Gastric Cancer Associated with *Helicobacter pylori*

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Abstract: In spite of a decline in incidence and mortality of gastric cancer over the last decades, it is still the fourth most common cancer and the second most common cause of cancer death in the world. The differences in prevalence of gastric cancer have been explained as a multifactorial process with an interaction involving both infection with *Helicobacter pylori* as a triggering factor and host genetic susceptibility as an important explanation for interindividual variation in gastric cancer risk. At present, there is no definitive host genetic risk marker, and evidence suggests that each proposed host risk factor should be evaluated in specific ethnic populations to define its importance. In this review, we discuss the most relevant up to date data on genetic polymorphisms that have been associated with an increased risk for the development of gastric cancer, its potential role in the development of this neoplasia, and its interplay with the virulence factors of the bacteria.

Introduction

1. Epidemiology of gastric cancer

Stomach cancer is the fourth most common cancer worldwide with 930,000 cases diagnosed in 2002. Despite a major decline in incidence and mortality over the last decades, stomach cancer is still the fourth most common cancer and the second most common cause of cancer death in the world, accounting for more than 803,000 deaths each year. It has been observed that there is a 10-fold variation in incidence between populations with the highest and lowest risk. For instance, the incidence of gastric adenocarcinoma is higher in East Asia, Central America, and South America than in most other parts of the world and is about twice as high among men. This neoplasia is rare before the age of 40, and its incidence peaks in the seventh decade of life. According to the National Cancer Institute, gastric cancer is more common in people over the age of 72 (National Cancer Institute) and the diagnosis of gastric cancers is frequently made based on dyspeptic and alarm symptoms. Unfortunately, sometimes alarm symptoms are not sufficiently sensitive to detect malignancies. Dysphagia, weight loss and a palpable abdominal mass appear to be major independent prognostic factors in gastric cancer, but when these symptoms appear, the patients are usually in advanced stages of cancer. The difference in the prevalence of gastric cancer throughout the world has been described from different points of view. One of the most accepted explanations is that the development of gastric cancer is multifactorial with an interplay involving both infection with *Helicobacter pylori* and host polymorphisms in a process initiated by specific *H. pylori* genotypes and the host immune response.

2. Gastric cancer and *Helicobacter pylori*

*H. pylori* are a Gram-negative spiral-shaped bacterium that persistently colonizes the human stomach. It is the most common chronic bacterial infection worldwide and is associated with diverse clinical outcomes that range from asymptomatic gastritis to more serious conditions, such as peptic ulcer disease and gastric cancer. In general, countries with a high incidence of stomach cancer have a high prevalence of *H. pylori* infection but in Europe only
a small fraction of those infected by *H. pylori* develop stomach cancer. In Japan, the country with the highest incidence of stomach cancer in the world, it has been estimated that of the 60 million people infected by *H. pylori*, only 0.4% had stomach cancer. The worldwide prevalence of *H. pylori* is more than 50% in the adult population and the incidence of *H. pylori* related diseases varies considerably throughout the world. After the discovery of *H. pylori*, it was reported that *H pylori*-positive subjects have a two to three-fold increased risk of developing gastric cancer when compared with *H pylori*-negative subjects. The risk was even higher in subjects infected with strains encoding the *H pylori cagA, vacAs1* and *babA2*, which are the main virulence genes related to this bacterium.

One of the most prominent differences in gene content among *H. pylori* strains is the presence or absence of a 40-kb region of chromosomal DNA known as the cag pathogenicity island (PAI). This island involves the cagA-encoded CagA protein, which is delivered into gastric epithelial cells via the bacterial type IV secretion system, where it undergoes tyrosine phosphorylation by Src and Abl kinases. Tyrosine-phosphorylated CagA then acquires the ability to interact with and deregulate SHP-2 phosphatase, the deregulation of this enzyme is involved in a variety of human malignancies (CagA also alter tight junctions and epithelial apical-basolateral polarity. These CagA activities may collectively contribute to the transformation of gastric epithelial cells. Despite the overwhelming evidence that *H. pylori* infection is a risk factor for noncardia gastric cancer, accumulating evidence shows that although *H. pylori* eradication is relatively simple to accomplish. Impacting the global burden of gastric cancer will be a more difficult challenge. *H. pylori* infection is a key risk factor for chronic atrophic gastritis, an established precursor of gastric cancer. There is increasing evidence of frequent elimination of the infection during progression of chronic atrophic gastritis and it has been proposed that there is a higher elimination of *H. pylori* during the development of the disease. *H. pylori, cagA+* strain, is an established risk factor for stomach cancer and *H. pylori* infection and cagA+ status have been inversely associated with a new diagnosis of Barrett’s esophagus. The findings are consistent with the hypothesis that *H. pylori* colonization protects against Barrett’s esophagus and that the association may be at least partially mediated through Gastroesophageal Reflux Disease (GERD).

It has been recently proven that *H pylori* infection is associated with significantly reduced risks of esophageal adenocarcinoma and adenocarcinomas of the esophagogastric junction but not with squamous cell carcinomas. Infection with cagA-positive *H. pylori* seems to play an essential role in the development of gastric carcinoma, although the bacteria alone cannot be considered a unique factor in the promotion of gastric cancer. Host susceptibility has also been involved in this process, the interaction of some *H. pylori* genotypes, in relation to host polymorphisms can lead to gastric cancer. When *H. pylori* is eliminated in patients treated for early stage gastric cancer, the risk of developing a second gastric cancer decreases by two-thirds.

### 3. Host genetic polymorphisms and cancer susceptibility

Host genetic factors play an important role in influencing disease risk, but identifying candidate genes is a major challenge that requires a fundamental understanding of the disease. The best-established risk factors for stomach cancer are *H. pylori* infection--by far the strongest established risk factor for distal stomach cancer--as well as male gender, a family history of stomach cancer, and smoking.

### 4. Gastric carcinogenesis

The precancerous process had been the subject of inquiry way before the scientific community was aware of *H. pylori* as a human pathogen. The histopathology of the precancerous stages has long been recognized: chronic gastritis, gland loss (atrophy), intestinal metaplasia (complete and incomplete), and epithelial dysplasia.
Progression of the process in high-risk populations has been documented.

Modified model of carcinogenesis. *H. pylori* acts as a pivotal factor in the development of gastritis. Increased chronic inflammation is influenced by genetic susceptibility factors (first stage genes) and progression of the disease is strongly influenced by second stage genes.

The discovery of *H. pylori* strongly supported the carcinogenesis model triggering the response with other factors having important roles in the progression thorough the carcinogenesis cascade. In the next paragraphs, some of the host factors involved will be discussed. The first group of genes related to host susceptibility is the “first stage genes”, i.e., which influence the first stage of the cascade and are related to a more intense inflammatory response after gastritis associated to *H. pylori* infection is clearly established in the stomach.

“Second stage genes” seem to have an important role after atrophic gastritis has been established. The inflammatory process leads to *in situ* mutations and strong activity of antitumor genes such as p53 and oncogenes such as c-fos, c-jun c-met, K-sam, and K-ras. Several pathogenic pathways suggesting how the infection could increase the risk of gastric cancer have been proposed, but it is thought that long-term chronic inflammation represents the primary mechanism.

*H. pylori* is specifically adapted to survive in the hostile acidic gastric environment and the colonization of the stomach by *H. pylori* results in the development of gastritis in virtually all infected subjects. The attachment of the bacteria to the epithelial cells induces an inflammatory response resulting in the recruitment of neutrophils followed by B and T lymphocytes, macrophages and plasma cells, most of which generate large amounts of reactive oxygen or nitrogen species, implicated in epithelial cell damage and carcinogenesis Gastric mucosal damage induced by *H. pylori* infection is also due to the bacterial virulence factors encoded by the *cag* pathogenicity island, such as the vacuolating cytotoxin A (VacA) and the CagA protein (CagA). The first is a secreted toxin that enters the epithelial cell membrane, induces vacuole formation and affects mitochondria leading to apoptosis. On the other hand, CagA, upon entering the epithelial cell, induces proliferation and motility signals, as well as production of cytokines [24]. *H. pylori*-induced gastritis is driven by a variety of bacterial factors that stimulate epithelial cell, macrophage, and DC activation, as well as a Th1-predominant lymphocyte response; the role of the Th17 versus Th1 response is an area of intense investigation.
5. First stage genes

Interleukin 1 Family

The association of *IL-1* polymorphisms to gastric cancer has a deep principle: alleles *IL-1B-31*C, *IL-1B-511*T, and *IL-1RN*2, lead to high-level expression of IL-1β, reduction of acid output, corpus-predominant colonization by *H. pylori*, pangastritis, atrophic gastritis, and increased risk of gastric cancer. The importance of the *IL-1beta* gene polymorphism with an increased risk of developing hypochlorhydria and gastric atrophy, which is considered a gastric cancer precursor, was first demonstrated in a Caucasian population and later reported in other populations. El Omar et al. found that risk increases progressively and that individuals with three or four polymorphisms in *IL-1*, *IL1-RN* or *IL10* and infected with *H. pylori* have a 27-fold increased risk of developing non-cardia cancer. The interaction between a host’s immunological defenses, and environmental and *H. pylori* virulence factors play an important role in the development of gastric cancer. Other researchers confirmed that the allelic variation in IL1-beta seems insufficient for the development of gastric cancer. Figueiredo et al. investigated combinations of bacterial and host genotypes in association with gastric cancer and found that a high proportion of gastric carcinoma patients were carriers of *IL-1 beta-511*T (69%) allele. The results on the association between *IL-1 ß* polymorphisms and gastric cancer risk remained inconclusive. An association between these polymorphisms was not found in some populations suggesting that this divergence may reflect the different genetic background related to ethnicity and the potential confounding variables, such as *H. pylori* status and family history of malignancy.

Interleukin 8 and TNFA

Since the watershed publication of El-Omar et al. linking polymorphisms in genes regulating the gastric inflammatory responses to gastric cancer risk due to *H. pylori*, many groups have been investigating other susceptibility loci ruled by polymorphic alleles, particularly those of the innate immune response. One of the most studied is Interleukin-8 (*IL-8*), which is a potent chemokine that may play a role in gastric cancer pathogenesis. Gastric cancer specimens have increased IL-8 protein levels, and many gastric cancer cell lines express high levels of *IL-8* mRNA and the protein. The *IL-8-251* polymorphism might be a host susceptibility factor for gastric carcinoma development and angiogenesis in gastric carcinogenesis but this association is likely to be ethnic-specific [68] because several studies have reported an association with gastric cancer. This association has not been confirmed in studies of Caucasian populations. Another of the studied genes is TNFA. In relation to this gene, the TNFA-308 allele, which is thought to increase the production of TNF-alfa, confers an increased risk for the development of gastric cancer. This polymorphism increases the risk for non-cardia gastric cancer by approximately two-fold.

NOD1, NOD2, COX2, ROS and RNS

The pathogen-associated intracellular recognition molecules NOD1 and NOD2 are important regulators of chronic inflammation. NOD1 appears to be involved in the activation of a key transcription factor, NF-kB, by the Cag pathogenicity island. Rosenstiel et al. reported that NOD1 and NOD2 were upregulated in the gastric epithelial cells of patients with chronic infection by *H. pylori*, the NOD2 variant R702W was more prevalent in patients with gastric lymphoma than in *H. pylori*-infected individuals with gastritis or gastric ulcers. Cyclooxygenase2 (COX-2) has long been known to be over-expressed in gastric cancer and in *H. pylori* infection. Studies of gastric cancer cases and controls with preneoplastic lesions from China showed an association between specific COX-2
genotypes with high level COX-2 expression and gastric cancer risk. On the other hand, Reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been implicated in increasing gastric inflammation and therefore carcinogenesis. The mechanism by which the bacteria induce gastric carcinogenesis is not defined. It has been reported that *H. pylori* produces ROS in addition to ROS/RNS by activated neutrophils. Some studies have revealed that *H. pylori*-induced ROS production affects gastric epithelial cell signal transduction, resulting in gastric carcinogenesis. ROS/RNS production in the stomach can damage DNA in gastric epithelial cells, thus increasing the risk of gastric carcinogenesis.

**Toll-like receptor 4**

Recognition of pathogens is mediated by a set of germline-encoded receptors that are referred to as pattern-recognition receptors (PRRs). These receptors recognize conserved molecular patterns, which are found in many species of microorganisms. An important PRR is Toll-like receptor 4 (TLR4), a transmembrane receptor that recognizes a range of ligands, including lipopolysaccharide (LPS), which is found in Gram-negative bacteria like *H. pylori*. TLR-4 polymorphisms have been associated with a variety of inflammatory conditions, where defective signaling through TLR-4 is thought to trigger an inappropriate inflammatory response. Two single nucleotide polymorphisms (SNPs) in the *TLR4* gene, Asp299Gly and Thr399Ile transitions, have been shown to lead to hyporesponsiveness to LPS, reduced epithelial TLR4 density and reduced inflammatory cytokine response to LPS [42]. TLR4 Asp299Gly and Thr399Ile polymorphisms have been reported to be a risk factor for gastric carcinoma or its precursors in Caucasian and Indian populations. Also the TLR4+3725 G/C polymorphism has been described as a risk factor of severe gastric atrophy in *H. pylori* seropositive Japanese. Hold et al. addressed the role of TLR with respect to *H. pylori* infection in gastric carcinogenesis by the study of patients previously investigated for cytokine polymorphisms and susceptibility to gastric cancer from Poland, Scotland, and the United States. An association between a polymorphism in TLR-4 and an increased risk of noncardia gastric cancer and its precursor lesions including achlorhydria was identified in that study. This association was specific for noncardia gastric cancer as it was not observed in esophageal or gastric cardia cases and remained even after correcting for the polymorphic variations in IL-1β and theIL-1 receptor previously documented by this group.

**Interleukin 10**

Interleukin-10 (IL-10) is an anti-inflammatory cytokine that downregulates the production of Th1-derived cytokines and seems to limit and terminate the inflammatory response by the blocking proinflammatory cytokine secretion. Some functional polymorphisms have been described for the IL-10 gene promoter. The single-nucleotide polymorphisms (SNP) at positions -1082 (G/A), −819 (C/T), and −592 (C/A) from the transcriptional start site are in linkage disequilibrium, and are responsible for three different haplotypes formed by the combination of ATA, GCC and ACC. The IL-10 haplotypes and cytokine production have been correlated with counterpointing results. A higher prevalence of gastric cancer in patients with the proinflammatory (low IL-10 secreting) haplotype ATA has been reported, but contrasting results have also reported an association between carcinoma and the anti-inflammatory (high IL-10 secreting) haplotype GCC. The study of Rad et al. showed that this contrastive observation might be explained by the finding that cagA + strains were more prevalent among GCC carriers. Further studies are needed to clarify the role of IL-10 polymorphisms in *H pylori* infection.
6. Second stage genes

External environmental exposures play an important role. These can give rise to a number of different genetic changes, the most common of which include chromosomal changes such as loss of heterozygosity (LOH), rearrangements, deletions, gains, and translocations; gene mutations such as base substitutions, small insertions and deletions, allelic loss, amplification and rearrangements; and epigenetic events such as alteration in DNA methylation. Loss of tumor suppressor function leads to the initiation and progression of cancer. Inactivation of tumor suppressor genes can result from both genetic mechanisms, such as mutation, and epigenetic mechanisms, such as DNA hypermethylation.

**E-cadherin**

E-cadherin is a calcium dependent cell-cell adhesion glycoprotein. Mutations in this gene are associated with gastric, breast, colorectal, thyroid and ovarian cancer. It has been reported that promoter hypermethylation of E-cadherin plays an important role in gastric carcinogenesis. Evidence shows that a heterozygous germline point or small frameshift mutations in the E-cadherin gene (CDH1, OMIM +192090) are associated with diffuse cancer [81]. Carriers of CDH1 germline mutations have an accumulative GC risk, before age 75, of 40–67% for men and 63–83% for women.

**P53**

The p53 gene has been called the genome guard is critical in maintaining orderly proliferation of cells. Normally, damage to cellular DNA initiates increased expression of p53 that may lead to the arrest of the cell cycle. This interruption allows DNA repair to occur before abnormal proliferation is produced. If DNA repair is not successful, then the cell undergoes apoptosis to avoid proliferation of mutated cells. When p53 mutates, DNA-damaged cells are not arrested in G1 and DNA repair does not take place, allowing other mutations to accumulate and conduce to neoplastic transformation and cancer. Mutation of p53 is probably the most significant genetic change characterizing the transformation of cells from normal to malignant [83]. With this principle, one of the most known polymorphisms in p53 have been studied in relation to gastric cancer and some polymorphisms in this gene have been associated with the development of distal GC in Mexican, Chinese, Korean and Japanese populations. A meta-analysis suggests that the p53 codon 72 polymorphism may be associated with gastric cancer particularly among Asians, and that the difference in genotype distribution may be associated with the location, stage, and histological differentiation of gastric cancer.

**Oncogenes**

Once activated, a proto-oncogene or its product is a tumor-inducing protein. Some of the well-known oncogenes are RAS, WNT, MYC, ERK, and TRK. Among these, one of the most studied is MYC, which is involved in multiple cellular functions, such as cell cycle regulation, cell growth arrest, cell adhesion, metabolism, ribosome biogenesis, protein synthesis, and mitochondrial function. It has a main role in several carcinogenesis processes in humans. The RAS family is responsible for cell proliferation and functions. They act as a switch that controls intracellular signaling networks in processes such as actin cytoskeleton integrity, proliferation, differentiation, cell adhesion, apoptosis and cell migration. Ras proteins are often deregulated in cancers, leading to increased invasion and decreased apoptosis. Mutations in the RAS family are common, and have been found in 20% to 30% of all human tumors, but no specific mutation has been consistently related to gastric cancer.
Conclusions

Host genetic susceptibility has been suggested as one of the most important possible explanations for interindividual differences in gastric cancer risk and even to tumor invasion. In the first stage, inflammation seems to play a critical role in the development of many types of cancer, including gastric cancer and genetic changes in gene coding some crucial mediators in the inflammatory response may play an essential role in Helicobacter pylori-infected individuals. In the second stage, participation of tumor suppressor proteins and oncogenes seems to define the course of the disease. There are currently no definitive genetic risk markers for gastric cancer risk that can be applied to all populations. We need to recognize that distal gastric cancer is a multifactorial event. We do not discuss the effect of the environment that may influence both bacteria and the host factors.

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