

REVIEW

Proteomic identification of biomarkers related to *Helicobacter pylori*-associated gastroduodenal disease: Challenges and opportunities

Ming-Shiang Wu,* Lu-Ping Chow,[†] Jaw-Town Lin* and Shyh-Horng Chiou[‡]

*Division of Gastroenterology, Department of Internal Medicine, National Taiwan University Hospital, [†]Graduate Institute of Biochemistry and Molecular Biology, College of Medicine, National Taiwan University, and [‡]Institute of Biochemical Science, National Taiwan University, Taipei, Taiwan

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Correspondence

Professor Ming-Shiang Wu, Department of Internal Medicine, National Taiwan University Hospital, No. 7, Chung Shan South Road, Taipei, Taiwan. Email: mingshiang@ntu.edu.tw

Abstract

Helicobacter pylori colonize the stomach of over half the world's population. While 80–90% *H. pylori*-infected individuals have clinically asymptomatic gastritis, 10–15% develop peptic ulcer, and 1–2% gastric malignancies. These variable clinical outcomes have led to an interest in prognostic indicators. The current disease paradigm suggests that host genetics and bacterial virulence both play important roles in modulating the final outcome of *H. pylori* infection. Elucidation of the interaction between host and bacterium is essential to clarify pathogenesis and to develop new strategies for prevention and treatment. Proteomic technology is a powerful tool for simultaneously monitoring proteins and protein variation on a large scale in biological samples. It has provided an unprecedented opportunity to survey a cell's translational landscape comprehensively, and the results may allow in-depth analyses of host and pathogen interactions. Using this high-throughput platform and taking advantage of complete sequences for both the *H. pylori* and the human genome in available databases, we have identified several crucial proteins that have pathogenic and prognostic potential. Among them, antibodies to AhpC and GroEs of *H. pylori* could be utilized for identification of patients who are at high risk of disease complications after *H. pylori* infection. Evolving proteomic technologies, together with appropriate clinical phenotyping and genotype information should enhance understanding of disease pathogenesis and lead to more precise prediction of variable disease outcomes. It will also facilitate development of biomarkers for diagnosis, treatment, and prevention of *H. pylori* infection.

Discovery of *Helicobacter pylori*: a new era in management of gastroduodenal diseases

The discovery and successful culture of *H. pylori* by Marshall and Warren in 1982 has revolutionized the diagnosis and treatment of gastroduodenal disease.¹ *H. pylori* is a microaerophilic spiral-shaped gram-negative bacterium that efficiently colonizes the human stomach. Epidemiological studies indicate that *H. pylori* infect at least half of the world's population. In developing countries, the infectious process occurs during childhood by intrafamilial transmission, and the prevalence rate is 60–80% in adulthood. In contrast, the annual incidence of *H. pylori* acquisition is much lower (~1%) in developed countries and is estimated to be 30–40% in adulthood.² Prolonged infection and inflammation because of persistent *H. pylori* colonization ultimately lead to chronic gastritis. A minority of patients go on to develop more severe gastric pathologies, such as peptic ulcer, gastric adenocarcinoma, and

gastric mucosa-associated lymphoid tissue lymphoma (MAL-Toma).² *H. pylori* is defined as a class I carcinogen for gastric cancer by the World Health Organization, and eradication of *H. pylori* is considered as the first-line treatment of peptic ulcer disease and MAL-Toma. In addition to its crucial role in gastroduodenal disease, *H. pylori* infection has also been implicated in the development of a proportion of idiopathic thrombocytopenic purpura and iron deficiency anemia.³ Furthermore, based on the observation that decreasing infection rate is associated with increasing occurrence of obesity and gastroesophageal reflux disease in developed countries, a beneficial role of *H. pylori* in esophageal disease was proposed.³ Two hormones, ghrelin and leptin, both originally discovered in the gastric mucosa, play an important role in the regulation of appetite. Recent reports demonstrated that plasma ghrelin but not plasma leptin is significantly lower in *H. pylori*-infected subjects than in respective controls. Moreover, this attenuation in plasma ghrelin levels by *H. pylori* was independent of gender and body mass index.⁴ This indicates

that some adverse appetite symptoms such as nausea and lack of appetite and possibly growth inhibition in *H. pylori*-infected children could be attributed to the suppression of ghrelin by this bacterium. Evidence is also accumulating that phylogeographic differences within *H. pylori* can be used to trace ancient human migrations and genetic diversity.⁵ In summary, infection with *H. pylori* has important health consequences, and investigation of its relationships to human diseases and population may provide a wealth of information important for medicine and public health.

Gastric cancer: an ongoing concern

On the basis of epidemiologic studies, the World Health Organization classified *H. pylori* as a class I (definite) carcinogen in humans in 1994.⁶ Several meta-analyses have shown a strong and consistent association between *H. pylori* infection and non-cardiac gastric cancer.^{7–10} An updated consensus indicated that chronic atrophic gastritis caused by *H. pylori* is the major precursor of gastric cancer.³ In a recent study, the attribution fraction of non-cardiac gastric cancer was 74% in developed countries and 78% in developing countries. These percentages represent 592 000 cases, or 5.5% of all cancers.¹¹

In addition to epidemiologic observations, data from animal studies have corroborated the causal relationship between *H. pylori* infection and gastric cancer.¹² Furthermore, biologic studies have clarified the underlying molecular mechanisms.^{13–17} To sum up, epidemiologic, animal, and biologic studies have provided consistent evidence to confirm the role of *H. pylori* infection in gastric carcinogenesis.¹⁸

The finding that *H. pylori*-induced chronic atrophic gastritis is the major cause of gastric cancer suggests that eradication of the bacterium may prevent this malignancy. Computer-simulation studies have confirmed the cost-effectiveness of eradication in high-risk subjects.^{19,20} However, clinical eradication trials using reduction of gastric cancer as the primary end-point remain inconclusive.²¹ Furthermore, unresolved issues complicate active testing and treating for *H. pylori* infection among asymptomatic carriers. Concerns include the enormous cost for developing countries to implement strategies, the inconclusiveness of data from randomized controlled studies, the potential induction of antimicrobial resistance, and the uncertain effect of eliminating this organism on the spectrum of modern disease. Although current evidence is insufficient to recommend universal testing and treatment, it is possible to identify highly susceptible individuals who are most likely to benefit from treatment.

The major scientific challenge after Nobel Prize: what mechanisms lead to diseases in certain *H. pylori*-infected subjects?

The 2005 Nobel Prize for Medicine awarded to Marshall and Warren was a formal recognition of their discovery that many peptic ulcers were a result of an infection that could be cured with antibiotics. Although *H. pylori* has been identified as a major pathogen leading to the development of a wide range of gastroduodenal disease, only a small portion of infected patients have more severe gastric pathology such as peptic ulcer (10–15%) and

gastric malignancies (1–3%).² This points to major scientific challenge: what mechanisms lead to cancer or ulcer in certain subjects and what mechanisms prevent the development of cancer or ulcer in other chronically infected subjects.

There are three main gastric phenotypes as a result of *H. pylori* infection: (i) mild pangastritis: not altering gastric physiology and not associated with significant disease; (ii) corpus-predominant gastritis: associated with gastric atrophy, hypochlorhydria, and increased risk of gastric cancer; and (iii) antral-predominant gastritis: associated with high gastric acid secretion and increased risk of duodenal ulcer disease.²² Notably, these three phenotypes of gastritis are not separate entities as there may be a progression from antral-predominant to corpus-predominant or to pangastritis. The current disease paradigm suggests that gastric immune and inflammatory responses because of interaction of host genetic and bacterial virulence factors determine the distribution and severity of gastritis and the final outcomes after *H. pylori* infection.²³ Accordingly, it is crucial to identify factors that determine the progression and serenity of gastric inflammation after infection. Many researches have now focused on the role of bacterial virulence factors, and *cag* pathogenicity island, *vacA*, *iceA*, *BabA*, *OipA*, and *SabA* are among the reported strain-specific virulence factors for *H. pylori*-related disease.²⁴ On the other hand, host genetic polymorphisms may directly influence inter-individual variation in the magnitude of cytokine response and contribute to an individual's ultimate clinical outcome. In the case of *H. pylori* infection, an array of pro- and anti-inflammatory cytokine polymorphisms affects the infectious outcomes. Potential candidate genes include interleukin (IL)-1 β , IL-8, IL-10, tumor necrosis factor- α , and Toll-like receptor 4.²² Despite marked progress in defining host and bacterial genotypes and their association with more severe gastric pathologies, geographic differences exist and a definitive model is elusive. Moreover, the underlying cellular mechanisms are far from clear. Further in-depth studies in this field are needed.

Evolving proteomics technology: new tools for investigation of *H. pylori*-related disease

Technical advances coupled with complete deciphering of the human and *H. pylori* genome sequences herald a new era in the identification of high-risk population and elucidation of pathogenesis.^{25,26} Among important contributions relating to *H. pylori*, global gene expression has been shown in *H. pylori*, whether interacting with host cells *in vitro*, in animal models, or in infected tissues.^{27,28} Additional high-throughput methods to investigate the bidirectional relationship include proteomics. These studies could delineate the cross-talk between the host and *H. pylori* and enhance the understanding of disease pathogenesis.

Proteomics is the study of the proteome (the collection of all the proteins expressed from the genome in all isoforms, polymorphisms, and post-translational modifications). The basic platform of proteomics technology is two-dimensional gel electrophoresis (2D-GE) and mass spectrometry (MS). 2D-GE separation of complex protein mixtures and the subsequent analysis of isolated proteins spots by MS allow fast and accurate identification of proteins. The comparison of spots from different samples separated on customized 2D gels facilitates the identification of

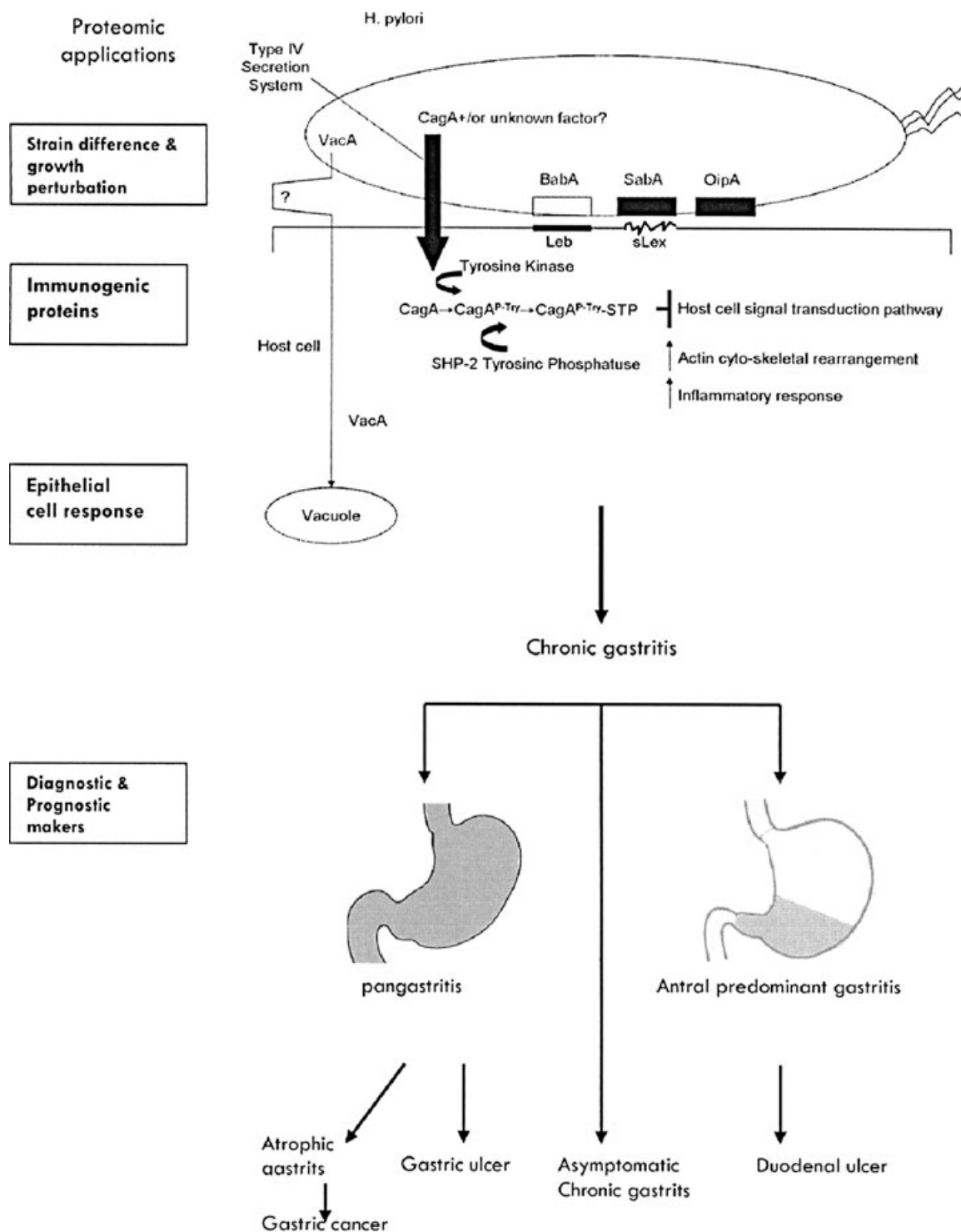


Figure 1 Overview of *Helicobacter pylori*–host interaction and the various outcomes after infection. Potential proteomic applications include: (i) proteomics-based characterization of intrinsic and extrinsic perturbations and strain differences of *H. pylori*; (ii) elucidation of epithelial cell response to *H. pylori* infection; (iii) identification of diagnostic/prognostic biomarkers; and (iv) uncovering of immunogenic proteins for vaccine targets. (Adapted from *Mol. Cell. Biochem.* 2003; 253:209–215 and Wu *et al.*, 2008.³⁹)

differences in protein expression, presence of isoforms, splice variants, and post-translational modifications by MS. Application of such technologies creates new possibilities in the elucidation of pathogenesis and the discovery of novel treatment target and early disease markers. In the field of *H. pylori*-related gastroduodenal disease, traditional biomarkers for gastroduodenal diseases include measurement of serum pepsinogen and gastrin level for gastric inflammation and carcinoembryonic antigen level for gastric cancer. However, these tests have limited sensitivity and specificity in identification of susceptibility to complications and in prognosis tailored to individuals. Proteomics is a potential tool for the discovery and application of novel biomarkers in increased precision in diagnosis and prognosis of *H. pylori*-related gastroduodenal disease. Because individual biomarkers will have limited sensitivity and specificity, proteomics may further provide the advantage to seek for a panel of complementary biomarkers that will have more robust operational characteristics. However, some limitations were noted in application of proteomics in the field of *H. pylori*-associated gastroduodenal diseases. These include ill-defined clinical phenotypes and genotypes and lack of consideration of host–bacterial interaction. Thus, combinations of proteomics technologies with information on clinical phenotypes and genotypes of host and bacteria will shed new light on *H. pylori*-related gastroduodenal diseases.

Proteomics identification of biomarkers related to *H. pylori*-associated gastroduodenal disease

Oxidative stress has been assumed to be one of the key mechanisms leading to *H. pylori*-related pathogenesis. Such oxidative stress may also exert bidirectional effects on host and pathogens. The *H. pylori*-induced oxidative stress can cause lesions in host cells, whereas *H. pylori* will also face the attack of oxygen-related free radicals by the host. To address the influence of oxidative stress and its underlying mechanisms, we have compared protein expression profiles of *H. pylori* incubated under normal microaerophilic (5% O₂) and aerobic stress (20% O₂) conditions.²⁹ These results revealed that more than 10 proteins were differentially expressed. Most notable, the protein expression level of UreE (an essential metallochaperone for urease activity) and alkyl hydroperoxide reductase (AhpC) with anti-oxidant potential were greatly reduced under oxygen tension. We have further characterized AhpC, which can display both anti-oxidative activates and molecular chaperone properties under different conditions.³⁰ Furthermore, patients with gastric ulcer and gastric cancer had higher titers of antibodies to AhpC in comparison with patients with gastritis and duodenal ulcer (unpublished data, Chuang *et al.*).

As gastric cancer and duodenal ulcer are clinically divergent disease outcomes, we compared two-dimensional immunoblots of an acid-glycine extract of *H. pylori* probed with serum samples from 15 patients with gastric cancer and 15 with duodenal ulcer to find gastric cancer-related antigens, which were subsequently identified by MS. Many protein spots were recognized by more than one serum, and 24 of these were better recognized by gastric cancer sera. The proteins showing higher frequency of recognition in gastric cancer group are threonine synthase, rod shape-determining protein, S-adenosylmethionine synthetase, peptide chain releases factor 1, DNA-directed RNA polymerase alpha

subunit, co-chaperonine GroES (monmeric and dimeric forms), response regulator OmpR, and membrane fusion protein. Of these proteins, GroES was identified as a dominant gastric cancer-related antigen with a much higher seropositivity of gastric cancer samples (64.2%, *n* = 95) compared with 30.9% for gastritis (*n* = 94) and 35.5% for duodenal ulcer (*n* = 124). GroES seropositivity was more commonly associated with antral gastric cancer than with non-antral ones (odds ratio = 2.7; 95% confidence interval, 1.1–6.7). In peripheral blood mononuclear cells, GroES stimulated production of IL-8, IL-6, granulocyte macrophage colony-stimulating factor, IL-1 β , tumor necrosis factor- α , cyclooxygenase-2, and prostaglandin E2. Moreover, when incubated with gastric epithelial cells, GroES induced expression of IL-8, cell proliferation, and upregulation of c-jun, c-fos, and cyclin D1 but caused downregulation of p27 (Kip1). Our results indicted that GroES of *H. pylori* is a novel gastric cancer-associated virulence factor and may contribute to gastric carcinogenesis through induction of inflammation and promotion of cell proliferation.³¹ In addition, we have found 11 proteins were strongly recognized by serum IgG from duodenal ulcer patients. Among them, translation elongation factor EF-G (FusA), catalase (KatA), and urease alpha subunit (UreA) were identified as duodenal ulcer-related antigens, showing a higher seropositivity in duodenal ulcer samples (*n* = 124) than in gastric cancer samples (*n* = 95) (FusA: 70.2% vs 45.3%, KatA: 50.8% vs 41.1%, and UreA: 44.45% vs 27.4%). A protein array containing the three duodenal ulcer-related antigens was further developed to provide a more rapid and more sensitive method for detecting serum antibody patterns of duodenal ulcer patients.³²

Conclusions

Elucidation of the interaction between the host and *H. pylori* helps our understanding of pathogenesis and the development of new prevention and treatment strategies. Proteomics technology is a tremendously powerful tool for simultaneously determining the presence of proteins and protein variation on a large scale in various biological samples. This new technology has provided unprecedented opportunity to survey a cell's translational landscape comprehensively and may allow in-depth analyses of host and pathogen interaction. Using this high-throughput platform and taking advantage of complete sequences for both the *H. pylori* and human genome in the database, some promising results and important information have been reported. Figure 1 summarizes the current status of proteomic applications in investigations of the *H. pylori*–host interaction and the various outcomes after infection.³³ The combination of new proteomics technologies with information on clinical phenotypes and genotypes will enhance the understanding of disease pathogenesis, lead to a more precise prediction of outcomes, and facilitate the development of effective biomarkers for diagnosis, treatment, and prevention of *H. pylori* infection.

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