***Helicobacter pylori* and Epigenetic Mechanisms Underlying Gastric Carcinogenesis**

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Key Words

Epigenetic mechanisms • Gastric carcinogenesis

Abstract

Gastric carcinogenesis is a multistep process triggered by *Helicobacter pylori* and characterized by accumulation of molecular alterations. Two mechanisms are implicated in cancer-related molecular alterations: genetic and epigenetic. The former includes changes in the DNA sequence, the latter occurs without changes of DNA sequence. However, the most important difference between genetic and epigenetic alterations is that epigenetic changes are potentially reversible by eliminating toxic agents. DNA methylation is the major epigenetic phenomenon of eukaryotic genomes and involves the addition of a methyl group to the carbon 5 position of the cytosine ring within the CpG dinucleotide. DNA methylation is needed for the normal development of cells, whereas aberrant methylation of CpG islands confers a selective growth advantage that results in cancerous growth. The stomach is one of the organs frequently showing aberrant methylation of DNA epithelial cells because of its accessibility to exogenous toxic agents such as *H. pylori* infection. Aberrant methylation of CpG islands occurs early in gastric carcinogenesis, tends to increase as the process advances and is prevalently related to the infection. In conclusion, gas-

tric cancer is mainly an epigenetic disease and *H. pylori*, acting through inflammatory mediators, may play a key role in the development of such molecular alterations.

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Gastric cancer (GC) remains a major clinical challenge due to its frequency, poor prognosis and limited treatment options. According to the International Gastric Cancer Society, >1,000,000 people are affected by GC every year and up to 800,000 people succumbed to GC estimating that the 5 year-relative survival rate is <20% [1]. GC is often resistant to radio- and chemotherapy and, indeed, surgery represents the only treatment with a curative potential. However, two-thirds of GC patients from westernized countries are still diagnosed in advanced stages, when surgery can be only palliative [2]. Therefore, one of the primary objectives of the World Health Organization and researchers is to establish programs for GC prevention. However, to be successful, this strategy depends on understanding the etiological factors and molecular alterations underlying gastric carcinogenesis.

The most important factor implicated in gastric carcinogenesis is *Helicobacter pylori* that has been designated a first-class carcinogen by the International Agency for Research on Cancer [3].

The causal link between *H. pylori* and the development of GC has been postulated based on epidemiological investigations (retrospective, case-control and prospective) and animal model studies [4–6]. According to a recent meta-analysis, concurrent or previous *H. pylori* infection is associated with an increased risk of GC versus uninfected people (OR 3.0; 95% CI 2.3–3.8). The risk is stronger when blood samples for *H. pylori* serology are collected 10 years before cancer diagnosis (OR 5.9; 95% CI 3.4–10.3) [7].

From a molecular viewpoint, GC may be considered the end result of a progressive accumulation of genotypic changes [8, 9]. Two mechanisms have been implicated in cancer-related molecular alterations: genetic and epigenetic. The former includes changes in DNA sequence (chromosomal rearrangement mutations, deletions, etc.), the latter occurs without changes of DNA sequence. In contrast to genetic alterations, epigenetic changes have not been extensively studied because they have been considered to play a minor role in carcinogenesis. Both genetic and epigenetic alterations are stably inherited over all cell divisions, although in tumoral cells 90% of heritable alterations are of epigenetic nature and 10% genetic [10].

However, the most important difference between genetic and epigenetic alterations is that epigenetic changes can be reversed by eliminating the toxic agents or with the use of therapeutic interventions and chemical agents, consequently, they are an important potential target for an innovative therapy [11]. Two mechanisms are implicated in epigenetic changes: histone acetylation and DNA methylation, which work in close concert and play a key role in chromatin structure and remodeling. The chromatin region is transcriptionally active when it is acetylated and hypomethylated, and is transcriptionally inactive when it is deacetylated and hypermethylated.

DNA methylation is the major epigenetic phenomenon of eukaryotic genomes and involves the addition of a methyl group to the carbon 5 position of the cytosine ring within the CpG dinucleotide [12, 13]. The eukaryotic genome is rich in CpG dinucleotides, but they are depleted during development by spontaneous deamination. The remaining CpG dinucleotides are prevalently found in the 5' promoter regions of the genes called 'CpG islands' and, are usually unmethylated. One of the fundamental aspects of DNA methylation is its role in regulation of gene expression and particularly in transcriptional repression due to the formation of a transcriptionally silent chromatin conformation [10, 14, 15].

Table 1. Frequency of hypermethylated genes in gastric carcinogenesis (synthesis of 39 studies)

	Normal %	CG %	IM %	AD %	GC %
Signal transduction					
APC	69	65	81	72	78
RASSF1A	0.4	0	0	0	23
Cell cycle regulation					
CDH1	38	85	57	58	61
CHFR	3.1	–	–	–	37
P14/ARF	9.4	30	20	31	33
p15/INK4B	9.6	–	11	–	58
p16/INK4A	4.9	2.7	7	11	38
Inflammatory response					
COX-2	4.1	1.4	8.8	3.8	27
Apoptosis					
DAP-K	55	35	44	34	54
DNA repair					
GSTP1	0	0	0	0	14
hMLH1	1.7	0	7	9.8	24
MGMT	25	15	8.8	10	23
Growth factor					
HPP1	3.1	–	–	–	50
Transcription factor					
RUNX3	9	8.1	28	28	65
Angiogenesis					
THBS1	1.9	18	49	34	30
TIMP3	20	19	44	28	43

Modified from Sato and Meltzer [22].

CG = Chronic gastritis; IM = intestinal metaplasia; AD = adenoma; GC = gastric cancer.

DNA methylation is needed for the normal development of cells, and is restricted to physiologic situations such as embryogenesis, balance between pluripotent and committed cells and cell differentiation. Aberrant methylation of CpG islands confers a selective growth advantage that results in cancerous growth, indeed, hypermethylation of CpG islands induces silencing of tumor suppressor genes, whereas hypomethylation induces activation of proto-oncogenes. Overall, in tumoral cells there is global DNA hypomethylation associated with regional hypermethylation [16].

The stomach is one of the organs that frequently undergoes DNA epithelial cell methylation of CpG islands. The cause remains unclear but it may be related to the easy accessibility of the tissue to exogenous agents. In addition to exogenous agents, gastric mucosa may also endogenously produce reactive oxygen species (ROS) and nitric oxide (NO) that can cause methylation of CpG islands [17].

Fig. 1. The multistep process of gastric carcinogenesis and the occurrence of epigenetic alterations over time.

GC is one of the tumors that has a high frequency of CpG island methylation. Recent studies on GC have identified a CpG island methylator phenotype (CIMP) that involves the targeting of multiple genes by promoter hypermethylation [18]. Kang et al. [19–21] analyzed the methylation profile of five genes in pre-neoplastic tissues, i.e., intestinal metaplasia and gastric adenoma, and in neoplastic lesions of gastric mucosa. The methylation index and the number of methylated genes significantly increased during the multistep process of carcinogenesis ($p < 0.001$). Since intestinal metaplasia is a typical abnormality of differentiation, it is probable that an epigenetic alteration is involved in its development.

In 2006, Sato and Meltzer [22] examined 39 studies concerning the methylation status of genes (overall 16) implicated in important cellular functions, i.e. signal transduction, cell cycle regulation, inflammation, angiogenesis, and so on (table 1). The methylation index of many genes (11 out of 16) significantly increased from chronic gastritis to GC (fig. 1). Therefore, aberrant CpG islands methylation occurs in the early stages of gastric carcinogenesis and tends to increase as the multistep process advances. Given the large number of cases, epigenetic changes appear to be the predominant mechanism in sporadic GC. However, since *H. pylori* is the major causative agent of GC, the question arises: How does it induce epigenetic alterations? And should this be the case, could *H. pylori* eradication therapy reverse epigenetic alterations?

Exposure of gastric epithelial cells to *H. pylori* results in a complex inflammatory and immune reaction with the generation of cytokines, reactive oxygen species and nitric oxide. These factors, probably by activating DNA methyltransferase, may hypermethylate CpG islands and induce gene silencing [23–25]. Thus, gastric DNA methylation may occur as a consequence of chronic exposure

to inflammatory mediators overproduced during *H. pylori* infection.

Ushijima et al. [26] recently analyzed the methylation of promoter CpG islands of 48 genes in 11 patients with *H. pylori* infection and in 11 healthy volunteers without *H. pylori* infection. Ten genes were unmethylated in both groups, whereas the remaining genes were prevalently methylated in the *H. pylori*-positive group. In three recent studies, Chan et al. [27–29] analyzed the methylation status of E-cadherin in relation to *H. pylori* infection. E-cadherin occurred prevalently in the methylated form in patients with *H. pylori* infection (53 vs. 6%, $p < 0.002$) and in the unmethylated state in *H. pylori*-negative patients (94 vs. 47%). After *H. pylori* eradication, the number of patients with methylated E-cadherin significantly decreased (19 vs. 4, $p < 0.004$), whereas the number of patients with unmethylated E-cadherin increased (22 vs. 34). Finally, during the follow-up performed at three time points after eradication therapy (0, 6 weeks and 3 years), E-cadherin remained in the methylated state in 10 patients with persistence of *H. pylori* infection. In contrast, in 25 patients who became *H. pylori*-negative, E-cadherin changed from a methylated to an unmethylated state in 10, but did not change in 2 who presented intestinal metaplasia, and changed from an unmethylated to a methylated state in 4. Thus, it appears that *H. pylori* infection can induce epigenetic alterations that may resolve if infection is treated within a reasonable time. On the other hand, the epigenetic alterations may persist and may even induce genetic alterations irrespective of eradication therapy if infection is long-standing [27–29].

Mounting evidence suggests that GC may origin from bone marrow-derived cells (BMDCs) locally recruited from blood vessels due to gastric mucosal inflammation [30–32]. Since BMDCs are very sensitive to epigenetic events, these data would also explain the epigenetic pro-

genitor model of cancer [33]. Indeed, chronic inflammation induces epigenetic alterations of recruited BMDCs that may develop genetic alterations if apoptosis fails. Thus, both epigenetic and genetic alterations may support cancer transformation and development. Finally, since epigenetic alterations are potentially reversible, they could be used to detect or predict cancer risk, to understand the cancer phenotype, and as a target for chemotherapeutic strategies.

The same methylation profile of CpG islands has been detected in cancerous tissue and in DNA derived from sputum, serum and urine. This data may suggest that methylation changes might reflect the risk an individual has of developing cancer, besides being a marker of cancer [34, 35]. In 54 patients, the agreement between DNA methylation of five common genes in serum and in cancer tissue was pretty good [36]. Miyamoto and Ushijima [37] evaluated a series of studies focusing on the serum DNA methylation status of some genes in patients with GC versus control subjects. Aberrant methylation of serum DNA in patients with cancer ranged from 10 to 83% versus 0% of a control group. A univariate analysis of clinicopathologic findings and methylation status of some genes involved in CIMP (p16, DNA mismatch repair gene hMLH1) and of 4 loci of CpG islands methylated in tumors (MINT1, MINT2, MINT25 and MINT31) showed a better survival with concordant methylation of multiple gene/loci [38]. Kanyama et al. [39] did not find any association between serum p16 aberrant methylation and histologic type, tumor invasion and metastasis. In-

terestingly, abnormal methylation was found in the serum of patients at all clinical stages suggesting that p16 hypermethylation could be a new marker for early detection of GC.

Treatment with DNA methylation inhibitors such as azacytidine and decitabine can restore the activity of tumor suppressor genes and decrease the proliferative rate of cancer cells. These agents, together with inhibitors of histone deacetylation, have proved to exert clinical activity in the treatment of some hematologic malignancies that are characterized by gene hypermethylation. Several clinical trials are currently ongoing in which cytosine analogues are being used to treat preneoplastic syndromes, particularly myeloid dysplastic syndrome. The use of these drugs administered either intravenously or subcutaneously may significantly decrease the rate of disease progression. Unfortunately, the results with solid tumors including GC have been disappointing [40].

In conclusion, GC is mainly an epigenetic disease and *H. pylori*, acting through inflammatory mediators, may play a key role in the development of such molecular alterations. In recent years much has been learnt, but much remains to be learnt to combat GC, which is one of the most frequent and lethal neoplasias worldwide. The study of epigenetic alterations offers great potential for the identification of biomarkers that can be used to detect and diagnose cancer in its earliest stages, to accurately assess individual risk and as targets for chemotherapeutic strategies.

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