Special Section

Etiology of Gastric Cancer: What Is New?

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Abstract

Recent advances in understanding of risk factors for gastric cancer have focused attention on genetic polymorphisms in both the human host and in *Helicobacter pylori*. Variation in genes for cytokines such as interleukin-1β and its receptor antagonist may allow identification of those individuals predisposed to mount an immune response that puts them at elevated risk for gastric cancer. Likewise, analysis of how genetic variation in the genome of *H. pylori* may modulate the action of virulence factors like CagA may prove useful in identification of persons for whom *H. pylori* eradication efforts would be most important. This review examines recent studies on interleukin-1β polymorphisms and *H. pylori* CagA variation with respect to their modulation of risk for gastric cancer. (Cancer Epidemiol Biomarkers Prev 2005;14(8):1865–8)

Introduction

Gastric cancer was the most common and most lethal cancer in the world during most of the 20th century. In recent decades, it has lost that dubious distinction because of gradual decrease in incidence in many countries of the most common type of gastric cancer and the increase to epidemic proportions of lung cancer. Gastric cancer still ranks as the fourth most common cancer and the second most frequent cause of cancer deaths, accounting for 10.4% of cancer deaths worldwide (1). In the United States, incidence of cancers of the distal portion of the stomach has been declining, whereas incidence of tumors of the gastric cardia has remained unchanged (2, 3) or increased (4). When analyzed by histologic subtype, the intestinal form of gastric cancer has been declining, but the diffuse subtype has increased, as a proportion of all gastric cancers (5). Unfortunately, not all studies are stratified by subtype and location, and assumptions must be made regarding tumor subtypes from the best data available regarding specific populations. Declining incidence of gastric cancer has been noted in many parts of the world, both in developing and developed countries (6, 7).

For many decades, the etiology of gastric cancer was totally obscure. Considerable efforts were made by international investigators to explore and test the hypothesis that *N*-nitroso compounds (mostly nitrosamines) were causal factors. Although small amounts of *N*-nitroso compounds were formed in the stomach, proof of causality totally evaded the investigators. Since 1983, the emphasis has gradually shifted from the search for environmental chemical carcinogens to the bacterial etiology. In 1994, the IARC classified the infection with *Helicobacter pylori* as a class I human carcinogen (8). More recently, the long suspected influence of genetic susceptibility has come to the forefront. The present state of the art points to the role of polymorphisms as major determinants of the etiologic forces. Such polymorphisms have been detected in the microbial agent as well as in the host. Their interaction apparently determines the risk for each individual. This review concentrates on newly acquired knowledge of the polymorphisms in the inflammatory cytokine genes of the host and on polymorphic genotypes of *H. pylori*.

Inflammatory Cytokines

In the absence of a direct mutagen or carcinogen in the bacterium, the chronic active inflammatory response to the infection elicited by *H. pylori* has been considered as a possible mechanism by which the infection may eventually lead to neoplasia. A chronic active inflammation may induce neoplasia by a variety of pathways that are still considered hypothetical. The immune response and the damage resulting from oxidative stress are two main proposed mechanistic candidates. Much remains to be clarified in these pathways. A major challenge is to explain why and how the infection and the resulting inflammation apparently selects which subjects enter and which do not enter into the neoplastic cascade. It is well known that patients with duodenal ulcer are not at increased gastric cancer risk in spite of their *Helicobacter*-induced chronic active antral gastritis. In the case of oxidative stress, it is suspected that antioxidant compounds (such as the micronutrients abundant in fruits) may protect epithelial cells against the carcinogenic forces launched by the bacteria.

It is therefore clear that the modulation of the inflammatory process in large part determines the (neoplastic versus nonneoplastic) outcome. One way in which such modulation may take place is the susceptibility (or resistance) of the host to the genotoxic forces brought about by the infection. One portion of this review focuses on the susceptibility markers linked to the inflammatory response, mainly the cytokines.

It has long been suspected that the gastric microenvironment may play a key role in the precancerous process. One obvious difference between neoplastic and nonneoplastic outcomes of the infection is the acid secretion of the gastric glands. Duodenal ulcer patients (not at increased cancer risk) have adequate or increased acid secretion; gastric ulcer patients (at increased cancer risk) tend to be hypochlorhydric. One proinflammatory cytokine (i.e., interleukin-1β, IL1β) is also a very potent inhibitor of acid secretion (100 times more potent than proton pump inhibitors). El-Omar et al. studied the *IL1B* gene cluster in gastric cancer patients and controls in Poland and found that the T/T genotype at the *IL1B* −511 single nucleotide polymorphism

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is associated with an elevated relative risk for gastric cancer (odds ratio, 2.6; 95% confidence interval, 1.7-3.9; ref. 9). Heterozygote carriers (*IL1B* −511 C/T) also had an elevated relative risk (odds ratio, 1.8; 95% confidence interval, 1.3-2.4). Genotypes of a variable number of tandem repeats polymorphism in the IL1β receptor antagonist gene (*IL1RN*), part of the same gene cluster, are also associated with increased risk: for the genotype *IL1RN* *2/*2 (homozygosity for the short allele), the odds ratio is 3.7 (95% confidence interval, 2.4-5.7). These seminal studies showed that the combination of proinflammatory and acid suppression functions of the gene cluster represent a potent carcinogenic influence for the host. A new avenue of research was opened and has been followed by numerous studies in several populations at risk. The findings have been confirmed in other populations of European ancestry: Northern (White) populations of the United States (10) and Portuguese (11, 12).

A study in Portugal took advantage of a screening program to detect gastric premalignant lesions in a cohort of shipyard workers subjected to gastroscopy and biopsies in 1998 (12). In them, both susceptibility polymorphisms in the host as well as the genotypes of the infecting *H. pylori* bacteria were determined. The analysis comparing gastric cancer with nonatrophic gastritis patients (without atrophy or metaplasia) revealed a markedly increased risk of gastric cancer when highly susceptible individuals were infected with the most virulent bacteria. Subjects who were carriers of the *IL1B* 511T polymorphism, infected with VacA *s1 Helicobacter* had a relative risk of 87 (95% confidence interval, 11-679) when compared with *IL1B* C homozygotes infected with VacA s2 *Helicobacter*. Similarly, CagA-positive bacteria infecting *IL1B* (−511) T carriers were associated with a relative risk of cancer 25 times greater than that of CagA-negative bacteria infecting *IL1B* (−511) C homozygote individuals. These results clearly illustrate that the effects of genetic and bacterial polymorphisms combine to increase gastric cancer incidence significantly.

The generalizability of the results on the cytokine susceptibility polymorphisms to non-European populations is under investigation. Studies in China in low-risk areas have reported similar results to those of the European findings, for the *IL1B* −511 association with gastric cancer (13). However, the same authors reported that in high-risk Chinese populations, the proportion of *IL1B* (−511) high-risk genotypes was similar in cases and controls. The reasons for the lack of association in high-risk regions are not clear. The high-risk cytokine alleles tend to be less frequent in controls from the low-risk populations compared with those of high risk: *1L1B* (−511) T allele frequency in controls from the low-risk area was 0.34 compared with 0.51 in the high-risk area. Similar higher prevalence of high-risk cytokine alleles in populations at high cancer risk compared with those in low cancer risk populations has been reported (14). It would seem that in high cancer risk populations a high proportion of subjects is very susceptible to cancer development. This may be one reason why the high susceptibility markers are less discriminatory in high cancer risk populations.

The potential of these cytokine polymorphisms as markers remains to be fully exploited. Although it is reasonable that the polymorphisms may be causative or contributory to cancer risk, this has not yet been proven and it awaits further epidemiologic and experimental confirmation. However, the discovery of markers for increased risk, whether causative or not, may facilitate screening for high-risk individuals.

Genetic Variation in the *Helicobacter* Genome

The remarkable genetic diversity of *H. pylori* has been well noted (15, 16), but whether and how this diversity may contribute to the wide variation in incidence rates of gastric cancer throughout the world is still being explored.

An important polymorphic virulence factor is the secreted vacuolating cytotoxin, VacA. The protein inserts itself into the membrane, forming an anion-selective pore (17). VacA causes depolarization of the epithelial cell's membrane potential (18), apoptosis (19-22), inhibition of epithelial cell attachment (23), and inhibition of T-cell activation (24). The *vacA* gene has two variable regions: the region coding for the signal peptide, which exists in *s1a*, *s1b*, *s1c*, or *s2* alleles and the middle region, which consists of *m1*, *m2a*, and *m2b* alleles (25, 26). Strains bearing *s1* and *m1* alleles have been long noted as being more virulent than *s2 m2* strains (27). Curiously, although all strains of *H. pylori* contain the *vacA* gene, not all secrete the protein. Secretion of VacA protein is associated with the presence of the CagA protein (28, 29).

The CagA protein was originally discovered as a highly immunodominant 128-kDa protein produced by some *H. pylori* strains (28, 29). Although not present in every isolate, where present, this marker is associated with more severe clinical outcomes, such as peptic ulcer disease and gastric adenocarcinoma (30-33). This *cagA* gene locus is a marker for the pathogenicity island (PAI), a 37-kb insertion into the glutamate racemase gene in the *H. pylori* chromosome. Other genes in the PAI encode proteins that form a type IV secretion apparatus, which serves to inject the CagA protein into gastric epithelial cells (34-38). Infection of CagA-positive strains of *H. pylori* of gastric epithelial cells is associated with the induction of cytokines such as IL-8 (39, 40), granulocyte-monocyte colony-stimulating factor, tumor necrosis factor-α, and nuclear factor-κB (41-43).

After the CagA protein is injected into gastric epithelial cells via the type IV secretion apparatus, the protein binds to the inner surface of the host plasma membrane and becomes tyrosine phosphorylated by host *Src* family tyrosine kinases (34-38). In *in vitro* studies, injection of gastric epithelial cells with CagA is accompanied by cell scattering and extension of cell processes resulting in the formation of the so-called ''hummingbird'' phenotype (34, 44, 45). The kinases identified as responsible for phosphorylation of CagA are c-Src and Lyn, two members of the Src kinase family, which are membrane anchored (45). The mechanism creating the hummingbird phenotype has been variously explained. One model (44) proposes that the presence of phosphorylated CagA inhibits the responsible kinase, c-Src, in a classic negative feedback loop. Inhibition of c-Src leads to dephosphorylation of cortactin, an actin-binding protein. The dephosphorylation of cortactin causes an alteration in its location within the cytoskeleton, causing cortactin to colocalize with filamentous-actin in the tip and base of cell projections, leading to cell scattering in the hummingbird phenotype.

Another model (46) for the generation of the hummingbird phenotype focuses on the interaction of phosphorylated CagA with a different enzyme: the Src homology 2-containing tyrosine phosphatase (SHP-2). SHP-2 contains two Src homology 2 (SH2) domains, both of which are required for CagA binding activity. Binding of phosphotyrosine moieties to the SH2 domains relieves inhibition of phosphatase activity, which alters host signal transduction pathways by activation of the phosphatase. Because SHP-2 is involved in regulating cell spreading, migration, and adhesion, the activation of SHP-2 caused by CagA is a rational mechanism for dysregulation of epithelial signal transduction pathways by CagA.

This model, taken in consideration with the variation in the COOH-terminal of the CagA protein (distinctive in some Asian strains), reveals a possible contributor to the high incidence of gastric cancer in Asian countries: variation in that portion of CagA affects the strength of SHP-2 binding. The COOH-terminal of CagA contains a variable number of EPIYA (glutamic acid-proline-isoleucine-tyrosine-alanine) motifs, which are potential sites of tyrosine phosphorylation (28, 29). Examining this region in Japanese strains, Yamaoka

et al. noted four variants that differ in molecular weight and number of EPIYA motifs (47, 48). Although 94% of 155 strains sequenced had three EPIYA motifs, seven of the strains had four EPIYA motifs and a longer replicated segment. The latter strains were associated with gastric cancer. In addition, Yamaoka et al. also reported a repeat segment that differs in sequence in Western versus Asian strains (47). Others have also reported variations in this region, with up to seven EPIYA motifs (49).

In site-directed mutagenesis experiments involving sequences with EPIYA motifs (50), several studies have focused attention on the importance of the EPIYA sequences which are contained within repeats [called EPIYA-D1 by Covacci et al. (29) but EPIYA-C or EPIYA D by Higashi et al. (50)]. These EPIYA motifs, reported to be the major phosphorylation sites in the CagA protein, are surrounded by regions that differ in Western and Eastern strains, as previously noted by Yamaoka et al. (47). Western and some Asian strains contain within the repeat a ''Western CagA-specific sequence,'' or WSS, and a subset of strains isolated in Asia contain the ''East Asian CagA-specific sequence,'' or ESS. In studies comparing ESS versus WSS strains, Higashi et al. found approximately equal phosphorylation in the ESS and WSS strains but a greater binding affinity for SHP-2 in the ESS strains compared with the WSS strains. Furthermore, this difference was associated with an increased ability in the Asian strains to alter gastric epithelial cell shape into the hummingbird phenotype (50). The site responsible for this difference between ESS and WSS strains was identified as the amino acid 5 positions towards the COOH-terminal from the tyrosine of the EPIYA C (phenylalanine in ESS and aspartic acid in WSS). Grades of inflammation, activity of gastritis and atrophy were higher in the ESS strains, and all strains isolated from gastric cancer patients were ESS strains (51). Argent et al. have recently reported that even strains bearing Western-patterned *cagA* genes can vary in EPIYA motifs and that this difference may have functional consequences (52). They found variation of three to six EPIYA phosphorylation motifs within the Western-type CagA, and the strains with the higher number of motifs were associated with more IL-8 secretion and more epithelial cell elongation. Those strains were also more frequently found in patients with gastric cancer.

We eagerly await further examination of the effect of the CagA protein on the signal transduction pathways of the epithelial cell. If variation in the COOH-terminal of the CagA protein modulates the effect of CagA on signal transduction in the gastric epithelial cell in the human stomach, it will be interesting to learn if this variation can have practical utility in screening in populations with high incidence of gastric cancer.

Epilogue

The acknowledged bacterial etiology of gastric cancer is offering opportunities to advance our understanding of cancer causation. The interplay of polymorphic variants of the agent (*H. pylori*) and those of the host (human inflammatory cytokines) may represent major forces whose interplay determines the (neoplastic versus nonneoplastic) outcome of the cellular injury. Clarifying the etiology of gastric cancer could throw light into the pathogenesis of other cancers, especially those in which chronic active inflammation is suspected to play a role, such as carcinoma of the cervix, liver and large bowel, and perhaps even prostate cancer. The lack of a carcinogenic role of active antral gastritis associated with duodenal ulcer should be investigated, as it may hold the clue for prevention. It indicates that a neoplastic outcome is not a necessary consequence of chronic active inflammation. Defense mechanisms such as antioxidants should be investigated for their potential in cancer prevention.

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