**Special Section**

**Helicobacter pylori and Gastric Cancer: What can be Learned by Studying the Response of Gastric Epithelial Cells to the Infection?**

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**Abstract**

The development of gastric adenocarcinoma is closely linked to chronic infection with the bacterial pathogen *Helicobacter pylori*. One *Helicobacter*-specific virulence factor in particular, the CagA protein, has emerged as a main effector molecule in the interaction of *H. pylori* with gastric epithelial cells and has been implicated in gastric carcinogenesis. This review highlights the latest insights that have been gained into the pathogenesis of the disease by transcriptional profiling approaches studying gene expression in normal gastric tissue and gastric cancer tissue from human biopsy material as well as animal models of *Helicobacter* infection. The potential role of CagA as a bacterial oncprotein is also discussed. (Cancer Epidemiol Biomarkers Prev 2005;14(8):1859–64)

**Introduction**

Gastric cancer is a leading cause of cancer-related deaths in many parts of the world (1). It is now clear that the single most important factor responsible for the development of gastric cancer is chronic infection with the bacterial pathogen *Helicobacter pylori* (2). This microbe infects the stomach of roughly half of the world’s population. It is typically acquired in childhood and persists in the gastric mucosa for the lifetime of the human host. Chronic infection with *H. pylori* causes disorders in ∼20% of infected individuals that range from gastritis to peptic ulcer disease to gastric cancer; however, the majority of infected individuals remain free of overt disease symptoms. At present, no definite predictive diagnosis can be made as to who will suffer infectious sequelae and who will live unaffected by this persistent infection.

How might *H. pylori* play a role in the development of gastric adenocarcinoma? The bacterium could have an indirect carcinogenic effect by triggering a strong inflammatory reaction followed by a cascade of molecular and morphologic changes in the inflamed epithelium that are characteristic of the progression to intestinal-type gastric cancer: chronic gastritis, chronic atrophic gastritis, intestinal metaplasia, and dysplasia (3). Alternatively, *H. pylori* or its products may have a direct effect on infected cells by manipulating key cellular processes such as cell cycle control, apoptosis, tumor suppression, maintenance of polarity, and cell-to-cell contacts. These two scenarios are not mutually exclusive and both may contribute to the role of *H. pylori* as a tumor initiator or promoter. This article attempts to highlight the progress that has been made recently towards better understanding the link between *H. pylori* and gastric carcinogenesis, with a special emphasis on recent technological advances that we believe will facilitate research in this field.

**H. pylori Targets the Mucus-Producing Pit Cell Lineage In vivo**

The interaction of *H. pylori* with epithelial cells forms the basis for the development of gastric pathology as this cell type triggers a proinflammatory response by secreting cytokines such as interleukin-8. The resulting recruitment of immune cells to the site of infection manifests as chronic gastritis, which in turn has been shown to initiate epithelial hyperproliferation in a T cell–dependent manner (4). Because epithelial cells play such a key role in the initial events of the infection, we were interested in studying their response to the infection in an intact mucosal environment *in vivo*. Our experimental approach was to use laser microdissection, which permits the harvesting of single cells or small clusters of cells from stained cryosections in a way that preserves the integrity of the RNA. Moreover, due to some recent methodologic improvements, RNA can now be extracted from very small numbers of cells and amplified in a way that faithfully preserves the representation of transcripts (5). Thus, we captured populations of the three major epithelial lineages of the murine gastric mucosa (i.e., the mucus-producing pit cell, acid-producing parietal cell, and pepsinogen-producing chief cell) from cryosections of infected and uninfected murine stomachs, which had been stained briefly with a histologic dye. The corresponding RNA, taken at specific times after *H. pylori* infection and amplified, was used to examine global gene expression. This experimental approach generated a large database resource that not only confirms and extends our current understanding of the basic biology of these cell types, but also, we believe, provides first detailed *in vivo* insights into the pathways that are turned on in response to *H. pylori* infection (6). Whereas ontological mining of the parietal and chief cell-specific transcriptomes mainly highlighted the physiologic functions previously attributed to these cells, the
mucus-producing cell transcriptome suggests a highly complex, multifaceted lineage (Fig. 1). Most notably, our analysis attributes novel functions in sampling and sensing the environment to this cell type, as evidenced by the expression of a large number of cell surface receptor–linked signal transduction molecules. Factors involved in the initiation of a defense response, such as cytokines, chemokines, and, in particular, molecules with roles in antigen processing and presentation via MHC class I, are also enriched in mucus-producing cells. All these functions liken the gastric mucus-producing cell to the specialized M-cell of the Peyer’s patch.

Only the mucus-producing cell type showed a transcriptional response to \textit{H. pylori} infection in the timeframe of our experiment (6). Neither chief nor parietal cell genes were deregulated at any one of the time points we analyzed between days 2 and 28 after infection. In the mucus-producing cell, we found strong evidence for a proinflammatory response: the cytokines interleukin-1\textsubscript{\textbeta}, tumor necrosis factor-\textalpha, and IFN-\gamma or their downstream target genes as well as the two chemokines, granulocyte macrophage colony-stimulating factor and RANTES, were up-regulated. In line with this observation, several transcription factors known to regulate the expression of proinflammatory genes, such as Jun and Fos, and the nuclear factor-\textkappaB pathway were also induced in response to infection. Another functional group of genes affected by the infection include factors involved in a mucosal defense response, such as the small proline-rich protein SPRR2A, trefoil factor 2, and prostaglandin E receptor 4. Differential regulation of vascular endothelial growth factor as well as lactoferrin and ferritin further suggest that \textit{H. pylori} modifies the microvasculature and iron availability in the colonized epithelium. In contrast to those groups of genes whose expression was higher in the gastric mucosa of infected animals, it was striking that most genes in the tumor suppressor category were repressed compared with uninfected animals. This finding indicates that tumor suppression is shut down, at least in the timeframe of our experiment, suggesting that \textit{H. pylori} affects growth control and apoptosis as early as the first weeks of infection, perhaps already setting the stage for the later development of gastric malignancy. We believe that this study provides us with the foundation for future analysis of the processes that bring about malignant transformation of these cells during chronic infection with \textit{H. pylori}. Having established the baseline transcriptome of the major gastric epithelial cells, we hope it will now be possible to track changes that occur over time in animal models of gastric cancer and its precursor lesions.

Evidence for Spasmolytic Polypeptide Expressing Metaplasia as a Precursor of Gastric Dysplasia

Another study recently used laser microdissection to link a specific metaplastic cell lineage (SPEM, or spasmolytic polypeptide expressing metaplasia) to the development of dysplasia in male C57BL/6 mice infected with \textit{Helicobacter felis} (7). The fundic glands of these infected mice develop atrophic changes characterized by the replacement of parietal cells by cells of the SPEM lineage. These cells are named after one of their markers, spasmolytic polypeptide (or trefoil factor 2), and occur also in other experimental models characterized by glandular atrophy and loss of parietal cells. The study by Nomura et al. (7) revealed 11 additional transcripts that are expressed by SPEM, but not surface mucus cells, indicating that they might be good markers for this lineage. Interestingly,
in situ hybridization with riboprobes against 10 of 11 transcripts stained regions of gastritis cystica profunda, a direct precursor lesion to mucosal dysplasia and neoplasia (7). From this, the authors conclude that dysplasia is derived from SPEM in this animal model thus challenging the concept that intestinal metaplasia (derived from mucin-producing goblet cells) is the direct precursor lesion to dysplasia. Morphologically, SPEM resembles a mucus cell lineage typical of the deep antral glands. In terms of the expression patterns of the 10 marker genes however, SPEM seems to be derived from fundic chief cells rather than mucus-producing cells, highlighting the superiority of transcriptome analysis over classic histologic stains in determining lineage relationships.

Evidence for Intestinal Metaplasia as a Precursor of Gastric Dysplasia

In contrast to these results, a different study examining gastric cancer and its precursor lesions at the whole genome level found that intestinal metaplasia could be the direct precursor in some forms of the human disease (8). Chen et al. identified a transcriptional signature in antral biopsies with intestinal metaplasia that they termed “intestinal-like” because of its similarities to the profiles of small intestine biopsies. Interestingly, this signature was also found in a distinct set of tumors characterized by the presence of intestinal metaplasia at the tumor edge, indicating that this set has actually arisen from intestinal metaplasia. The intestinal-like expression pattern was seen with equal frequency in the histologically defined intestinal and diffuse subtypes of gastric cancers. Based on their findings, the authors hypothesize that intestinal-like gastric cancers, although not necessarily the intestinal-type cancers as defined by histology, arise from gastric mucosal cells that have previously undergone intestinal metaplasia and thus retain the molecular signature of the intestinal enterocyte, whereas the gastric-like cancers arise by an alternative pathway (8). This study thus also challenges the current concept of gastric carcinogenesis, which has been based on histologic classification alone.

It is interesting to note that in the report by Chen et al. (8), no tumor-associated transcripts correlated with H. pylori infection, implying that the role H. pylori infection plays in carcinogenesis precedes the development of overt cancers or that the bacterium has an indirect role in carcinogenesis. A very general but important conclusion drawn from this study is the enormous heterogeneity of gene expression patterns in gastric cancer compared with other solid tumors, implying multiple alternative mechanisms of tumor initiation and progression. The one feature that all tumors included in this study had in common was the loss of transcripts characteristic of normal stomach physiology, indicating a complete dedifferentiation of cells in all types of gastric cancer.

Risk Factors in Gastric Carcinogenesis

The risk of developing gastric cancer is influenced by both host and bacterial determinants. One of the recognized host factors is mucosal gastrin, a hormone that stimulates gastric epithelial cell proliferation in vitro (9). Transgenic mice overexpressing gastrin (INS-GAS mice) spontaneously develop gastric cancer, a process that can be greatly accelerated by concomitant H. felis infection.
(10) or H. pylori infection (11). Male gender and polymorphisms in the human interleukin-1β promoter associated with enhanced interleukin-1β expression further augment the risk for atrophic gastritis and gastric adenocarcinoma in infected individuals (12, 13), as do high levels of other proinflammatory cytokines (reviewed in ref. 14).

Several bacterial determinants also contribute to gastric carcinogenesis, the best understood being the Cag pathogenicity island (Cag-PAI), a genomic insert of ~40 kb that encodes a type IV secretion system. Infection by H. pylori carrying this island markedly heightens the risk for atrophic gastritis and distal adenocarcinoma (15, 16). The contribution of various genes encoded by the Cag-PAI to H. pylori-associated pathogenesis is currently the focus of a number of experimental model systems both in vivo and in vitro. Whereas inactivation of the cagE gene, which encodes a structural component of the type IV secretion system, significantly reduces acute and chronic inflammation in a time frame of several weeks after infection in the gerbil model (17), it delays but does not prevent progression to gastric carcinoma in the INS-GAS model (11). In a study from our own laboratory exploring the contribution of cagE and the entire Cag-PAI to the transcriptional response of cultured AGS cells, we found that a cagE mutant fails to induce nuclear factor-κB and tumor necrosis factor-α signaling and other signaling pathways typically activated during an inflammatory response (18), consistent with the findings in gerbils. We further quantified the contribution of various virulence determinants to the response of polarized T84 cells: the vast majority of gene expression changes upon infection with H. pylori are dependent on the Cag-PAI; an isogenic mutant lacking the entire island elicited only a very limited response (Fig. 2; ref. 19). Remarkably, 80% of the transcriptional changes were dependent on the expression of a single PAI-encoded gene, cagA. The CagA protein is an effector molecule that is translocated from an adherent bacterium into the host cell cytosol by means of the type IV secretion system.

Is CagA a Bacterial Oncoprotein? Does It Play a Direct Role in the Development of Gastric Carcinoma?

A number of studies have pointed to a link between carriage of CagA+ strains and an increased risk of gastric cancer, and a meta-analysis that included 16 studies with 2,284 cases and 2,770 controls has recently confirmed this once more: infection with CagA+ strains of H. pylori increases the risk for gastric cancer over the risk associated with H. pylori infection alone (2.87- and 2.28-fold, respectively; ref. 20). This is particularly true of CagA variants found in East Asia (21), where the prevalence of gastric cancer is higher than in the Western hemisphere. It is noteworthy to point out that the association among H. pylori, CagA+ status, and gastric cancer may be markedly underestimated. First, most epidemiologic studies miscalculate the prevalence of past infection because data is collected after the development of gastric atrophy and cancer, which often occur years after H. pylori infection may have cleared. In studies that control for some of these biases, the relationship between H. pylori or CagA+ status and gastric cancer can be much higher (22). For example, a recent study in Germany by Brenner et al. (23) applied three exclusion criteria to minimize bias against possible clearance of the infection in the course of disease development and found that this increased the odds ratio of noncardia gastric cancer from 3.7 (95% confidence interval, 1.7-7.9) to 18.3 (95% confidence interval, 2.4-136.7) for any H. pylori infection

![Figure 3](image-url). After entering the host cell, CagA disrupts the apical junctions and activates RTK-like signaling. Left, hypothetical diagram and confocal immunofluorescence image (inset) of H. pylori injecting CagA (red) into the host cell through a type 4 secretion system, in the vicinity of the tight junctions. The confocal immunofluorescence image (left inset) shows an H. pylori bacterium expressing GFP (green) adhered to the cell surface. Antibodies to CagA (red) detect injected CagA in the vicinity of the attached bacterium. This induces recruitment of the tight junction protein ZO-1 (blue) to the bacterial attachment site (39). CagA interacts with tight junction components through its NH2-terminal domain in a phosphorylation independent manner. It also activates RTK-like signaling through its COOH-terminal domain after Src-induced tyrosine phosphorylation in a manner reminiscent of the adaptor protein Gab1 (diagram adapted from ref. 31). Right, H. pylori interacting with a polarized epithelial monolayer of Madin-Darby canine kidney cells in vitro. The bacteria (red) preferentially adhere in the vicinity of tight junctions (ZO-1, green) and also modify the distribution of junction proteins (arrows).
and from 5.7 (95% confidence interval, 2.6-12.8) to 28.4 (95% confidence interval, 3.7-217.1) for CagA-positive *H. pylori* infections.

A second reason for potentially underestimating the role of CagA is that its biological effects depend not only on the presence of the *cagA* gene in the *H. pylori* chromosome but also on the ability of each strain to effectively deliver CagA protein into the host cell cytoplasm. We now know that CagA delivery into the host cell requires the assembly of a functional microinjection device, the abovementioned type IV secretion system, which is capable of interacting with the host cell plasma membrane and of translocating CagA directly from the bacteria to the host cell (reviewed in ref. 24). Only recently have epidemiologic studies begun to test *H. pylori* clinical isolates for their ability to deliver functional CagA into host cells (25) and to further classify strains according to CagA subtypes (26).

In addition to its epidemiologic link to cancer, CagA is proving to have powerful biological activities reminiscent of oncproteins and capable of modifying epithelial cell behavior. Once inside the host cell, the COOH terminus of CagA serves as a substrate for Src family tyrosine kinases and becomes tyrosine phosphorylated at sites containing the 5-amino-acid motif EPIYA (27, 28). There can be considerable variation in the number and the phosphorylation state of these domains and this may also contribute to pathogenesis (25, 29). Downstream events after CagA EPIYA-tyrosine phosphorylation are less clear in vivo, but several studies in cultured cells suggest that CagA may be functionally mimicking eukaryotic adaptor proteins of the Gab family (30). These adaptor proteins modulate the assembly of signaling complexes in the vicinity of activated receptor tyrosine kinases (RTK; ref. 31; reviewed in ref. 32). CagA, like Gab1, for example, has been shown to bind and activate SH2 domain containing proteins like the tyrosine phosphatase SHP2 (33) and Grb-2 (34). CagA, like Gab1, also has been reported to associate with at least one activated RTK, c-Met, the receptor for hepatocyte growth factor (35). Gab1, like CagA can be tyrosine phosphorylated by src family kinases (36). It is intriguing to speculate that CagA may function as a kind of Gab functional homologue, although it does not share any amino acid homology with Gab1. Future explorations into CagA’s structural and functional features should shed light on this issue. It is important to note that the pathways and proteins activated by phosphorylated CagA are known or potential oncogenes. For example, activated mutant alleles of c-met are associated with a number of human tumors, including intestinal-type gastric cancer (37, 38), growth factor receptor binding protein 2, and SHP2, are oncogenes implicated in leukemia, but aberrant expression and signaling have also been observed in gastric cancer.

Further insight into CagA’s biological properties is the discovery that CagA acts within a specific subcellular context (i.e., the apical junctions of epithelial cells; refs. 39, 40). The apical junctional complex comprises the tight and adherens junctions of epithelia and is composed of structural proteins that regulate cell-cell adhesion between neighboring cells and maintain the integrity of the epithelial barrier as well as regulatory proteins that act as a signaling hub for pathways controlling cell proliferation, cell differentiation, and cell polarity. CagA translocated into epithelial cells specifically associates with tight junction components and recruits tight junction proteins to sites of bacterial attachment (ref. 39, Fig. 3). Although the RTK-like signaling effects of CagA depend on the COOH-terminal phosphorylation domains of CagA, the ability of CagA to localize and associate with junction components is independent of its phosphorylation (39) and depends on the NH₂ terminus of the protein. Furthermore, a significant proportion of changes induced by CagA at the transcriptional level in polarized epithelial cells are phosphorylation independent (ref. 19; Fig. 2). This raises the possibility that CagA either has multiple functions in the eukaryotic cell, or more likely usurps a physiologic link between RTK signaling and the apical junctions. Because perturbations of the mechanisms that control the proper assembly and maintenance of junctions in epithelia have been repeatedly shown to contribute to cancer progression (40–42), chronic disruption of junction function, and RTK-like signaling by CagA may be responsible for its association with gastric carcinoma.

Conclusions

Although its biological activities suggest a direct oncogenic role for CagA, one must note that CagA acts on host cells many years before cancers arise, and CagA signaling is a reversible phenomenon that changes cell phenotype but not genotype. Because tumor progression requires the accumulation of genetic changes, how can CagA contribute to the development of gastric cancer as an epigenetic, noninherited signal? This is a question that will need close scrutiny in the future and likely will bring us back to the complex interplay between specific signaling processes used by *H. pylori* to communicate with host cells and the chronic inflammatory responses elicited by the host.

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