Gastric Cancer Prevention by Demethylation

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Abstract

Niwa and colleagues report in this issue that treatment with the DNA demethylation agent 5-aza-2’-deoxycytidine decreases the incidence of gastric cancers in an animal model of Helicobacter pylori–promoted gastric cancer. This provocative study underscores the importance of changes in DNA methylation that contribute to the origin of inflammation-related cancers. The findings also raise the exciting possibility of cancer prevention by altering DNA methylation events early during tumorigenesis. Cancer Prev Res; 6(4); 253–6. ©2013 AACR.

Worldwide, an estimated 16% of cancers, accounting for approximately 2 million new cases per year, are related to infectious agents, including hepatitis B and C viruses, human papillomavirus, Epstein–Barr virus, and Helicobacter pylori (H. pylori; ref. 1). The latter agent, a spiral bacterium adapted to reside in the human stomach, promotes the development of gastric B-cell lymphoma and gastric adenocarcinoma, a disease that will kill an estimated 10,990 Americans in 2013 (2). Annually, approximately 660,000 new gastric cancer cases worldwide are related to H. pylori infection (1).

Multiple mechanisms have been proposed for the promotion of gastric cancer by H. pylori. The Cag pathogenicity island, a 40-kb locus present in some strains, is associated with an increased risk of distal gastric cancer, compared with strains lacking the cag island (3, 4). The cagA gene, found at the terminal region of the island, encodes a CagA oncoprotein, which is injected into gastric epithelial cells via the bacterial type IV secretion system, whereupon CagA becomes phosphorylated by Src family kinases and activates SHP-2 tyrosine phosphatase, disrupting cell signaling pathways. Phosphorylated CagA binds to PAR1 (5). Crk adaptor proteins, c-Met, and ZO-1. PAR1 participates in the establishment and maintenance of epithelial cell polarity, whereas Crk proteins are involved in regulation of the actin cytoskeleton, cell proliferation, and migration (6). The hepatocyte growth factor receptor c-Met affects cell motility, and CagA deregulates c-Met signaling (7). CagA also binds to the tight junction scaffolding protein ZO-1 and alters the apical-junctional complex (8). Which one or more of these functions may be responsible for the oncogenic effects of CagA is not clear, but gastrointestinal and other malignancies develop when CagA is expressed in transgenic mice but only if CagA tyrosine phosphorylation capability is maintained (9).

In addition to these CagA-dependent functions, H. pylori infection exerts more general influences related to oxidative and nitrosative effects that may contribute to carcinogenesis. Chronic inflammation is accompanied by an influx of neutrophils and macrophages, which generate and release reactive oxygen species and reactive nitrogen intermediates, leading to further increased inflammation and DNA damage. H. pylori’s secreted virulence factor, HP-NAP (neutrophil activating protein), induces the assembly of neutrophil NADPH oxidase components from the cytoplasm and membranes. Although in principle, assembled NADPH complexes can be targeted to either phagosomes or the extracellular space, in the case of H. pylori infection, complexes are preferentially targeted to the latter location (10), leading to a release of superoxide that enhances inflammation. This effect is advantageous to H. pylori, as local tissue damage is enhanced, which releases nutrients. Macrophages, in addition to their role of producing interleukin (IL)-12 that activates T-helper cell 1 (TH1) cells, also function as effector cells by generation of nitric oxide (NO) catalyzed by inducible nitric oxide synthase (iNos). Following H. pylori infection, iNos is upregulated in human gastric mucosae (11), and this effect is accompanied by increases in the production of the DNA adducts 8-nitroguanine and 8-oxo-7,8-dihydro-2’-deoxyguanosine (8-oxo-DG, also called 8-OHdG; ref. 12). H. pylori infection also generates H$_2$O$_2$ within gastric epithelial cells by upregulating spermine oxidase, the enzyme that oxidizes spermine to release H$_2$O$_2$, leading to DNA damage detectable as 8-OHdG (13). Mispairing of 8-OHdG during DNA replication may lead to G > T transversion mutations (14).

Another consequence of H. pylori infection related to carcinogenesis is alteration in patterns of DNA methylation in gastric epithelial cells. aberrant methylation of the CDH1 promoter has been associated with the presence of H. pylori infection in dyspeptic patients (15). Subsequent studies reported aberrant methylation of other genes, including...
LOX, HAND1, THBD, HRA5LS, FLNC, ARCl, CDKN2A, and TWIST1, associated with H. pylori infection (16, 17). Aberrant methylation has been found to be partially reversible after H. pylori is eradicated (18, 19). Using the Mongolian gerbil (Meriones unguiculatus) model of H. pylori–induced gastric cancer, Niwa and colleagues in a previous study proposed that inflammation, rather than the H. pylori per se, promoted hypermethylation, because animals treated with the immnosuppressant cyclosporine A did not undergo hypermethylation of the monitored genes, even though levels of H. pylori remained constant (20).

In this issue, the same investigators now report that treatment of gerbils with the demethylating agent 5-aza-2’-deoxycytidine (5-aza-dC) reduces the incidence of neoplasia in an animal model of inflammation-promoted gastric cancer (treatment of gerbils with both H. pylori and the carcinogen N-methyl-N-nitrosourea, or MNU; ref. 21). Following 5-aza-dC treatment, cancer incidence declined from 55.2% to 23.3%. Evaluation of methylation levels of 6 methylation-prone promoters revealed a partial reversal in methylation following the use of 5-aza-dC, whereas global methylation levels declined slightly. Previously, the same investigators had measured increases in expression of Il1b, Nos2, and Tnf accompanying H. pylori infection in this model (20). In the current article, they report that in gerbils treated with 5-aza-dC, the expression of Il1b and Nos2 in gastric mucosae declined to 42% and 58% of untreated levels. In contrast, Tnf expression increased to 187% of untreated levels. Notably, these dysregulatory changes in response to 5-aza-dC occurred without a significant change in the levels of mononuclear or polymorphonuclear cell infiltration. About side effects of treatment, testicular atrophy was observed, but no histologic changes were detected in the small intestine, liver, or kidneys. The investigators note that this is the first report of prevention of an inflammation-induced cancer by a demethylation agent.

Alterations in DNA methylation are likely to be key early steps in the process of carcinogenesis. According to the Epigenetic Progenitor Model of Cancer (22), tumors arise from epigenetic disruption of progenitor or stem cells, and epigenetic changes, especially alterations in DNA methylation, may lead to aberrant inactivation of tumor suppressor genes and activation of oncogenes. Epigenetic changes are polyclonal, which is consistent with the field defect, long noted in gastric and other tumor types (23). Therefore, it is reasonable that prevention efforts directed at the primary epigenetic dysfunction may be effective. Clinically, 5-aza-dC (decitabine), is now being used either alone or in drug combinations for the treatment of various malignancies, including non–small cell lung cancer (24), acute myeloid leukemia (25), and myelodysplastic syndrome (26). Considerations of costs and side effects are significant challenges to the prophylactic use of these drugs in the near future. Despite these limitations, the ramifications of the study conducted by Niwa and colleagues inspire hope for better drugs or therapies for reversal of methylation changes sustained during the precancerous process.

A possible mechanism for inflammation-induced dysregulation of DNA methylation has been described by O’Hagan and colleagues as DNA repair gone awry (27). Following DNA damage, histone H2AX becomes phosphorylated as γ-H2AX (28) and stabilizes interaction of repair proteins with chromatin. O’Hagan and colleagues found increased avidity of binding of DNA methyltransferase 1 (DNMT1) and the NAD+–dependent class III histone deacetylase (SIRT1) to chromatin following treatment of human embryonic carcinoma cells with H2O2. DNA damage produced by ionizing radiation or UV light did not reproduce the effect. Immunoprecipitation experiments revealed that DNMT1 and SIRT1 became part of a complex containing DMNT3B and members of the polycomb repressive complex 4 (EZH2, SUZ12, and EED2). The multiprotein complexes (or “silencing complexes”) preferentially targeted GC-rich regions, such as those found in some promoters. Those GC-rich regions were, as might be expected, enriched in 8-oxo-dG. Following H2O2 treatment, a set of genes with high transcription rates had those rates reduced, in association with binding of the silencing complex. In contrast, in the same short time frame (29 minutes following treatment), a set of genes with low basal transcription rates showed increased DNA methylation associated with the binding of the complex. Tumor-associated increases in promoter methylation occur more frequently in genes with low basal transcription rates (29). To test whether the same avid binding of SIRT1 and EZH2 occurred in vivo, the investigators examined a mouse model of an inflammation-related colon cancer (Min mice infected with Bacteroides fragilis) and found tighter binding of those 2 proteins in the distal portion of the mouse colon, where inflammation is greater. Coimmunoprecipitation experiments also showed more DNMT1 complexed with EZH2 in the tissue where the severity of inflammation was greater. Low expression genes with CpG island-containing promoters (including Fbn1, Sec6l, Sfrp5, and Sox17, which undergo tumor-specific DNA methylation) showed enrichment of EZH2 and DMNT1 at the promoter CpG islands from the inflamed tissue. These results are consistent with a model of tumor-specific DNA methylation resulting from or secondary to a process adapted to repair DNA damage arising from oxidative stress, especially in GC-rich regions such as CpG islands. High expression genes seem somehow protected against DNA methylation, whereas low expression genes are not and consequently sustain such methylation.

Work from our group has indicated that the presence of the cagA gene in the infecting H. pylori strain may also be related to methylation effects in the gastric mucosa of the host. H. pylori strains isolated from subjects residing in regions of high and low risk for gastric cancer were characterized for the presence of cagA. Gastric biopsy DNA from the corresponding subjects was analyzed for aberrant methylation at 4 candidate gene promoters (RPRM, APC, MGMT, and TWIST1). In a multivariate analysis, the presence of cagA was independently associated with elevated levels of methylation at promoters of RPRM, APC, and MGMT (30). Whether this association is a result of the greater
inflammatory response induced by cagA-positive strains (31) or to one of the other effects of cagA described earlier is not yet clear.

The current study by Niwa and colleagues is another example of the use of the Mongolian gerbil model for H. pylori–promoted gastric cancer, as this model recapitulates most closely the human disease, among small animal models. Wild-type mice do not develop gastric cancer after inoculation with H. pylori, and even transgenic models such as INS-GAS mice do not fully reproduce human gastric cancer as well as the gerbil. Future efforts to understand inflammation-promoted gastric cancer may include sequencing of the gerbil genome, as well as the development of species-specific reagents. Studies such as the current investigation also highlight the importance of studying microbial and host constituents in a conjoined fashion rather than in isolation. A holistic approach will permit more detailed insights into the complex pathways that lead to gastric adenocarcinoma.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Grant Support
The authors’ work is supported in part by funding from the National Center for Research Resources (U11 RR024975-01), by NIH R01 Grants (CA28842 and CA116087), by NIH R01 Grants (DK58587 and CA77955), and NIH Grant P30DK058404.

Received February 25, 2013; accepted February 25, 2013; published online April 4, 2013.

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