxin (*vacA*) gene. *J Gastroenterol Hepatol* **18**, 190–195.
- Cheng, A. M., Saxton, T. M., Sakai, R., Kulkarni, S., Mbamalu, G., Vogel, W., Tortorice, C. G., Cardiff, R. D., Cross, J. C. & other authors (1998). Mammalian Grb2 regulates multiple steps in embryonic development and malignant transformation. *Cell* **95**, 793–803.
- Choi, K. D., Kim, N., Lee, D. H., Kim, J. M., Kim, J. S., Jung, H. C. & Song, I. S. (2007). Analysis of the 3' variable region of the *cagA* gene of *Helicobacter pylori* isolated in Koreans. *Dig Dis Sci* **52**, 960–966.
- Chomvarin, C., Namwat, W., Chaicumpar, K., Mairiang, P., Sangchan, A., Sripa, B., Tor-Udom, S. & Vilaichone, R. K. (2008). Prevalence of *Helicobacter pylori* *vacA*, *cagA*, *cagE*, *iceA* and *babA2* genotypes in Thai dyspeptic patients. *Int J Infect Dis* **12**, 30–36.
- Churin, Y., Al-Ghoul, L., Kepp, O., Meyer, T. F., Birchmeier, W. & Naumann, M. (2003). *Helicobacter pylori* CagA protein targets the c-Met receptor and enhances the motogenic response. *J Cell Biol* **161**, 249–255.
- Coussens, L. M. & Werb, Z. (2002). Inflammation and cancer. *Nature* **420**, 860–867.
- Covacci, A., Telford, J. L., Del Giudice, G., Parsonnet, J. & Rappuoli, R. (1999). *Helicobacter pylori* virulence and genetic geography. *Science* **284**, 1328–1333.
- Datta, S., Chattopadhyay, S., Balakrish Nair, G., Mukhopadhyay, A. K., Hembram, J., Berg, D. E., Rani Saha, D., Khan, A., Santra, A. & other authors (2003). Virulence genes and neutral DNA markers of *Helicobacter pylori* isolates from different ethnic communities of West Bengal, India. *J Clin Microbiol* **41**, 3737–3743.
- De Souza, D., Fabri, L. J., Nash, A., Hilton, D. J., Nicola, N. A. & Baca, M. (2002). SH2 domains from suppressor of cytokine signaling-3 and protein tyrosine phosphatase SHP-2 have similar binding specificities. *Biochemistry* **41**, 9229–9236.
- Ekstrom, A. M., Held, M., Hansson, L. E., Engstrand, L. & Nyren, O. (2001). *Helicobacter pylori* in gastric cancer established by CagA immunoblot as a marker of past infection. *Gastroenterology* **121**, 784–791.
- El-Etr, S. H., Mueller, A., Tompkins, L. S., Falkow, S. & Merrell, D. S. (2004). Phosphorylation-independent effects of CagA during interaction between *Helicobacter pylori* and T84 polarized monolayers. *J Infect Dis* **190**, 1516–1523.
- Erzin, Y., Koksai, V., Altun, S., Dobrucali, A., Aslan, M., Erdamar, S., Dirican, A. & Kocazeybek, B. (2006). Prevalence of *Helicobacter pylori* *vacA*, *cagA*, *cagE*, *iceA*, *babA2* genotypes and correlation with clinical outcome in Turkish patients with dyspepsia. *Helicobacter* **11**, 574–580.
- Falush, D., Wirth, T., Linz, B., Pritchard, J. K., Stephens, M., Kidd, M., Blaser, M. J., Graham, D. Y., Vacher, S. & other authors (2003). Traces of human migrations in *Helicobacter pylori* populations. *Science* **299**, 1582–1585.

- Fischer, W., Puls, J., Buhrdorf, R., Gebert, B., Odenbreit, S. & Haas, R. (2001). Systematic mutagenesis of the *Helicobacter pylori* cag pathogenicity island: essential genes for CagA translocation in host cells and induction of interleukin-8. *Mol Microbiol* **42**, 1337–1348.
- Fodde, R. & Brabletz, T. (2007). Wnt/ β -catenin signaling in cancer stemness and malignant behavior. *Curr Opin Cell Biol* **19**, 150–158.
- Forman, D., Newell, D. G., Fullerton, F., Yarnell, J. W., Stacey, A. R., Wald, N. & Sitas, F. (1991). Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigation. *BMJ* **302**, 1302–1305.
- Franco, A. T., Israel, D. A., Washington, M. K., Krishna, U., Fox, J. G., Rogers, A. B., Neish, A. S., Collier-Hyams, L., Perez-Perez, G. I. & other authors (2005). Activation of β -catenin by carcinogenic *Helicobacter pylori*. *Proc Natl Acad Sci U S A* **102**, 10646–10651.
- Fu, H. Y., Asahi, K., Hayashi, Y., Eguchi, H., Murata, H., Tsujii, M., Tsuji, S., Azuma, T. & Kawano, S. (2007). East Asian-type *Helicobacter pylori* cytotoxin-associated gene A protein has a more significant effect on growth of rat gastric mucosal cells than the Western type. *J Gastroenterol Hepatol* **22**, 355–362.
- Ghose, C., Perez-Perez, G. I., Dominguez-Bello, M. G., Pride, D. T., Bravi, C. M. & Blaser, M. J. (2002). East Asian genotypes of *Helicobacter pylori* strains in Amerindians provide evidence for its ancient human carriage. *Proc Natl Acad Sci U S A* **99**, 15107–15111.
- Guillemin, K., Salama, N. R., Tompkins, L. S. & Falkow, S. (2002). cag pathogenicity island-specific responses of gastric epithelial cells to *Helicobacter pylori* infection. *Proc Natl Acad Sci U S A* **99**, 15136–15141.
- Hatakeyama, M. (2004). Oncogenic mechanisms of the *Helicobacter pylori* CagA protein. *Nat Rev Cancer* **4**, 688–694.
- Hatakeyama, M. (2006). *Helicobacter pylori* CagA – a bacterial intruder conspiring gastric carcinogenesis. *Int J Cancer* **119**, 1217–1223.
- Higashi, H., Tsutsumi, R., Fujita, A., Yamazaki, S., Asaka, M., Azuma, T. & Hatakeyama, M. (2002a). Biological activity of the *Helicobacter pylori* virulence factor CagA is determined by variation in the tyrosine phosphorylation sites. *Proc Natl Acad Sci U S A* **99**, 14428–14433.
- Higashi, H., Tsutsumi, R., Muto, S., Sugiyama, T., Azuma, T., Asaka, M. & Hatakeyama, M. (2002b). SHP-2 tyrosine phosphatase as an intracellular target of *Helicobacter pylori* CagA protein. *Science* **295**, 683–686.
- Higashi, H., Nakaya, A., Tsutsumi, R., Yokoyama, K., Fujii, Y., Ishikawa, S., Higuchi, M., Takahashi, A., Kurashima, Y. & other authors (2004). *Helicobacter pylori* CagA induces Ras-independent morphogenetic response through SHP-2 recruitment and activation. *J Biol Chem* **279**, 17205–17216.
- Higashi, H., Yokoyama, K., Fujii, Y., Ren, S., Yuasa, H., Saadat, I., Murata-Kamiya, N., Azuma, T. & Hatakeyama, M. (2005). EPIYA motif is a membrane-targeting signal of *Helicobacter pylori* virulence factor CagA in mammalian cells. *J Biol Chem* **280**, 23130–23137.
- Hoppler, S. & Kavanagh, C. L. (2007). Wnt signalling: variety at the core. *J Cell Sci* **120**, 385–393.
- Huang, J. Q., Zheng, G. F., Sumanac, K., Irvine, E. J. & Hunt, R. H. (2003). Meta-analysis of the relationship between cagA seropositivity and gastric cancer. *Gastroenterology* **125**, 1636–1644.
- Ikenoue, T., Maeda, S., Ogura, K., Akanuma, M., Mitsuno, Y., Imai, Y., Yoshida, H., Shiratori, Y. & Omata, M. (2001). Determination of *Helicobacter pylori* virulence by simple gene analysis of the cag pathogenicity island. *Clin Diagn Lab Immunol* **8**, 181–186.
- Jamora, C. & Fuchs, E. (2002). Intercellular adhesion, signalling and the cytoskeleton. *Nat Cell Biol* **4**, E101–E108.
- Kanada, R., Uchida, T., Tsukamoto, Y., Nguyen, L. T., Hijiya, N., Matsuura, K., Kodama, M., Okimoto, T., Murakami, K. & other authors (2008). Genotyping of the cagA gene of *Helicobacter pylori* on immunohistochemistry with East Asian CagA-specific antibody. *Pathol Int* **58**, 218–225.
- Karin, M. (2006). Nuclear factor- κ B in cancer development and progression. *Nature* **441**, 431–436.
- Kausar, F., Khan, A. A., Hussain, M. A., Carroll, I. M., Ahmad, N., Tiwari, S., Shouche, Y., Das, B., Alam, M. & other authors (2004). The cag pathogenicity island of *Helicobacter pylori* is disrupted in the majority of patient isolates from different human populations. *J Clin Microbiol* **42**, 5302–5308.
- Kersulyte, D., Mukhopadhyay, A. K., Velapatino, B., Su, W., Pan, Z., Garcia, C., Hernandez, V., Valdez, Y., Mistry, R. S. & other authors (2000). Differences in genotypes of *Helicobacter pylori* from different human populations. *J Bacteriol* **182**, 3210–3218.
- Kidd, M., Lastovica, A. J., Atherton, J. C. & Louw, J. A. (1999). Heterogeneity in the *Helicobacter pylori* vacA and cagA genes: association with gastroduodenal disease in South Africa? *Gut* **45**, 499–502.
- Kurashima, Y., Murata-Kamiya, N., Kikuchi, K., Higashi, H., Azuma, T., Kondo, S. & Hatakeyama, M. (2008). Deregulation of β -catenin signal by *Helicobacter pylori* CagA requires the CagA-multimerization sequence. *Int J Cancer* **122**, 823–831.
- Maeda, S., Yoshida, H., Ikenoue, T., Ogura, K., Kanai, F., Kato, N., Shiratori, Y. & Omata, M. (1999). Structure of cag pathogenicity island in Japanese *Helicobacter pylori* isolates. *Gut* **44**, 336–341.
- Matsumoto, Y., Marusawa, H., Kinoshita, K., Endo, Y., Kou, T., Morisawa, T., Azuma, T., Okazaki, I. M., Honjo, T. & other authors (2007). *Helicobacter pylori* infection triggers aberrant expression of activation-induced cytidine deaminase in gastric epithelium. *Nat Med* **13**, 470–476.
- Miehke, S., Kirsch, C., Agha-Amiri, K., Gunther, T., Lehn, N., Malfertheiner, P., Stolte, M., Ehninger, G. & Bayerdorffer, E. (2000). The *Helicobacter pylori* vacA s1, m1 genotype and cagA is associated with gastric carcinoma in Germany. *Int J Cancer* **87**, 322–327.
- Mimuro, H., Suzuki, T., Tanaka, J., Asahi, M., Haas, R. & Sasakawa, C. (2002). Grb2 is a key mediator of *Helicobacter pylori* CagA protein activities. *Mol Cell* **10**, 745–755.
- Miwa, H., Go, M. F. & Sato, N. (2002). *H. pylori* and gastric cancer: the Asian enigma. *Am J Gastroenterol* **97**, 1106–1112.
- Moese, S., Selbach, M., Brinkmann, V., Karlas, A., Haimovich, B., Backert, S. & Meyer, T. F. (2007). The *Helicobacter pylori* CagA protein disrupts matrix adhesion of gastric epithelial cells by dephosphorylation of vinculin. *Cell Microbiol* **9**, 1148–1161.
- Mukhopadhyay, A. K., Kersulyte, D., Jeong, J. Y., Datta, S., Ito, Y., Chowdhury, A., Chowdhury, S., Santra, A., Bhattacharya, S. K. & other authors (2000). Distinctiveness of genotypes of *Helicobacter pylori* in Calcutta, India. *J Bacteriol* **182**, 3219–3227.
- Murata-Kamiya, N., Kurashima, Y., Teishikata, Y., Yamahashi, Y., Saito, Y., Higashi, H., Aburatani, H., Akiyama, T., Peek, R. M., Jr & other authors (2007). *Helicobacter pylori* CagA interacts with E-cadherin and deregulates the β -catenin signal that promotes intestinal transdifferentiation in gastric epithelial cells. *Oncogene* **26**, 4617–4626.
- Naito, M., Yamazaki, T., Tsutsumi, R., Higashi, H., Onoe, K., Yamazaki, S., Azuma, T. & Hatakeyama, M. (2006). Influence of EPIYA-repeat polymorphism on the phosphorylation-dependent biological activity of *Helicobacter pylori* CagA. *Gastroenterology* **130**, 1181–1190.

- Neel, B. G., Gu, H. & Pao, L. (2003). The 'Shp'ing news: SH2 domain-containing tyrosine phosphatases in cell signaling. *Trends Biochem Sci* **28**, 284–293.
- Nilsson, C., Sillen, A., Eriksson, L., Strand, M. L., Enroth, H., Normark, S., Falk, P. & Engstrand, L. (2003). Correlation between *cag* pathogenicity island composition and *Helicobacter pylori*-associated gastroduodenal disease. *Infect Immun* **71**, 6573–6581.
- Odenbreit, S., Puls, J., Sedlmaier, B., Gerland, E., Fischer, W. & Haas, R. (2000). Translocation of *Helicobacter pylori* CagA into gastric epithelial cells by type IV secretion. *Science* **287**, 1497–1500.
- Ogiwara, H., Graham, D. Y. & Yamaoka, Y. (2008). *vacA* i-region subtyping. *Gastroenterology* **134**, 1267.
- Parkin, D. M. (2001). Global cancer statistics in the year 2000. *Lancet Oncol* **2**, 533–543.
- Parkin, D. M. (2004). International variation. *Oncogene* **23**, 6329–6340.
- Parkin, D. M. (2006). The global health burden of infection-associated cancers in the year 2002. *Int J Cancer* **118**, 3030–3044.
- Parkin, D. M., Bray, F., Ferlay, J. & Pisani, P. (2005). Global cancer statistics, 2002. *CA Cancer J Clin* **55**, 74–108.
- Parsons, J. T. (2003). Focal adhesion kinase: the first ten years. *J Cell Sci* **116**, 1409–1416.
- Peruzzi, B. & Bottaro, D. P. (2006). Targeting the c-Met signaling pathway in cancer. *Clin Cancer Res* **12**, 3657–3660.
- Ren, S., Higashi, H., Lu, H., Azuma, T. & Hatakeyama, M. (2006). Structural basis and functional consequence of *Helicobacter pylori* CagA multimerization in cells. *J Biol Chem* **281**, 32344–32352.
- Rhead, J. L., Letley, D. P., Mohammadi, M., Hussein, N., Mohagheghi, M. A., Eshagh Hosseini, M. & Atherton, J. C. (2007). A new *Helicobacter pylori* vacuolating cytotoxin determinant, the intermediate region, is associated with gastric cancer. *Gastroenterology* **133**, 926–936.
- Rieder, G., Merchant, J. L. & Haas, R. (2005). *Helicobacter pylori* Cag-type IV secretion system facilitates corpus colonization to induce precancerous conditions in Mongolian gerbils. *Gastroenterology* **128**, 1229–1242.
- Saadat, I., Higashi, H., Obuse, C., Umeda, M., Murata-Kamiya, N., Saito, Y., Lu, H., Ohnishi, N., Azuma, T. & other authors (2007). *Helicobacter pylori* CagA targets PAR1/MARK kinase to disrupt epithelial cell polarity. *Nature* **447**, 330–333.
- Salih, B. A., Abasiyanik, M. F. & Ahmed, N. (2007). A preliminary study on the genetic profile of *cag* pathogenicity-island and other virulent gene loci of *Helicobacter pylori* strains from Turkey. *Infect Genet Evol* **7**, 509–512.
- Saribasak, H., Salih, B. A., Yamaoka, Y. & Sander, E. (2004). Analysis of *Helicobacter pylori* genotypes and correlation with clinical outcome in Turkey. *J Clin Microbiol* **42**, 1648–1651.
- Satomi, S., Yamakawa, A., Matsunaga, S., Masaki, R., Inagaki, T., Okuda, T., Suto, H., Ito, Y., Yamazaki, Y. & other authors (2006). Relationship between the diversity of the *cagA* gene of *Helicobacter pylori* and gastric cancer in Okinawa, Japan. *J Gastroenterol* **41**, 668–673.
- Segal, E. D., Cha, J., Lo, J., Falkow, S. & Tompkins, L. S. (1999). Altered states: involvement of phosphorylated CagA in the induction of host cellular growth changes by *Helicobacter pylori*. *Proc Natl Acad Sci U S A* **96**, 14559–14564.
- Selbach, M., Moese, S., Hauck, C. R., Meyer, T. F. & Backert, S. (2002). Src is the kinase of the *Helicobacter pylori* CagA protein in vitro and in vivo. *J Biol Chem* **277**, 6775–6778.
- Selbach, M., Moese, S., Hurwitz, R., Hauck, C. R., Meyer, T. F. & Backert, S. (2003). The *Helicobacter pylori* CagA protein induces cortactin dephosphorylation and actin rearrangement by c-Src inactivation. *EMBO J* **22**, 515–528.
- Selbach, M., Moese, S., Backert, S., Jungblut, P. R. & Meyer, T. F. (2004). The *Helicobacter pylori* CagA protein induces tyrosine dephosphorylation of ezrin. *Proteomics* **4**, 2961–2968.
- Singh, K. & Ghoshal, U. C. (2006). Causal role of *Helicobacter pylori* infection in gastric cancer: an Asian enigma. *World J Gastroenterol* **12**, 1346–1351.
- Stein, M., Rappuoli, R. & Covacci, A. (2000). Tyrosine phosphorylation of the *Helicobacter pylori* CagA antigen after *cag*-driven host cell translocation. *Proc Natl Acad Sci U S A* **97**, 1263–1268.
- Stein, M., Bagnoli, F., Halenbeck, R., Rappuoli, R., Fantl, W. J. & Covacci, A. (2002). c-Src/Lyn kinases activate *Helicobacter pylori* CagA through tyrosine phosphorylation of the EPIYA motifs. *Mol Microbiol* **43**, 971–980.
- Tomb, J. F., White, O., Kerlavage, A. R., Clayton, R. A., Sutton, G. G., Fleischmann, R. D., Ketchum, K. A., Klenk, H. P., Gill, S. & other authors (1997). The complete genome sequence of the gastric pathogen *Helicobacter pylori*. *Nature* **388**, 539–547.
- Tsukashita, S., Kushima, R., Bamba, M., Nakamura, E., Mukaisho, K., Sugihara, H. & Hattori, T. (2003). β -Catenin expression in intramucosal neoplastic lesions of the stomach. Comparative analysis of adenoma/dysplasia, adenocarcinoma and signet-ring cell carcinoma. *Oncology* **64**, 251–258.
- Tsutsumi, R., Higashi, H., Higuchi, M., Okada, M. & Hatakeyama, M. (2003). Attenuation of *Helicobacter pylori* CagA SHP-2 signaling by interaction between CagA and C-terminal Src kinase. *J Biol Chem* **278**, 3664–3670.
- Tsutsumi, R., Takahashi, A., Azuma, T., Higashi, H. & Hatakeyama, M. (2006). Focal adhesion kinase is a substrate and downstream effector of SHP-2 complexed with *Helicobacter pylori* CagA. *Mol Cell Biol* **26**, 261–276.
- Uchida, T., Kanada, R., Tsukamoto, Y., Hijiya, N., Matsuura, K., Yano, S., Yokoyama, S., Kishida, T., Kodama, M. & other authors (2007). Immunohistochemical diagnosis of the *cagA*-gene genotype of *Helicobacter pylori* with anti-East Asian CagA-specific antibody. *Cancer Sci* **98**, 521–528.
- Uemura, N., Okamoto, S., Yamamoto, S., Matsumura, N., Yamaguchi, S., Yamakido, M., Taniyama, K., Sasaki, N. & Schlemper, R. J. (2001). *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med* **345**, 784–789.
- Viala, J., Chaput, C., Boneca, I. G., Cardona, A., Girardin, S. E., Moran, A. P., Athman, R., Memet, S., Huerre, M. R. & other authors (2004). Nod1 responds to peptidoglycan delivered by the *Helicobacter pylori* *cag* pathogenicity island. *Nat Immunol* **5**, 1166–1174.
- Vilaichone, R. K., Mahachai, V., Tumwasorn, S., Wu, J. Y., Graham, D. Y. & Yamaoka, Y. (2004). Molecular epidemiology and outcome of *Helicobacter pylori* infection in Thailand: a cultural cross roads. *Helicobacter* **9**, 453–459.
- Yamaoka, Y., Kita, M., Kodama, T., Sawai, N., Kashima, K. & Imanishi, J. (1997). Induction of various cytokines and development of severe mucosal inflammation by *cagA* gene positive *Helicobacter pylori* strains. *Gut* **41**, 442–451.
- Yamaoka, Y., Orito, E., Mizokami, M., Gutierrez, O., Saitou, N., Kodama, T., Osato, M. S., Kim, J. G., Ramirez, F. C. & other authors (2002). *Helicobacter pylori* in North and South America before Columbus. *FEBS Lett* **517**, 180–184.
- Zhou, J., Zhang, J., Xu, C. & He, L. (2004a). *cagA* genotype and variants in Chinese *Helicobacter pylori* strains and relationship to gastroduodenal diseases. *J Med Microbiol* **53**, 231–235.
- Zhou, W., Yamazaki, S., Yamakawa, A., Ohtani, M., Ito, Y., Keida, Y., Higashi, H., Hatakeyama, M., Si, J. & other authors (2004b). The diversity of *vacA* and *cagA* genes of *Helicobacter pylori* in East Asia. *FEMS Immunol Med Microbiol* **40**, 81–87.