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Chronic *Helicobacter pylori* Infection and DNA-Damaged Stem Cells: A Recipe for Disaster

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Helicobacter pylori is the most common bacterial infection worldwide. Biological costs incurred by chronic colonization include increased risk for gastric adenocarcinoma [1]. Approximately 660,000 new cases of gastric cancer/year are attributable to *H. pylori*; eradication of this pathogen significantly decreases the risk of developing cancer in infected individuals. Nevertheless, only a small percentage of colonized persons develop neoplasia; enhanced risk is related to *H. pylori* strain differences, host responses governed by genetic diversity, and/or specific interactions between host and microbial determinants [2]. Universal test and treat strategies for *H. pylori* are not feasible due to the high prevalence of infection combined with the expense and side effects of antibiotic therapy [1]. These observations, in conjunction with evidence that carriage of certain strains is inversely related to esophageal adenocarcinoma and atopic diseases [1,3], underscore the importance of identifying mechanisms that regulate interactions of *H. pylori* with its human host which promote carcinogenesis.

One *H. pylori* strain-specific virulence locus that augments gastric cancer risk is the *cag* pathogenicity island, which encodes a type IV secretion system (TFSS) [4–6]. The product of the *cagA* gene (CagA) is translocated by the TFSS into epithelial cells, undergoing targeted tyrosine phosphorylation by Src and Abl kinases at motifs (termed A, B, or C/D) containing the amino acid sequence EPIYA [7–10]. Phosphorylated CagA activates a cellular phosphatase (SHP-2) and ERK mitogen-activating protein kinases (MAPK) leading to morphological aberrations that mirror changes induced by growth factor stimulation [11,12]. Moreover, non-phosphorylated intracellular CagA can exert effects with carcinogenic potential including activation of β-catenin [13,14].

DNA damage resulting from inflammation-associated reactive oxygen and nitrogen species (RONS) also is contributory towards the development of pre-malignant lesions within *H. pylori*-infected gastric mucosa. Loss of a key enzyme, alkyladenine DNA glycosylase (AAG) that repairs DNA damage induced by RONS, augments the severity of atrophy and foveolar hyperplasia in the stomachs of mice challenged with *H. pylori* [15]. *H. pylori* can also directly induce DNA damage in gastric epithelial cells via activation of a pathway mediated by spermine oxidase (SMO). SMO catabolizes the formation of the polyamine spermine, which produces H_2O_2, leading to DNA damage [16]. Chaturvedi et al. recently reported that *H. pylori* CagA can induce SMO-mediated DNA damage *in vitro* and in rodent models of infection, results that were mirrored by findings in human subjects colonized with *H. pylori cagA*$^+$ strains [16]. Of great importance, *H. pylori*-infected rodents that developed dysplasia harbored a subset of gastric epithelial cells in which SMO production and DNA

damage were high, but which were resistant to apoptosis, thereby representing a cellular population poised for neoplastic transformation (Figure 1).

The gastric epithelium is organized into functionally monoclonal glandular units. Homeostasis is regulated via stem cells, which legislate glandular function via infrequent asymmetric divisions with subsequent differentiation of progeny into epithelial cell lineages. Stem cells are tightly regulated by Wnt proteins, which activate β-catenin. Barker *et al.* previously demonstrated that the Wnt target gene *Lgr5*, encoding an orphan G protein-coupled receptor, marks a multipotent stem cell population responsible for long-term renewal of gastric epithelium [17]. Further, a subpopulation of Lgr5-expressing cells possesses the ability to generate gastric organoid cultures *in vitro*, which could be maintained for up to 9 months [17].

Helicobacter pylori can interact intimately with stem cell populations. In transgenic mice that over-express Le^b, *H. pylori* adhere directly to gastric epithelial cells [18,19]. Genetic ablation of parietal cells in Le^b-expressing transgenic mice permits the gastric epithelial progenitor (GEP) stem cell population to expand, accompanied by an expansion of *H. pylori* colonization and inflammation within the glandular epithelium [20, 21]. Further, delineation of the GEP transcriptome has identified several pathways that are over-represented in this lineage which are of particular biological importance for carcinogenesis, including Wnt/β-catenin [22]. Epidemiologic data supporting the presence of aberrant β-catenin signaling in the majority of gastric malignancies, in conjunction with evidence that cancer-associated *H. pylori cagA*$^+$ strains activate this pathway has fueled efforts to define how chronic inflammation mediates stem cell damage.

In this issue, Uehara *et al.* examined the relationship between *H. pylori* colonization, gastric cancer and DNA damage within $Lgr5^+$ cells using specimens harvested from patients, an important approach since the human stomach represents the primary endogenous ecological niche for *H. pylori*. Lgr5-positive cells, as determined by immunohistochemistry and immunofluorescence, were primarily localized to the gastric antrum within the mucous neck region, without localization to surface or pit regions. To bolster the specificity of Lgr5 as a stem cell marker, sections were also stained for another putative stem cell marker, CD44. Virtually all $Lgr5^+$ cells also co-expressed CD44. To replicate these findings in an experimental *in vivo* system, Lgr5-positive cells were also identified within murine gastric mucosa with a similar topographical pattern of distribution was identified.

Gastric mucosa from colonized subjects with cancer contained significantly increased populations of $Lgr5^+$ cells, compared with uninfected patients, with cancer; however, $Lgr5^+$ cell abundance was the same when all gastric cancer patients (infected and uninfected) were compared with all non-cancer patients. Thus, the presence of *H. pylori* influenced the expansion of stem cell populations in subjects who developed gastric malignancy.

Based upon these associations, the authors then delved into mechanisms that may underlie the development of cancer in these subjects. Buoyed by the finding of a strong relationship between the number of $Lgr5^+$ cells and the severity of acute inflammation, Uehara *et al.* next quantified DNA damage using 8OHdG as a surrogate marker of oxidative DNA damage, stratifying their results based on Lgr5 cellular positivity. Similar to findings focused on Lgr5 *per se*, significantly higher levels of 8OHdG were detected in $Lgr5^+$ epithelial cells compared with Lgr5-negative cells among *H. pylori*-infected persons with gastric cancer. Of interest, within the lamina propria, levels of 8OHdG were higher in $Lgr5^+$ cells in uninfected subjects who did not have gastric cancer. Further, in contrast to epithelial $Lgr5^+$ cells, lamina propria $Lgr5^+$ cells co-expressed CD45 and vimentin, suggesting a possible leukocyte lineage derivation.

This study has offered important information regarding how a chronic bacterial infection may affect carcinogenesis within the context of inflammation, providing a framework for future studies focused on these relationships. As with all original research, new questions and directions for future experiments ensue from these results. Subsequent studies should focus on the microbial elements required for the induction of DNA damage in stem cells. As described previously, the translocated effector protein CagA can directly induce DNA damage in gastric epithelial cells (Figure 1) [16]; thus a rational next step would be to stratify *H. pylori*-colonized persons into those that harbor *cagA*$^+$ versus *cagA*$^-$ strains. *In vitro* studies can be performed to determine if the ability of CagA to induce DNA damage is dependent upon the phosphorylation status of translocated CagA. If so, CagA proteins from East Asian *H. pylori* strains, which are more potent in inducing cellular morphologic aberrations, could be compared to CagA proteins derived from Western *H. pylori* strains. A logical extension could incorporate a more detailed investigation of other stem cell populations that may be affected. In the intestine, Lgr5 marks a highly proliferative population of stem cells. In contrast, Powell *et al.* recently reported that Lrig1 (Leucine-rich repeats and immunoglobulin-like domains 1) is a transmembrane protein that marks a distinct population of quiescent stem cells which functions as a tumor suppressor [23]. Lrig1 is expressed in the stomach; therefore, it would be additionally important to define the effects of *H. pylori* infection on this stem cell population. Finally, studies of mice infected with *H. pylori* or with the related mouse-adapted *Helicobacter* species, *H. felis*, have demonstrated that bone marrow derived cells (BMDCs) home to and engraft in sites of chronic gastric inflammation, particularly within foci where tissue injury induces excessive apoptosis and overwhelms the population of endogenous tissue stem cells [24,25]. Within the inflamed stomach, BMDCs degenerate into adenocarcinoma, suggesting that gastric epithelial carcinomas can originate from marrow-derived sources [24, 25]. Are the stem cells identified in the current study endogenous to the stomach or are they derived from other sites?

Studies such as the one performed by Uehara *et al.* are important in that they have utilized the natural niche of *H. pylori* as a nidus to demonstrate biologically relevant relationships between elements of the microbiota, host responses, and disease. Investigations that focus on specific interactions between *H. pylori* and its host can provide models for general patterns that may be extended to other malignancies that arise from inflammatory foci and facilitate a deeper understanding of how chronic inflammation leads to malignant degeneration.

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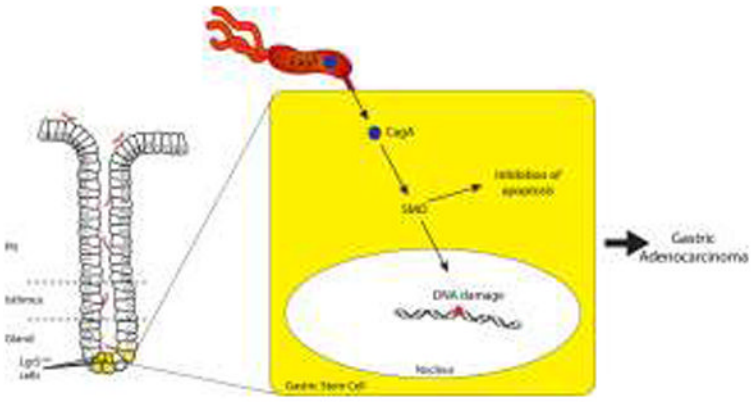

Fig. 1.

Hypothetical model of DNA damage induced by intracellular delivery of *H. pylori* CagA into gastric stem cells